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INFLUENCE OF SYSTEMIC INFLAMMATORY RESPONSE TO APPEARANCE OF NEW FOCI OF CHRONIC INFLAMMATION

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UTICAJ SISTEMSKOG INFLAMATORNOG ODGOVORA NA POJAVU NOVIH ŽARIŠTA HRONIČNE INFLAMACIJE

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ABSTRACT

Changes in the body in the presence of a chronic inflammatory process, even of a low intensity, lead to the change in the body's reactivity, having a negative impact on the development, course and clinical prognosis of newly emerging inflammatory processes. Structural changes in the vascular network in the focus of chronic inflammation and following cellular reactions that occur under the action of chemokines and cytokines are the basis for the maintenance and development of the phlogogenic process, including subsequent structural changes in tissues. The failure to resolve the inflammation leads not only to the persistence of the process in the primary focus, but also to the formation of a multitude of the so-calledpathological circles, included at the system level, causing the imbalance among proinflammatory, anti-inflammatory and pro-resolving factors. As a result, conditions are formed for the emergence of new foci of the inflammation in other organs and tissues and in the case of their realization, new vicious circles are formed that contribute to the maintenance and progression of the inflammation. The complex application of etiotropic, pathogenetic and sanogenetic principles of the treatment allows intensifying of the formation of specialized pro-resolving factors with the elimination of their relative insufficiency, contributing to the reduction of newly formed vessels and to the restoration of the normal cellular composition of the tissue as well as to the resolution of inflammation.

***Keywords**: specialized permissive mediators, chronic cytokine response, imbalance of counter-regulatory factors*

SAŽETAK

Promene u telu u prisustvu hroničnog inflamatornog procesa, čak i malog inteziteta, dovode do promene u reaktivnosti tela i imaju negativan uticaj na razvoj, tok i kliničku prognozu novih inflamatornih procesa. Strukturne promene u vaskularnoj mreži u žarištu hronične inflamacije koje prate ćelijske reakcije koje nastaju pod dejstvom hemokina i citokina su osnova za uspostavljanje i razvoj flogogenog procesa, uključujući sledeće strukturne promene u tkivima. Neuspeh da se reši problem inflamacije dovodi ne samo do postojanosti samog procesa u primarnom žarištu već i do stvaranja mnoštva takozvanih patoloških promena uključenih na sistemskom nivou, prouzrokujući disbalans između proinflamatornih i anti-inflamatornih faktora. Kao rezultat toga, javljaju se uslovi za nastanak novih žarišta inflamacije u drugim organima i tkivima i u slučaju njihovog ostvarenja, novi začarani krugovi nastaju koji doprinose održavanju i napredovanju inflamacije. Kompleksna primena etiotropnih, patogenetskih i sanogenetskih principa lečenja omogućava pojačano stvaranje specijalnih anti-inflamatornih faktora uz eliminaciju njihove relativne insuficijencije, doprinosi redukciji novo formiranih sudova i obnovi normalnog ćelijskog sastava tkiva kao i suzbijanju inflamacije.

***Ključne reči:** specijalni permisivni medijatori, hronični citokini odgovor, disbalans kontra regulatornih faktora*

ABBREVIATIONS

OA - osteoarthritis
PAMP - pathogen-associated molecular pattern
DAMP - damage-associated molecular pattern
SPM - specialized pro-resolving mediators
PRR - pattern recognition receptors
TLR - toll-like receptors
NLR - nod-like-receptors
TNF - tumor necrosis factor

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INTRODUCTION

There are people who get sick very rarely, for example, due toviral infections. At the same time, other people regularly get sick due to any randomly encountered viral infection, and their disease usually lasts for a long time, with complications. This happens most often in patients with comorbid pathology.

In the modern world, there are more and more patients with comorbid pathology. In such patients, with the appearance of a new, for example, somatic disease with the standard approach to its treatment, there is a high probability of transition to the chronic form of the course. In this connection, their choice of tactics and scope of medical measures become more complicated, including the subsequent period of time with an exacerbation of the course of one of the diseases. Thus, the prognosis for the complete cure of acquired diseases is constantly deteriorating.

Many researchers recognize that subclinical manifestations of the low-intensity inflammatory process, for example, in osteoarthritis, have a significant negative impact on the prognosis of this disease [20].

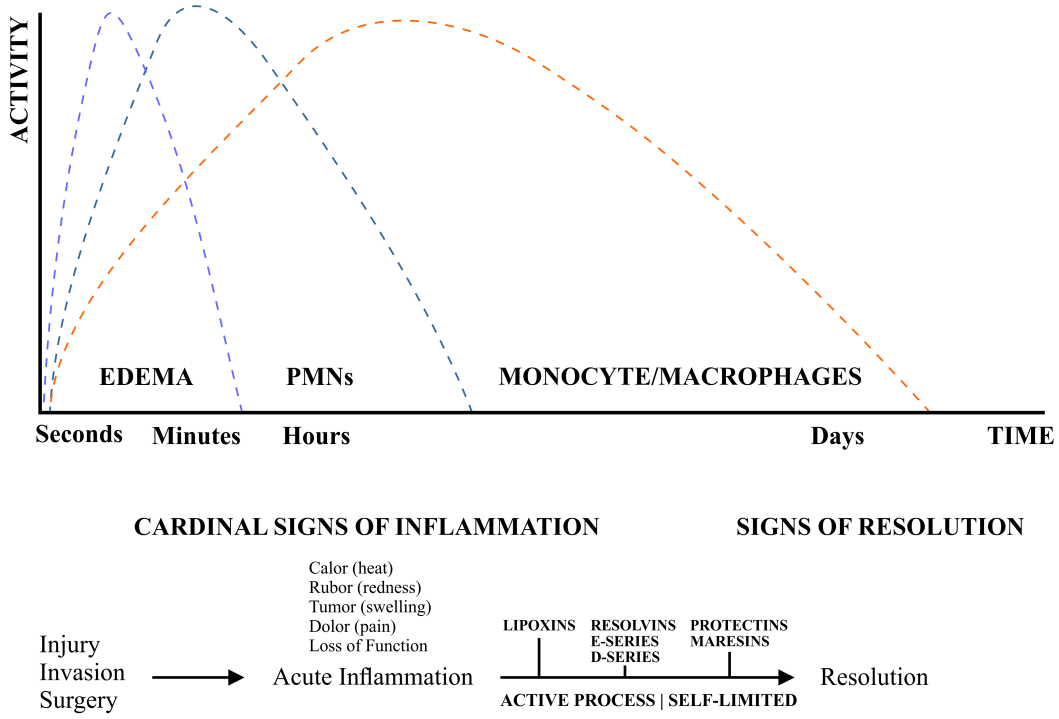
An attempt to systematize the accumulated experience on the influence of chronic inflammation in the body on the appearance of new foci and maintaining the course of the inflammatory process in them was the basis for writing this article.

RESEARCH ON THE EFFECTS OF SYSTEMIC INFLAMMATORY RESPONSE

Modern studies of the development mechanisms of many socially significant diseases have shaped the idea that their pathogenetic basis is a chronic systemic inflammation. The list of these comorbid forms of pathology is quite large including diseases of the cardiovascular system (atherosclerosis, myocardial infarction, stroke, etc.), obesity, neurodegeneration, type 2 diabetes, depression, oncology chronic kidney disease, chronic lung disease, etc. (22, 24, 28).

It is known that the development of acute inflammation occurs under a fairly tight control of counter-regulatory factors that function according to the principle of negative feedback, ensuring the coordination of phases of this adaptive response in time (5). In this case, it is customary to distinguish the initiation phase and the resolution phase (Figure 1).

Figure 1. Structure and functions of proinflammatory mediators



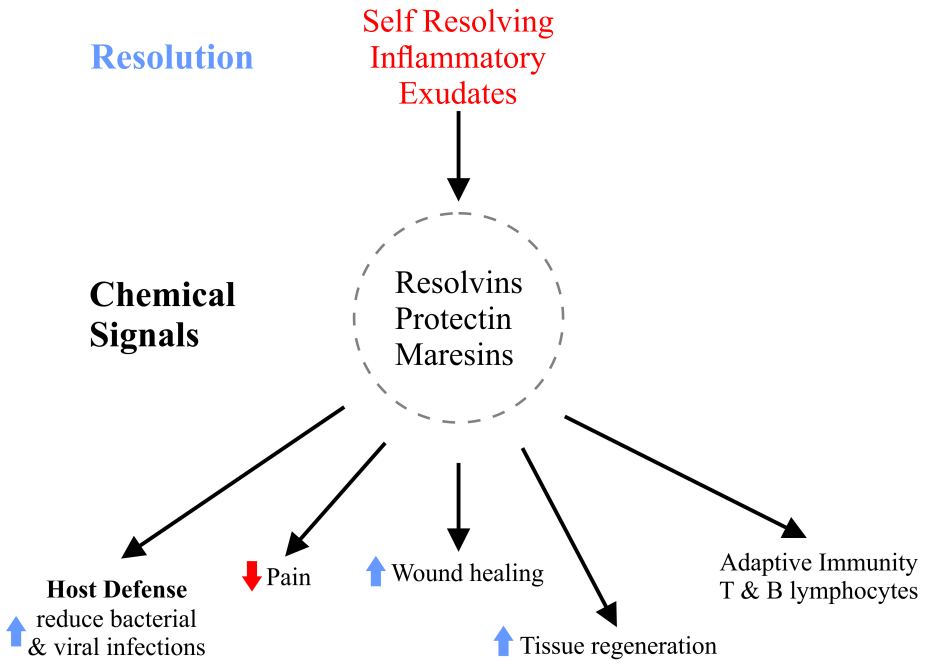
Acute inflammation was formed by an evolutionary protective-adaptive response, aimed not only to eliminate the cause, but also to eliminate the consequences of its effect, i.e. for the restoration of damaged tissues, it is strictly limited and leads to a complete resolution, which allows the return to homeostasis (25). In this regard, recently, the interest of researchers has been focused on the study of the mechanisms of flow of the resolution phase. According to the data obtained, it is the activity of development of the resolution phase that largely determines the outcome of inflammation and the restoration of function at the tissue level. C.D. Buckley, D.W. Gilroy, C.N. Serhan (2014) (4) consider the main events in this process:

1. Removal of the pathogen-associated molecular pattern (PAMP) and the damage-associated molecular pattern (DAMP).
2. Destruction of proinflammatory mediators and blockage of ways to implement their actions.
3. Suppression of emigration of polymorphonuclear leukocytes and their apoptosis.
4. Recruitment of alternatively activated macrophages for participation in efferocytosis and removal of debris.
5. Restoration of the structural integrity and normal cellular composition of the tissue.

By replacing the previous ideas that inactivating proinflammatory inflammatory mediators are enough to complete it, the understanding has emerged that resolving inflammation is a complex, coordinated, actively proceeding and controlled process. At the same time, G. Fredman, I. Tabas (2017) in their review, state (Figure 2) (12), that the resolution process is controlled by endogenous mediators of different chemical nature:

1. Specialized pro-resolving mediators (SPM), which include lipoxins, resolvins, protectins and maresins.
2. Protein mediators, such as annexin A15 and IL-10.
3. Gases, primarily carbon monoxide (CO) and hydrogen sulfide (H2S).
4. Nucleotides, such as adenosine and inosine.
5. It is quite obvious that the resolution mediators have not only local, but also the systemic effects.

Figure 2. The interaction of factors of the inflammatory process

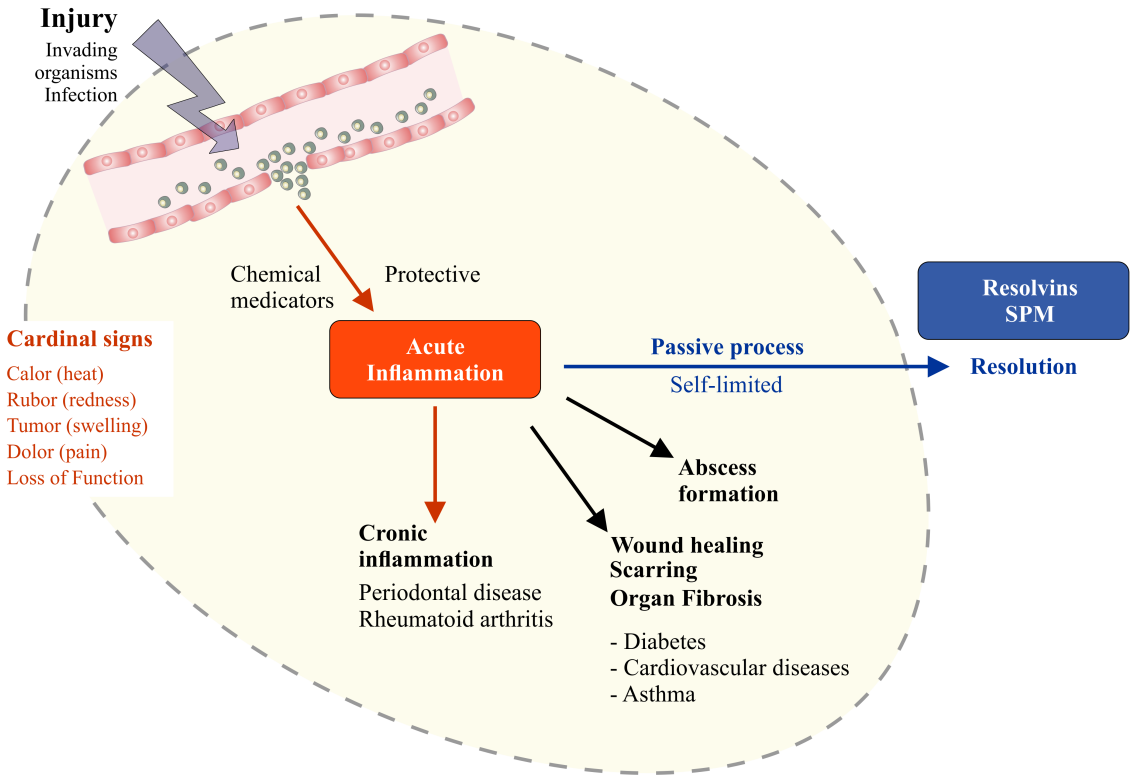


Resolution SPM signal links to system needs

From the above mentioned, it follows that various violations in the management or implementation of the system resolution mechanisms will lead to local "deficiency" of this phase in the foci of inflammation of any localization. Depending on the severity of this "deficiency", it can lead to various negative consequences: an increase in the duration of the process, up to the onset of chronic inflammation, and deterioration in the result of this protective-adaptive reaction, for example, in the form of scar tissue formation, fibrosis and dysfunction of the organ or tissue.

Imbalance of these two groups of the physiological processes, proinflammatory on the one hand, anti-inflammatory and resolving inflammation on the other, with insufficiency (absolute or relative) of resolution permissions mechanisms forms the basis of any chronic inflammation (22, 27), (Figure 3).

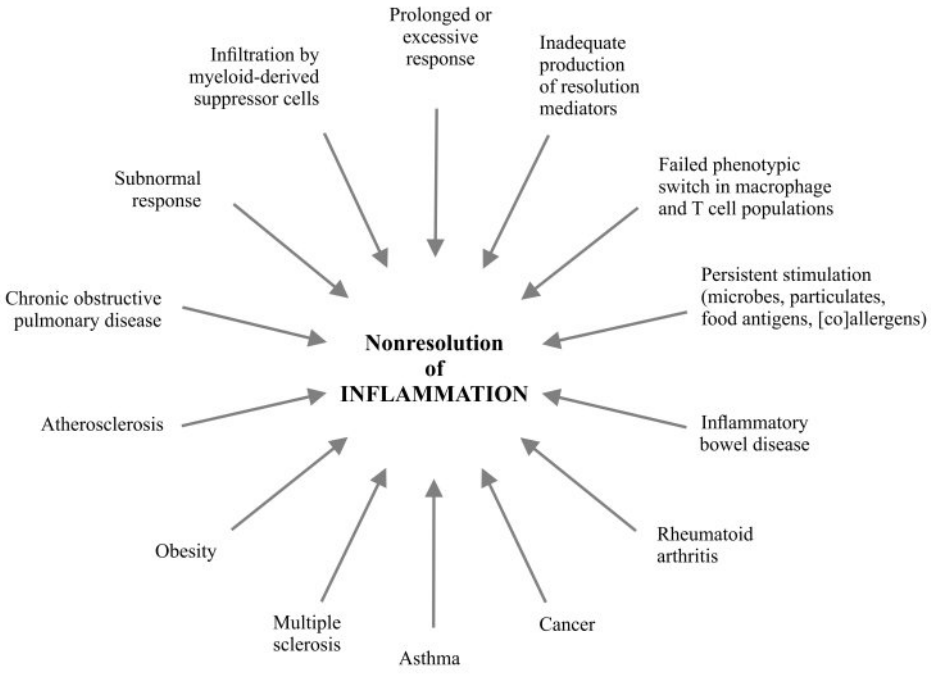
Figure 3. Ways to resolve acute inflammation



The duration and severity of inflammation are determined by the interaction (that is, the efficiency and consistency of the flow of multidirectional processes): on the one hand, there is an increase in the phlogogenic reaction aimed at isolating and eliminating the cause, and on the other hand, their restriction of inflammation and tissue repair. The overall magnitude and duration of inflammation depend on competing physiological processes, namely, on pro-inflammatory mechanisms that enhance the programs of inflammation and endogenous inhibition, which in turn control the resolution of inflammation (28). Thus, the imbalance with a predominance of pro-inflammatory phenomena may develop as a result of relative or absolute insufficiency of anti-inflammatory factors and mechanisms that allow inflammation.

The predominance of pro-inflammatory effects may develop despite the opposition of anti-inflammatory and inflammatory factors controlling the severity of their self-limiting mechanisms. The imbalance between the groups of counter-regulatory factors with a predominance of pro-inflammatory over anti-inflammatory and final inflammation is typical for the formation of a chronic variant of the course of inflammation and may develop due to many reasons (15, 25), (Figure 4).

Figure 4. Type of chronic (unresolved) inflammation



The totality of reasons for the unresolved process is united by the occurrence of absolute or relative insufficiency of the mechanisms of the resolution phase of inflammation. Modern researchers have focused on the regulators of the resolution of inflammation, known as "specialized pro-resolving mediators", due to the emergence of the ability to control the process of inflammation through drug and non-medication effects on the "target". There is an active process of accumulating data that the insufficiency of one or the other specialized permitting mediator (SPM) just plays a crucial role in the emergence of a range of socially significant diseases: atherosclerosis and other cardiovascular diseases, osteoarthritis, obesity, type 2 diabetes, chronic obstructive diseases of the lungs, ulcerative colitis, tumors, and so on (24). Disruption of the resolution mechanisms, in turn, can be caused by the following factors: nutritional deficiency of essential fatty acids (EPA eicosapentaenoic acid, DHA docosahexaenoic acid) that are substrates for specialized pro-resolving mediators, polymorphism of enzymes involved in their synthesis, irregularity when receiving specialized pro-permit mediators, etc. Thus, such a typical phenomenon as the coexistence of a whole spectrum of diseases in one patient, united by a common pathological process, becomes understandable and justified.

The generality of the pathogenesis of comorbid forms of pathology suggests a pattern of dissemination of such foci of chronic non-infectious inflammation with a sufficiently long non-resolution of this process. The initially developed inflammatory focus is limited, but if the inflammation is not resolved and continued, against the background of the organism's altered reactivity due to the development of systemic phenomena, conditions are created for the emergence of new "lesions". The complex of systemic phenomena that occurs under the action of cytokines is well known as the acute phase response, or the "pre-immune response". Common manifestations of the inflammation include hyperthermia, arthralgia and myalgia, sleep disturbances, loss of appetite, changes in functioning of the physiological systems (respiration, circulation, digestion, urination, etc.), as well as changes in the laboratory parameters: an increase in the erythrocyte sedimentation rate, leukocytosis, dysproteinemia (C-reactive protein, amyloid-A and P, transferrin, ceruloplasmin, immunoglobulins, enzymes, etc.) (14). The purpose of these reactions is to restore homeostasis and eliminate the cause of its violation, however, with the unresolved inflammation and its continuation, these phenomena can cause alteration of other tissues and organs, accompanied by the development of low-intensity (mild) inflammation in them, with the appearance of disorders of their function, which, it would seem, have no connection with the site of the initial lesion (3). As a result, even minor changes in the tissue with the formation of DAMP under normal conditions, that is, an event that occurs constantly, can lead to the emergence of a new independent chronic source of inflammation and the production of pro-inflammatory factors.

The progress in understanding the pathogenesis of osteoarthritisassociated with advances in molecular biology, discovered that the mediators of acute phase inflammatory response cytokines and prostaglandins are able to activate chondrocytes, which in turn increase the production and secretion of metalloproteinases that destroy cartilage and participate in the formation of alarminov (a molecular fragment associated with the damage of DAMP) such as fibronectin, hyaluronan, soluble heparan sulfate, β-defensin-2 and protein

groups with high mobility. In turn, DAMP, through the interaction with the pattern recognition receptors (PRR), such as toll-like receptors (TLR) on the surface of immune cells or with PRR in the cytoplasm of cells (nod-like-receptors (NLR)), activate the mechanisms of the innate immune response and trigger the development of non-infectious inflammation (16, 19).

For example, according to K.A. Scheibner, M.A. Lutz, S. Boodoo, M.J. Fenton, J.D. Powell, M.R. Horton (2006) (20), the resulting low molecular weight hyaluronan mediated by toll receptors, activates the expression of inflammatory genes in epithelial cells, endothelial cells, fibroblasts, dendritic cells and macrophages. The activated genes are responsible for chemokine synthesis (MIP-1α, MIP-1β, KC, RANTES, MCP-1, and IFN-inducible protein-10), cytokines (IL-8, IL-12, and TNF-α), as well as inducible NO α-synthase and plasminogen activator inhibitor 1 (23). This work has also shown the value of the ratio of pro-inflammatory factors (low molecular weight hyaluronan) to anti-inflammatory factors (high molecular weight hyaluronan). The violation of this ratio with the predominance of pro-inflammatory factors has been possible not only due to the enhanced formation of low molecular weight hyaluronan, but also due to its insufficient elimination. Thus, the cause of "chronization" of the process is a developing imbalance between the phases of the inflammatory reaction, with a predominance of pro-inflammatory phenomena in conditions of insufficient anti-inflammatory and resolving factors At the same time, only destructive-dystrophic processes are possible in a non-vascularized tissue by the analogy with how it initially occurs in articular cartilage. An excellent illustration is a well-known fact that "typical" inflammation develops only in vascular tissues. Therefore, it is considered that in neovascularized tissues, cartilage and cornea, the inflammation begins with the growth of blood vessels. Until vascularization occurs, no inflammation will occur: in the absence of blood vessels, exudation is absent, there is no cell emigration, and, as a result, the cell infiltration is absent (9, 11). As it's known, the main role in angiogenesis is played by endothelial cells, which trigger and control the entire process (10, 12, 21).

CLINICAL SIGNIFICANCE

It is known that there is a large number of studies proving the existence of relationships between angiogenesis and chronic inflammation in many different diseases: psoriasis, diabetes mellitus, Crohn's disease, rheumatoid arthritis, tumors, vessels are also found in the membrane of the hernial protrusion during intervertebral hernia. They demonstrated that the relationship between obesity and osteoarthritis, seemingly non-inflammatory diseases with the inflammation and vascular neoplasm, can be both direct and reverse (10). At the same time, structural changes in the vascular network in the focus of chronic inflammation are characterized not only by the formation of new vessels, but also by remodeling of the existing ones. It was also found that capillaries are capable of structurally and functionally transforming into the venular vessels, with a change in the phenotype of endothelial cells. The functional features were characterized by an increase in the sensitivity of the vessels to the action of the pro-inflammatory mediator P of the substance increasing the permeability of their wall. This feature suggests the possibility of its participation in the formation of the "circulus vitiosus", when small amounts of the inflammatory mediators to which normal vessels do not react, are able to support the increased permeability, promoting exudation and continuing inflammation (10, 13, 26).

Subsequent cellular reactions that occur under the action of chemokines and cytokines support development of the inflammation, through profound structural changes, with the formation of the pathological tissue, the so-called pannus in the cartilage, the underlying bone and the synovial membrane of the joint. A number of studies have demonstrated that the severity of synovitis directly correlates with the clinical symptoms and it also has an unfavorable prognostic value (18, 19).

Another process involved in the formation of new structures is the endothelial-mesenchymal transition, which consists in changing the endotheliocyte phenotype to myofibroblast. A similar phenomenon is observed in the outbreak of chronic (unresolved) inflammation in relation to epithelial cells, which can also transform into mesenchymal cells. The formation of scar tissue and fibrosis in chronic inflammation is associated with this phenomenon (6). The basis of these structural transformations is the change in the functional activity of a variety of cells: endotheliocytes, pericytes, fibroblasts, epithelial cells, macrophages, lymphocytes, and so on. The activation of these cells leads, along with vascular neoplasm, increased wall permeability and edema, to the emigration with infiltration of the tissue with inflammatory cells, an increase in the number and activity of fibroblasts with the development of fibrosis and other degenerative-destructive changes. The result is the formation of a new, non-essential tissue function that differs in its structural and functional characteristics from the normal one (6, 27). As a result, a change in the mechanical properties of the tissue can lead to its traumatization and damage even under the normal loads (16). The combination of these phenomena, in turn, enhances and "chronizes" (prolongs) the local inflammatory response, and, as a result, helps to maintain a systemic inflammatory response. In this way, another "circulus vitiosus", which has already been involved in the progression of the disease, is formed.

The important role of the endothelium should be noted not only in the progression, but also in the completion of inflammation due to the production of anti-inflammatory factors when interacting with polymorphonuclear leukocytes, macrophages and other cells, in replenishing the arising tissue defect and restoring the normal tissue structure (22, 23, 24).

It should be borne in mind that all the above-described phenomena and processes are ambiguous and often contradictory, which create difficulties in the choice and formation

of a medical strategy and tactics. An example of this difficulty is the use of modern pharmacological drugs in the treatment of rheumatoid arthritis. Since rheumatoid arthritis is a chronic inflammatory disease, prostaglandins, leukotrienes (LTB4), and TNFα play an important role in its pathogenesis. The action of modern drugs is aimed at suppressing and preventing their formation, which can help reduce the severity of inflammation and alleviate the symptoms of the disease, but at the same time, their use can lead to a lack of mechanisms for the completion of inflammation (1).

This kind of objection may be applied to other approaches to treatment. This includes serious doubts about the use of angiostatic agents for the treatment of arthritis. In connection with the huge role of angiogenesis in the occurrence of structural changes in the joint and the progression of the disease, it would seem logical to suggest using it in the treatment of angiostatic agents (7). However, these drugs cause only the inhibition of angiogenesis, without affecting the cause of vascular neoplasm and, therefore, ultimately preserve and maintain the achieved vascularization. In connection with the above, it follows that the winning strategy should be to activate the mechanisms of sanogenesis, i.e. in stimulating the completion of inflammation through its resolution. This means that it is necessary to activate the formation of those chemokines and other factors of the completion of inflammation (specialized resolving lipid mediators) that contribute to the reduction of newly formed vessels and the restoration of normal cellular tissue composition (2, 10, 17, 21).

CONCLUSION

The emergence of new foci of inflammation and their transition to a chronic course in patients with comorbid pathology is due, among other things, to the imbalance of counter-regulatory pro-inflammatory, anti-inflammatory and anti-resolving factors of a chronic systemic response. For a successful, controlled completion of the inflammatory process, it is necessary to ensure a systematic approach to the rehabilitation of patients, taking into account the basic medical principles, allowing thebreak of a complex set of pathological circles. The complexity of the impact allows you to potentiate the healing effects at all hierarchical levels in a living organism. It is necessary to take into account the understanding (representation) of the therapeutic effect influence on the phase of inflammation : on the initiation and development phase of the inflammatory response or on the resolution phase of the inflammation.

The etiotropic principle ensures that the impact is directed towards eliminating the cause and effects of its action, which means that it is necessary to take measures to identify and rehabilitate all foci of chronic inflammation in patients with comorbid pathologies, and the effectiveness of the treatment will be directly proportional to the success of each.

The pathogenetic principle ensures that the main mechanisms for the development and progression of the disease are affected, i.e. on the suppression and disconnection of "circulus vitiosus". The difficulty of applying this principle in practice is that among the huge numbers of vicious circles that are formed at all hierarchical levels of the organization, one must be able to choose certain basic, key points of influence as targets, on which the functioning of the basic mechanisms of the disease progression depends. We propose optimization of the choice based on the phenotyping of patient subgroups.

The simultaneous application of these two principles will influence most effectively the phase of the initiation and development of the inflammation.

Transition to chronic inflammation requires creation of conditions to strengthen the mechanisms for resolving the inflammation to eliminate inconsistency between strength of the mechanisms of the initial phase and the phase of completion. Thus, the prerequisite for achieving the desired therapeutic effect is the necessity and necessity of using and applying the sanogenetic principle, which includes measures aimed at the early completion of inflammation and the most complete restoration of damaged tissues.

In turn, in addition to carrying out "anti-inflammatory" therapeutic measures, it is necessary to take into account that the basis of the onset, development and progression of the disease involves non-inflammatory pathogenetic mechanisms, for which you should also use the principles described above for the formation of the treatment algorithm. There is no doubt that throughout the treatment, it is necessary to use the symptomatic treatment that will help speed up the healing process.

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ANTIHYPERLIPIDEMIC ACTIVITIES AND HEMATOLOGICAL PROPERTIES OF ETHANOL EXTRACT OF BLIGHIA SAPIDA KOENIG BARK IN ALLOXAN-INDUCED DIABETIC RATS

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ANTIHIPERLIPIDEMIJSKA AKTIVNOST I HEMATOLOŠKE KARAKTERISTIKE ETANOLNOG EKSTRAKTA BLIGHIA SAPIDA KOENIG BARK KOD PACOVA SA DIJABETESOM INDUKOVANIM ALOKSANOM

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ABSTRACT

Blighia sapida (BS) has been shown to be rich sources of antioxidant, thus, we evaluated effects of B. sapida Koenig stem bark ethanol extract (BSE) on lipid metabolism and hematological indices in diabetes rats.

Thirty male rats were divided into six groups of five rats each. Diabetes was elicited by intraperitoneal injection of alloxan (65 mg/kg body weight) once and orally administered with glibenclamide (5 mg/kg), B. sapida extract (50, 100 and 150 mg/kg body weight (bw) once daily for 21 days. Serum lipid profile, markers of hepato-renal toxicity and hematological indices were examined using automated analyzer. Data were analyzed using one-way ANOVA and $p < 0.05$ was considered to be statistically different.

Diabetic untreated animals showed considerably elevated total cholesterol $p < 0.05$, also, significant increase in AST, ALT, ALP, urea and creatinine compared to control. Triglycerides, LDL-c, VLDL-c, AI and CRI decreased with extract administration and HDL-c increased considerable compared to untreated diabetic rats. Furthermore, significant lower hemoglobin (Hb) levels, packed cell volume (PCV), red blood cells (RBCs) levels, white blood cells (WBCs) compared to normal animals was recorded in the untreated group. These changes were returned to normal after the administration of extract 50, 100 and 150 mg/kg body weight. Hence, these effects were most prominent in the animals treated with 150 mg/kg body weight of B. sapida bark.

This indicates that B. sapida stem bark possess anti-hyperlipidemic activity and improved the biochemical parameters within the hematological profile of diabetic rats.

Keyword: *Blighia sapida, antihyperlipidemic, hematological profile, diabetes*

SAŽETAK

Poznato je da je Blighia sapida (BS) bogati izvor antioksidanasa, stoga smo ispitivali efekte etanolnog ekstrakta B. sapida Koenig stem bark (BSE) na metabolizam lipida i hematološke indekse kod pacova sa dijabetesom.

Trideset pacova muškog pola bilo je podeljeno u šest grupa, po pet pacova u svakoj. Dijabetes je indukovan intraperitonealnom injekcijom aloksana (65 mg/kg telesne mase) tokom 21 dana i oralno je primenjivan glibenklamid (5mg/kg) i B. sapida ekstrakt (50, 100 i 150 mg/kg telesne mase (tm)) jednom dnevno tokom 21 dana. Serumski lipidni profil, markeri hepato-renalne toksičnosti i hematološki indeksi su odredjivani korišćenjem automatskog analizatora. Podaci su analizirani pomoću jednofaktorske analize varijanse (ANOVA) i $p<0.05$ se smatralo statistički značajnim.

Netretirane životinje sa dijabetesom su imale značajno povišen ukupni holesterol ($p < 0.05$) i takodje značajno povećanje AST, ALT, uree i kreatinina u poređenju sa kontrolom. Trigliceridi, LDL-c, VLDL-c, AI i CRI su se smanjili nakon administracije ekstrakta, a HDL-c se značajno povećao u odnosu na netretirane pacove sa dijabetesom. Osim toga, značajno niži nivo hemoglobina (Hb), hematokrit (PCV), broj eritrocita (RBCs), broj leukocita (WBCs) u poređenju sa zdravim životinjama je zabeležen u netretiranoj grupi. Ove vrednosti su se vratile u normalu nakon administracije 50, 100 i 150 mg/kg tm ekstrakta. Ovi efekti su bili najizraženiji kod životinja tretiranih sa 150 mg/kg tm B. sapida bark.

Ovo ukazuje da B. sapida stem bark poseduje antihiperlipidemijsku aktivnost i da poboljšava biohemijske parametre u okviru hematološkog profila kod dijabetičnih pacova.

Ključne reči: *Blighia sapida, antihiperlipidemijska aktivnost, hematološki profil, dijabetes*

ABBREVIATIONS

ALT: alanine transaminase;
AST: aspartate transaminase;
ALP: alkaline transaminase;
DM: diabetes mellitus;
BS: Blighia sapida;
TG: Triglycerides;
TC: Total cholesterol;
LDL-c: low density lipoprotein cholesterol;
HDL-c: high density lipoprotein cholesterol;
VLDL-c: very low density lipoprotein;
Hb: hemoglobin;
MCHC: mean corpuscular hemoglobin concentration
MCV: mean corpuscular volume;
RBC: red blood cells,
PCV: packed cell volume;
WBC: white blood cells.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disturbance defined by hyperglycemia caused by lack of insulin or disruption of insulin signaling as a result of lack of hypoglycemic agent or insensitivity of insulin hormone. DM is related to aberrant metabolism of macromolecules [1, 2, 3]. The illness happens as a results of exocrine gland β-cells injury, resulting in decreased secretion of insulin. It might conjointly arise once the receptors are resilient to the roles of insulin [4]. Hyperglycemia reoccurrence during diabetes leads to body proteins being glycated, that successively results in complications affecting body organs and arteries [5]. Stiffening of red blood cells could be liable for and or related to, large vessel disease in diabetes state [6]. Management option for DM is predicated on insulin therapy and oral hypoglycemic agents that have several facet effects [7]. In diabetes state, the effects and locations of involvement in biochemical activity are varied and elevated serum total triglyceride level, increase level of transaminase, creatinine and urea are of concerned [6]. Additional major issue, that confounds diabetic state, leading to death is hyperlipidemia [8]. Different ways to this fashionable pharmacotherapy of diabetes mellitus are desperately required, attributed to the lack of accessible therapies to manage all the pathological basis of the ailment, in addition to the mammoth cost and poor accessibility for several populations in the developing world [6]. Therefore, plants use as medical aid for diabetes is inspired and acclaimed, though, a number of them are lacking scientific examination.

Blighia sapida K.D. Koenig, also known as 'Akee apple' in English, belongs to the plant family called Sapindaceae and it is noted for its highly characteristic reddish fruits. The species in this family include *B. sapida* K.D. Koenig, *B. unijugata* Baker, and *B. welwitschii* (Hiern) Radlk. It is recognized as 'isin' in Yoruba, 'gwanja kusa' in Hausa and 'okpu' in Igbo [9]. *B. sapida* K.D. Koenig is distributed throughout Nigeria [10]. *B. sapida* could be a therapeutic herbal plant ordinarily utilized by trado-medical healers in Nigeria, and highly appreciated in West Africa for the management of various diseases like diabetes mellitus [11]. The fruit has inhibitory effect against α-glucosidase and α-amylase as reported by Kazeem et al. [12]. *B. sapida* root extract has been shown to possess normoglycemic impact [13]. The stem bark has been shown to ameliorate pancreatic β-cell dysfunction in diabetic rats [11]. Therefore, this study was focused on analyzing the effects of *Blighia sapida* K.D. Koenig stem bark on antihyperlipidemic and hematological parameters in diabetic rats.

METHODS

Plant material

Fresh stem bark peelings of *B. sapida* Koenig was obtained at a farm in the suburbs of Abeokuta, Ogun State, Nigeria. The plant was identified and authenticated by a senior taxonomist at the university herbarium with herbarium approval number (UHAE/2016/020). All plant names in this manuscript are formatted according to the latest revision in "The Plant List" (www.theplantlist.org).

Plant extracts preparation

Stem bark was air-dried in the laboratory at temperature (25 ± 2°C), pulverized using an electrical blender and the powders obtained stored till further use. The small grained sample (100 g) was extracted with solvent combination of 70% ethanol for 48 h. The extract was filtered and thereafter evaporated to dryness using rotary evaporator [14]. The concentrated extract was stored at 4 °C until further analysis. The extraction yield is as follows.

$$\text{Percentage yield} = \frac{\text{Weight of the extract}}{\text{Weight of powdered stem bark}} \times 100\ \%\ [15]$$

The percentage yield of the extraction was 19.1 %.

Experimental Animals

Six-week-old male Wistar rats with an initial mean body weight of 150 ± 50 g were obtained from Animal house Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. The animals were divided into six groups of five each and adapted to investigational condition for two weeks. The animals were housed in clean metabolic cages that provided free access to water and rat pellets (Ladokun Feeds, Nigeria). The principles of Animal Care (Public Health Services, 1986) was monitored through the duration (for twenty-one days) of the experiment. The standards of National Institute of Health (NIH publication 85-23, 1985) for experimental maintenance and handling of animals was conformed to [16]. The ethical committee of the Afe Babalola University approved this study with approval number (ABUAD/ACA/121). The rats in this study followed the rules of the established Animal Ethical Committee of Afe Babalola University.

Animal grouping

The animals were randomly divided into six groups of 5 animals namely;

Normal control group (distilled water),
Diabetic untreated group (65 mg/kg Alloxan, intraperitoneal)
Diabetic + 5 mg/kg bw
Diabetic + 50 mg/kg bw BSE, oral gavage
Diabetic + 100 mg/kg bw BSE, oral gavage
Diabetic + 150 mg/kg bw BSE, oral gavage

Dose dependent study was previously carried out in our laboratory with three different doses of BSE (50, 100 and 150 mg/kg body weight) based on ethnobotanical survey conducted by [11].

Induction of diabetes

In induction of diabetes, alloxan (65 mg/kg body weight) was dissolved in sterile physiological saline and intraperitoneal injected (5.6 mL) into the animals in the diabetic control, diabetic + glibenclamide (5 mg/kg body weight), diabetic + 50 mg/kg body weight of BSE (2.5 mL), diabetic + 100 mg/kg body weight of BSE (5.5 mL), diabetic + 150 mg/kg body weight of BSE (8.5 mL) groups to induce β-cell dysfunction. This was done in morning after the rats have been fasted overnight, while the animals in normal control group received a similar volume of distilled water. Forty-eight hours after induction, fasting blood glucose (FBG) of all rats were measured by collecting blood from the tail vein using a portable glucometer. Animals with a FBG level ≥ 200 mg/dl were considered as diabetic while animals with a FBG level < 200 mg/dl were disqualified [17].

Collection of blood

Under di ethyl anaesthesia, the neck area of the rats was quickly cleared to expose the jugular veins. Blood samples from each animal was collected after 21 days through their jugular vein and preserved until further processing. The blood sample was collected into a dry tube and allowed to clot for 30 min before centrifuging at 3500 rpm for 10 min to collect the serum for further study [18].

Biochemical parameters

Serum lipid concentrations, in additional to aspartate and alanine aminotransferases and alkaline phosphatase as well as urea and creatinine concentrations were assayed using commercially available kits (DRG Diagnostics, USA) according to manufacturer's protocol.

Low density lipoprotein-cholesterol was estimated according to equation as shown below:

LDL-cholesterol (mg/dl) = TC- HDL- (TG/5)

Non-HDL-cholesterol (mg/dl) = TC – HDL-cholesterol

Whereas, TG/5 is equivalent to the concentration of very low density lipoprotein. VLDL means very low density lipoprotein, TG means triacylglycerol

Atherogenic index (AI) was calculated and expressed as:

$$\text{Atherogenic index (AI)} = \frac{\text{TC-HDL-cholesterol}}{\text{HDL-cholesterol}}$$

Coronary artery risk index (CRI) was deduced using the formula below.

$$\text{Coronary artery risk index (CRI)} = \frac{\text{TC (mg/dl)}}{\text{HDL-cholesterol (mg/dl)}}$$

Whereas, HDL means high density lipoprotein-cholesterol, TC means total cholesterol

Hematological analysis

The hematological parameters like packed cell volume (PCV), hemoglobin (Hb), WBC count and WBC percentage composition, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were analysed by means of an automated analyzer (Sysmex K-2 IN, Japan). Total white blood cell counts (WBC) was analysed by the hemocytometer method, whereas smears were ready and marked by using Leishman technique and numbered by the longitudinal counting method to resolve differential count. Packed cell volume (PCV) was analyzed by the micro-haematocrit method while hemoglobin (Hb) levels was assessed by the cyano methemoglobin method [19].

Data analysis

Data are presented as the mean ± SD (n=5). The data were analyzed by one-way analysis of variance via a statistical software package (SPSS, Version 20.0, IBM Corporation, NY, USA) one-way ANOVA using Duncan multiple range *post-hoc* test (DMRT). Values were considered to be significantly different at $p < 0.05$.

RESULTS

The effect of *Blighia sapida* stem bark ethanol extract on fasting blood glucose level of alloxan-induced diabetic rats is presented in Table 1. Fasting blood glucose levels in all animals induced with alloxan was significantly increased, compared to the normal control group, $p < 0.05$. The fasting blood glucose levels were however, reduced considerably in diabetic + 50 mg/kg body weight of BSE, diabetic + 100 mg/kg body weight of BSE and diabetic + 150 mg/kg body weight of BSE groups in addition to glibenclamide treated animals.

Table 1: Effects of ethanol extract *Blighia sapida* stem bark on the fasting blood glucose level of alloxan-induced diabetic rats

Groups	Initial fasting blood glucose level (mg/dl)	Fasting blood glucose at 48 h after induction (mg/dl)	Final fasting blood glucose at 21 days after induction (mg/dl)
Control	86.10 ± 0.14^{a}	85.65 ± 0.10^{a}	87.20 ± 1.14^{a}
Diabetic control	86.48 ± 1.01^{a}	287.10 ± 1.01^{b}	364.10 ± 2.10^{d}
Diabetic + glibenclamide	84.02 ± 1.96^{a}	244.20 ± 1.45^{e}	88.40 ± 1.0^{a}
Diabetic + 50 mg/kg B.S	85.46 ± 1.42^{a}	287.10 ± 1.12^{e}	102.10 ± 1.10^{c}
Diabetic + 100 mg/kg B.S	84.49 ± 1.20^{a}	306.46 ± 1.24^{d}	96.96 ± 2.10^{b}
Diabetic + 150 mg/kg B.S	86.10 ± 1.32^{a}	386.12 ± 2.12^{d}	88.201 ± 1.10^{a}

Data are presented as the mean ± SEM of 5 animals. Values with different superscript letters (a-e) along a column for a given parameter are significantly different from each other between groups at P < 0.05.

Table 2: Effect of administration of ethanol extract of *Blighia sapida* stem bark on Serum lipid profiles, atherogenic and coronary risk indices of alloxan-induced diabetic rat

Groups	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Non-HDL (mg/dl)	HDL (mg/dl)	AI	CRI
Control	56.28±1.04^{a}	40.21±0.02^{a}	19.39±0.01^{a}	8.04±0.01^{a}	27.44±0.98^{a}	28.84±0.01^{a}	0.95±0.02^{a}	1.95±0.01^{a}
DC	95.84±0.12^{e}	104.26±0.14^{d}	64.91±0.21^{d}	20.85±0.12^{d}	85.76±1.22^{e}	10.08±0.02^{e}	8.51±1.01^{d}	9.51±1.22^{d}
DC + gliben-clamide	62.20±0.14^{d}	53.12±1.12^{c}	33.39±1.11^{c}	10.62±0.04^{c}	44.01±0.67^{c}	18.19±0.30^{d}	2.42±0.33^{c}	3.42±0.31^{c}
Diabetic rats + 50mg/kg BSE	64.32±1.20^{c}	54.36±1.18^{c}	33.33±1.08^{c}	10.87±0.38^{c}	44.20±0.01^{c}	20.12±0.14^{c}	2.20±0.41^{c}	3.19±0.58^{c}
Diabetic rats + 100mg/kg BSE	69.92±1.10^{b}	44.10±0.06^{b}	34.98±0.04^{b}	8.82±0.25^{a}	44.80±0.45^{b}	25.12±0.06^{b}	1.74±0.30^{b}	2.78±0.28^{b}
Diabetic rats + 150mg/kg BSE	55.89±1.48^{a}	40.82±1.06^{a}	18.74±0.05^{a}	8.16±0.01^{a}	26.91±1.12^{a}	28.98±1.01^{a}	0.93±0.01^{a}	1.93±0.02^{a}

Data are presented as the mean ± SEM of 5 animals. Values with different superscript letters (a-e) along a column for a given parameter are significantly ($P < 0.05$) different from each other. TC, Total cholesterol; TG, Triglyceride; LDL-cholesterol, Low density lipoprotein-cholesterol; HDL-cholesterol; High density lipoprotein-cholesterol, AI; atherogenic index, CRI; coronary artery index.

Serum lipid concentrations, calculated atherogenic index (AI) and coronary risk index (CRI) scores are displayed in Table 2. High serum concentrations of TC, TG, LDL-cholesterol and non-HDL-cholesterol levels in addition to analyzed AI and CRI scores with progressive decrease in serum HDL-cholesterol were observed within diabetic untreated group compared to the normal control. Administration with *B. sapida* stem bark ethanol extract to diabetic animals considerably and dose-dependently abridged TC, TG and LDL-cholesterol levels, AI and CRI in 50 ,100 and 150 mg/kg bw of BSE and glibenclamide groups compared with the diabetic untreated. Although considerably increase in serum HDL-cholesterol level was observed in the *B. sapida* K.D. Koenig stem bark ethanol extract treated groups compared to the diabetic untreated.

Serum ALT, AST, ALP, urea and creatinine levels are presented in Table 3. Concentrations of serum ALT, AST, ALP, urea and creatinine levels were considerably increased within the diabetic untreated compared to the normal control. On the other hand, administration of *B. sapida* stem bark ethanol extract to diabetic animals progressively ameliorated these changes in 50, 100 and 150 mg/kg bw of BSE and glibenclamide treated groups.

Hemoglobin (Hb) levels, packed cell volume (PCV) count, red blood cells (RBCs), white blood cells (WBCs), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E) are displayed in Table 4. Hemoglobin (Hb) levels, packed cell volume (PCV), red blood cells (RBCs) levels, white blood cells (WBCs), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E) were evidently reduced in diabetic untreated compared to normal control. Though, administration of 50-150 mg/kg bw of BSE groups demonstrated a considerably increase on the biochemical parameters mentioned above. Glibenclamide treatment conjointly considerably increase these biochemical parameters.

DISCUSSION

In this study, we examined the effect of oral administration of *B. sapida* Koenig stem bark ethanol extract in diabetic rats through twenty-one days' post-treatment period. The typical characteristics of diabetic dyslipidemia include high serum cholesterol, triglyceride (hypertriglycerolemia), LDL-cholesterol concentrations and low HDL-

Table 3: Effect of administration of ethanol extract of *Blighia sapida* stem bark on selected biomolecules of alloxan-induced diabetic rat

Groups	AST (µ/l)	ALT (µ/l)	Urea (mg/dl)	Creatinine (mg/dl)	ALP (µ/l)	Total Bilirubin (mg/dl)
Control	96.48±0.14^{a}	62.20±1.02^{a}	26.11±1.16^{a}	4.00±1.22^{a}	164.14±2.10^{a}	6.10±1.10^{a}
Diabetic Control	180.20±2.12^{e}	100.20±1.42^{e}	56.43±2.43^{e}	10.39±2.08^{e}	482.01±4.07^{e}	18.14±2.10^{d}
Diabetic rats + Glibenclamide	140.40±1.20^{d}	84.20±2.04^{d}	42.12±3.46^{d}	6.04±1.12^{d}	240.11±3.17^{d}	10.42±1.19^{c}
Diabetic rats + 50mg/kg BS	110.20±1.05^{c}	76.40±1.04^{c}	38.36±4.69^{c}	6.47±1.12^{c}	210.14±1.64+c	8.14±2.48^{b}
Diabetic rats + 100mg/kg BS	102.34±34^{b}	72.49±1.80^{b}	30.48±2.04^{b}	5.29±1.15^{b}	180.13±3.01^{b}	8.04±1.10^{b}
Diabetic rats + 150mg/kg BS	96.84±1.04^{a}	61.84±1.42^{a}	26.92±3.11^{a}	4.13±1.06^{a}	168.89±3.19^{a}	6.32±2.10^{a}

Data are presented as mean ± SEM of 5 animals. $^{a-e}$ Values with different superscript letters along a column for a given parameter are significantly different from each other group of animals. BS, *Blighia sapida*; ALT, Alanine transaminase; AST, Alanine transaminase; ALP, Alkaline phosphate.

Table 4: Effect of administration of ethanol extract of *Blighia sapida* stem bark on haematological parameters of alloxan-induced diabetic rat

Parameters	Control	Diabetic control	DC + Glibenclamide	Diabetic rats + 50 mg/kg BSE	Diabetic rats + 100 mg/kg BSE	Diabetic rats + 150 mg/kg BSE
PCV (%)	55.10±1.01^{a}	27.10±1.01^{e}	42.00±0.10^{d}	48.10±1.01^{c}	51.20±1.04^{b}	55.84±2.02^{a}
Hb (g/dl)	11.98±0.50^{a}	6.14±0.02^{e}	8.20±0.20^{d}	9.58±0.32^{c}	10.62±0.04^{b}	11.64±0.52^{a}
WBC (x w^{3}/µl)	2.42±0.22^{a}	0.45±0.14^{e}	1.26±0.28^{d}	1.69±0.46^{c}	2.00±0.07^{b}	2.44±0.84^{a}
N (%)	49.20±1.12^{a}	26.20±2.10^{e}	36.21±0.14^{d}	39.40±1.10^{c}	46.24±0.12^{b}	49.62±0.45^{a}
L (%)	30.11±0.01^{a}	20.20±0.12^{d}	24.03±0.12^{c}	27.20±1.12^{b}	28.20±1.02^{b}	30.44±1.20^{a}
M (%)	8.01±0.14^{a}	4.28±0.18^{d}	6.01±0.19^{c}	6.99±0.14^{c}	7.46±0.12^{b}	8.46±0.26^{a}
E (%)	3.01±0.11^{a}	0.92±0.13^{d}	1.49±0.16^{c}	1.94±0.08^{c}	2.46±0.02^{b}	3.68±0.04^{a}
RBC (xw^{11}/l)	3.20±0.31^{a}	0.80±0.04^{d}	1.45±0.02^{c}	1.64±0.42^{c}	2.48±0.12^{b}	3.48±0.06^{a}
MCHC (g/dl)	34.10±0.06^{a}	22.10±0.04^{d}	28.41±1.10^{c}	29.10±0.12^{c}	32.41±0.02^{b}	34.46±0.12^{a}
MCV (fl)	79.01±1.10^{a}	54.12±0.16^{e}	63.49±0.10^{d}	69.10±0.52^{c}	75.96±0.48^{b}	79.60±0.09^{a}
MCH (pg)	35.11±1.20^{a}	24.01±0.06^{e}	28.42±0.04^{d}	30.20±0.44^{c}	32.69±0.16^{b}	35.64±1.10^{a}

Data are presented as mean ± SEM of 5 animals. Values with different superscript letters (a-e) along a column for a given parameter are significantly ($P < 0.05$) different from each other group of animals. Haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs), white blood cells (WBCs), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E).

cholesterol (hypercholesterolemia) contents [20]. Likewise, the increase levels of LDL-C and atherogenic index by the alloxan indicates predisposition to cardiovascular risk [21]. The significant elevation in cholesterol, triacylglycerol and LDL-cholesterol concentrations in the diabetic rats treated with distilled water may be owing to improved mobilization of free fatty acids from the peripheral fat depots. This obvious hyperlipidemia that describe the diabetic state may hence be considered as a consequence of uninhibited actions of lipolytic hormones on the fat depots [22]. Aberrant lipid metabolism, resulting in amassing of LDL-C, VLDL and total cholesterol in addition to reduced HDL-cholesterol, is often related to diabetes mellitus [23]. Elevated levels of LDL, VLDL, atherogenic index, coronary artery index and total cholesterol are thought of as main menace for cardiovascular disease (CVD). On the contrary, elevated HDL-cholesterol that functions in the transport of cholesterol from the periphery to the liver reduces the chance of CVD [24]. Oral intervention of *B. sapida* stem bark ethanol extract and the standard drug both averts dyslipidemia, decreases the chance of developing atherogenesis and coronary artery disease and amplified serum HDL-cholesterol level. This is in accordance with newly published studies [8, 25, 26, 27].

Aminotransaminses are significant and key enzymes involved in the breakdown of amino acids into α-keto acid, which are directed for complete metabolism via the Krebs cycle and electron transport chain. They are considered exact biomarkers for liver damage [28]. In hepatocyte injury, there is impairment in the biomembrane of liver cells which leads to permeability of cytoplasmic enzymes such as AST and ALT which leak into the circulatory system and result in significant elevation in their activities in serum. Moreover, modification of membrane bound alkaline phosphatase (ALP) affects membrane permeability and produces imbalance in the transport of metabolites [29]. Elevated serum transaminase, alanine aminotransferase (ALT), aspartate aminotransaminse (AST), alkaline phosphatase (ALP), urea and creatinine levels are thought of as biomarkers of hepato-renal damage, related to liver disease and hyperglycaemia [30, 31]. Treatment with *B. sapida* stem bark ethanol extract at 50 - 150 mg/kg bw significantly reduced ($p < 0.05$) ALT, AST, ALP, urea and creatinine levels, indicating that *B. sapida* bark might ameliorates alloxan-induced injury in diabetic rats. The considerably lower serum creatinine and urea levels in the *B. sapida* stem bark ethanol extract treated groups compared with diabetic untreated, indicates its doable impact on the betterment of diabetes induced kidney injury [32, 33].

Vital reductions in haemoglobin (Hb) levels, packed cell volume (PCV), red blood cells (RBCs), white blood cells (WBCs), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E) were observed ($p < 0.05$) in the diabetic untreated rats. Substantial significant ($p < 0.05$) increase in haematological parameters occurred upon administration of the *B. sapida* stem bark ethanol extracts at 50 - 150 mg/kg doses compared to the diabetic untreated rats. Constant trend was observed in the levels of parameters mention above for glibenclamide treated rats. Though these effect were more pronounced in the 150 mg/kg bw of BSE group [6, 34]. This may be as a result of the presence of metabolites like saponins, phenols and alkaloids [35 36, 37]. Blood examination could be a great means of evaluating the well-being standing of animals because it performs significant physiological and biological roles in organisms [38]. However, in diabetic state, the extra glucose present reacts with haemoglobin to produce glycated haemoglobin. This therefore shown that diabetic untreated rats displayed aberrations in the hematological profiles. A number of these aberrations may result in destruction of developed red blood cells resulting in the small Hb counts accompanied with the drop in the RBC and PCV [39, 40]. Extremely low values

of RBC, Hb and hematocrit might specify anemia [34, 39]. Furthermore, modulatory effect and confined toxicity might ensue as noted within the lymphocytes and neutrophils of the diabetic untreated animals. Administration of the *B. sapida* stem bark ethanol extract stimulates positive changes in the hematological profile of the diabetic rats. Hence, upsurge in RBC by the extract is a sign of its restorative impact on alloxan-induced anemia whereas the alteration in level of lymphocytes by the extract could specify an anti-infection activity [36, 40, 41].

CONCLUSION

The results from this study indicates that *B. sapida* ethanol stem bark extract possesses robust anti-diabetic activity via improving dyslipidemia and ameliorate anemic condition in diabetic rats. Hence, ethanol extract of *B. sapida* K.D. Koenig stem bark could be a possible anti-diabetic natural product with no significant side effects. Further studies to isolate the bioactive principles using HPLC, complete safety assessment as well as activities on metabolizing enzymes and pro-inflammatory biomarkers should be carried out *in vivo*.

STATEMENT

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Statement of Ethics

The handling of animals was conformed to the standards of National Institute of Health (NIH publication 85-23, 1985) for experimental animal maintenance. The ethical committee of the Afe Babalola University approved this study with approval number (12/SCI03/015). The rats in this study followed the rules of the established Animal Ethical Committee of Afe Babalola University.

Disclosure Statement

Authors declare no conflict of interest.

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Authors Contribution

OAO design the study, ODI carried out the study, ABO wrote the manuscript, ABO and ODI carry out analysis and interpretation of data, BOA and BEO assisted with and supervised the manuscript writing, BEO did the first proof reading and BOA and BEO did the second proof reading. OAO, BOA and BEO supported the manuscript preparation, made conceptual contributions on data analysis, manuscript drafting and critically revised the manuscript. The authors have read and approved the final manuscript.

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THE IMPACT OF THE GENE VARIANTS FV LEIDEN, FII G20210A, MTHFR C677T AND PAI-1 4G/5G ON PREGNANCY LOSS IN WOMEN FROM CENTRAL SERBIA

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UTICAJ GENSKIH VARIJANTI FV LEIDEN, FII G20210A, MTHFR C677T I PAI-1 4G/5G NA GUBITKE TRUDNOĆA KOD ŽENA IZ CENTRALNE SRBIJE

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ABSTRACT

Thrombophilia is a condition of enhanced functionality of the haemostatic system with an increased tendency for thrombosis, and it can be a congenital, acquired, or complex defect. Pregnancy can be the cause of acquired transitory thrombophilia, which may lead to complications if inherited thrombophilia is also present.

The aim of this study was to determine the genetic structure of the population based on the frequency of the gene variants factor V Leiden G1691A, factor II G20210A, methylenetetrahydrofolate reductase C677T, and plasminogen activator inhibitor-1 4G/5G, as well as to investigate the predictive value of these gene variants in repeated miscarriages.

The study included 87 female patients from Central Serbia with an average age of 32.7±4.5 years with inherited thrombophilia and previous miscarriages, with or without intrauterine foetal death. The exclusion criteria included the existence of gynaecological and infectious aetiology and the deficit of factors important for the coagulation process.

The resulting genotypes were in Hardy-Weinberg equilibrium. The frequency of genotypes with mutated alleles was significantly higher in this group of patients than in the control group for all variants except factor II G20210A. The most commonly mutated alleles were the plasminogen activator inhibitor-1 4G allele (0.61) and methylenetetrahydrofolate reductase T allele (0.47). Double mutation of plasminogen activator inhibitor-1 4G/5G and methylenetetrahydrofolate reductase C677T was dominant in patients with recurrent pregnancy loss (46.15%).

The presence of a combination of genetic variants of the plasminogen activator inhibitor-1 4G/5G and methylenetetrahydrofolate reductase C677T is a significant predictor of spontaneous abortions in women with inherited thrombophilia in Central Serbia.

Keywords: *pregnancy complications, FV Leiden, FII G20210A, MTHFR C677T, PAI-1 4G/5G*

SAŽETAK

Termin trombofilija se upotrebljava za urođene, stečene i kompleksne poremećaje hemostaznog sistema kod kojih postoji sklonost ka trombozama. Stečeni prolazni razlog trombofilije je trudnoća koja zajedno sa urođenom trombofilijom može dovesti do komplikacija.

Cilj ove studije je utvrditi genetičku strukturu populacije na osnovu genskih varijanti za faktor V Leiden G1691A, faktor II G20210A, metilen tetrahidrofolat reduktazu C677T i inhibitor aktivatora plazminogena -1 4G/5G i ispitati prediktivnu vrednost ovih genskih varijanti u odnosu na ponovljene spontane pobačaje.

Studijom je obuhvaćeno 87 pacijentkinja sa teritorije centralne Srbije starosti 32, 7±4, 5 godina, sa urođenom trombofilijom i prethodnim spontanim pobačajima, i sa ili bez intrauterine smrti fetusa. Isključujući kriterijumi su bili postojanje ginekološke ili infektivne etiologije spontanih pobačaja ili deficit faktora važnih u procesu koagulacije i antifosfolipidni sindrom.

Dobijeni genotipovi su bili u Hardi-Vajnbergovom ekvilibrijumu. Frekvence genotipova sa mutiranim alelima su bile značajno više za sve varijante, osim za faktor II G20210A u grupi ispitanica u odnosu na kontrolu. Najčešće prisutni mutirani aleli su bili 4G alel za inhibitor aktivatora plazminogena -1 (0, 61) i T- alel za metilen tetrahidrofolat redukazu (0, 47). Nosioci dvostrukih mutacija za inhibitor aktivatora plazminogena-1 4G/5G i metilen tetrahidrofolat reduktazu C677T su bili dominatni kod ispitanica sa rekurentnim gubicima trudnoća (46, 15%).

Prisustvo kombinacije genskih varijanti inhibitor aktivatora plazminogena-1 4G/5G i metilen tetrahidrofolat reduktaze C677T predstavlja značajan prediktor spontanih pobačaja kod žena sa urođenom trombofilijom na teritoriji centralne Srbije.

Ključne reči: *komplikacije trudnoće, FV Leiden, FII G20210A, MTHFR C677T, PAI-1 4G/5G.*

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ABBREVIATIONS

FVL- factor V Leiden
FII- factor II
MTHFR- methylenetetrahydrofolate reductase
PAI-1- plasminogen activator inhibitor-1
SERPINC1- serpin family C member 1
PROC- protein C
PROS1- protein S
AT III- antithrombin III
RPL- recurrent pregnancy loss
IUGR- intrauterine growth retardation
IUFD- intrauterine foetal death
PL- pregnancy loss
APCRV- activated protein C resistance V
APSy- antiphospholipid syndrome
HHC- hyperhomocysteinemia
DNA- deoxyribonucleic acid
PCR- polymerase chain reaction
A- adenine
G- guanine
C- cytosine
T- thymine
FAM- 6-carboxyfluorescein
HEX- hexachloro-fluorescein
HWE- Hardy-Weinberg equilibrium
APC- activated protein C

INTRODUCTION

In physiological conditions, haemostasis allows the normal flow of blood in blood vessels and prevents and stops bleeding after the vessels suffer any damage. Maintenance of normal haemostasis enables the dynamic balance between antithrombin and prothrombin mechanisms (1, 2). Several factors are involved in this process: the vascular endothelium, platelets, coagulation factors, coagulation inhibitors, and the fibrinolytic system (3). Violation of the haemostatic balance can lead to a haemorrhagic or thrombotic disorder. Thrombophilia is a specific condition with an increased tendency towards thrombosis. Disorders in haemostasis, which are related to the occurrence of thrombophilia, may be congenital (occurring due to genetic mutations), acquired (such as antiphospholipid syndrome), or complex (diet or lifestyle habits interact with the genetic predisposition to thrombophilia) (2). The genetic risk factors for thrombophilia include the presence of variants in the genes encoding coagulation inhibitors (SERPINC1, PROC, PROS1), leading to the deficiency of anticoagulant proteins (AT III, protein C, protein S); variants in the genes encoding clotting factors (FVL, NM_000130.4: c.1601G>A, p.Arg534Gln and FII G20210A, NM_000506.4: c.*97G>A); variants in the genes encoding other members of the fibrinolytic system (PAI-1 4G/5G, NM_000602.4: c.-820_-817G (4_5)); and other genetic polymorphisms that contribute to the occurrence of thrombosis (MTHFR C677T, NM_005957.4: c.665C>T, p.Ala222Val) (2-7).

During pregnancy and puerperium, all three components of Virchow's triad are present: hypercoagulability, venous thrombosis, and injury to blood vessels (8). Hypercoagulability and hypofibrinolysis in pregnancy reduce the risk of blood loss during implantation, placentation, and the third delivery stage (9). The resulting physiological changes during pregnancy may interact with congenital and acquired thrombophilia conditions, therefore increasing the risk of thromboembolic complications. Thrombosis of placental blood vessels, which can lead to a heart attack and placental insufficiency, is considered to be the cause of RPL in women with thrombophilia. Additionally, damage to placental vascularization can lead to IUGR, IUFD, placental abruption, and preeclampsia (10, 11). The research in this area has shown the importance of appropriate thromboprophylaxis in order to increase the birth rate (11).

The aim of this study was to examine the population genetic structure of patients from the territory of Central Serbia (Šumadija district) where previous unsuccessful pregnancies due to the presence of thrombophilia were recorded. The frequency of the genetic variants FV Leiden, FII G20210A, MTHFR C677T and PAI-1 4G/5G was determined, and the possible association and predictive value of present genetic markers were examined in relation to previous miscarriages.

MATERIAL AND METHODS

This study included 87 women from Central Serbia diagnosed with thrombophilia in of the past 3 years who were hospitalized at the Clinic of Hematology, Clinical Center Kragujevac, Serbia. The average age of the patients was 32±4 years (range, 22 to 41 years). The study was conducted according to the ethical principles of the Declaration of Helsinki and based on the decision of the Ethics Committee of the Institution (No. 01-12294). The inclusion criteria for the study were PL with/without IUFD and the presence of one or more of the following variants: FV Leiden G1691A, FII G20210A, MTHFR C677T, and PAI-1 4G/5G. The exclusion criteria for this study were the presence of gynaecological or infectious aetiology; protein S, protein C, AT III, or APCRV deficiencies; APSy; or HHC.

Peripheral blood samples (with 3.8% sodium-citrate as the anticoagulant) or buccal mucosal cells taken by sterile swabs were used for DNA extraction. Genomic DNA was isolated using the DNA-Sorb-A and DNA-Sorb-B kits (SACACE Biotechnologies, Italy), according to the manufacturer's standard protocol. The different solutions included in the kits (lysis, washing, sorbent and DNA eluent) isolated DNA of the desired purity and concentration and ready for use in further molecular genetics analysis.

The detection of gene variants in FV Leiden, FII G20210A, MTHFR C677T, and PAI-1 4G/5G was carried out by real-time PCR on an SaCycler-96 Real-Time PCR System (model SaCycler-96 RUO, Sacace Biotechnologies, Italy). The following commercial kits were used: Duplica$\alpha^{RealTime}$Factor II G20210A Genotyping Kit, Duplica$\alpha^{RealTime}$Factor V G1691A Genotyping Kit, Duplica$\alpha^{RealTime}$MTHFR C677T Genotyping Kit, and Duplica$\alpha^{RealTime}$PAI-1 Genotyping Kit (Euroclone Diagnostica, Italy). Each of these kits was designed to identify the mentioned gene variants using two provided reaction mixes, the amplification mix (with Hot Start Taq DNA polymerase, nucleotides, $MgCl_2$ and buffer) and the oligo mix (with primers and fluorogenic probes), according to the manufacturer's instructions.

Based on specific recognition and amplification of the target sequences by PCR, normal and mutated alleles were separated. In general, the probes designed to detect the wild-type and mutated alleles were labelled at the 5' end with the fluorophores FAM and HEX, respectively. The results were interpreted based on the presence of only FAM signal (homozygous wild type), only HEX signal (homozygous mutant), or both signals (heterozygous).

The summary data, which considered the presence of previous thrombotic events, pregnancy complications, demographic factors and genetic analysis results, were collected and inserted into a Microsoft Office Excel file. The statistical analysis was performed using SPSS Inc/PASW Statistics 18 for Windows (SPSS, Chicago, USA). The possible relations between PL and genetic variants were tested using the Wilcoxon test. Deviation from HWE for each group of genotypes was analysed using the HWE calculator (http://www.oege.org/software/hwe-mr-calc.shtml), with χ^2 <3.84 and the accepted significance at p<0.05. The prevalence of each variant was compared with the data from the control group, a healthy Serbian population with previously published gene frequencies (5, 17, 18), using the chi-square test with a 95% confidence interval (CI) and significance at p<0.05. Multivariate binary logistic regression analysis was used to examine the significant predictors of previous miscarriages.

RESULTS

In the studied group of 87 patients with hereditary thrombophilia, there were 161 pregnancy losses, and 52 of the patients reported recurrent abortions (with a maximum of 6 recorded spontaneous abortions in one woman). In 21 cases, IUFD was present with pregnancy loss. The mean number of adverse pregnancy outcomes (spontaneous abortions + IUFD) was 1.89 ± 0.89.

The most commonly mutated alleles present in the study population were 4G of the PAI-1 gene (60.92%, 106/174 alleles) and the T allele of the MTHFR gene (47.13%, 82/174 alleles). The frequencies of mutant FVL and FII G20210A alleles were 9.77% (17/174) and 2.87% (5/174), respectively (Table 1).

Based on the measured and expected allelic and genotype frequencies of the variants FV Leiden G1691A, FII G20210A, MTHFR C677T and PAI-1 4G/5G, it was found that the population did not deviate from HWE (Table 1).

The estimated frequencies of mutated alleles in the analysed group of patients were all higher than those in the healthy control population in Serbia, but the chi-square test showed that only the increased presence of the mutated FV Leiden and MTHFR alleles was statistically significant (p<0.001 and p<0.01, respectively) (Figure 1).

Table1. The genotypes' and allelic frequencies of inherited trombophilic risk factors in the analyzed group of women with pregnancy complications from Central Serbia

Genes	The genotypes			Allelic Frequency (%)		Deviation from HWE	
	Wild type (%)	Heterozygote (%)	Homozygote (%)			X^2	*P*
Factor V	G/G	G/A	A/A	Wild type G	Mutated A	**1.17***	**0.2800**
	80.56	19.54	0.0	90.23	9.77		
Factor II	G/G	G/A	A/A	Wild type G	Mutated A	**0.09***	**0.7612**
	94.25	5.75	0.0	97.13	2.87		
MTHFR	C/C	C/T	T/T	Wild type C	Mutated T	**1.52***	**0.2182**
	31.03	43.68	25.29	52.87	47.13		
PAI-1	5G/5G	5G/4G	4G/4G	Wild type 5G	Mutated 4G	**3.19***	**0.0885**
	19.54	39.08	41.38	39.08	60.92		

* HWE received for value of χ^2 <3.84; *P* < 0.05 for one degree of freedom – 1df

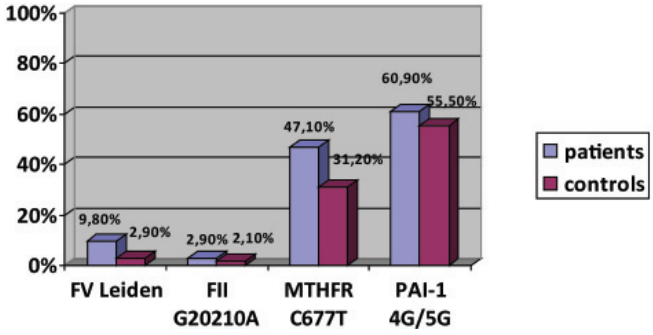

Figure 1. Comparison of allelic frequencies in the analyzed group of patients and healthy population

The most common genotypes with mutant alleles were for the variants 4G/5G in the PAI-1 gene (80.46%: 39.08% heterozygous and 41.38% homozygous) and C677T in the MTHFR gene (25.29% heterozygous and 43.68% homozygous) (Table 1). Statistically higher genotype frequencies in the study population than in the healthy population in Serbia were observed for all the variants except G20210A in the FII gene.

In 35.63% of the cases in the analysed group of patients (31/87), only one mutated variant was present. The rest of the patients carried different combinations of compound genotypes, with 4 types being observed in more than one case (Figure 2). The dominant combination of genetic variants was PAI-1 4G/5G + MTHFR C677T (45.98%, 40/87) (Figure 2).

In the group of patients with recurrent abortions, the most frequent combination was the presence of the double variants PAI-1 4G/5G + MTHFR C677T (46.15%, 24/52).

The Wilcoxon test showed only a weak positive correlation between miscarriages and the MTHFR C677T variant (0.217*, $P< 0.05$). The impact of all four variants in the analysed group of patients with PL with/without IUFD was determined by multivariate binary logistic regression analysis. The results showed that the variants 4G/5G in the PAI-1 gene (OR 0.364; 95% CI 0.134-0.973, $P<0.05$) and C677T in the MTHFR gene (OR 2.482; 95% CI 1.006-6.124, $P<0.05$) were segregated as significant predictors of the frequent occurrence of miscarriages (Table 2).

Table 2. Multivariate Binary Logistic Regression analysis of influence of examined genetic factors on pregnancy loss

Examined risk factors	Multivariate analysis	
	†OR (95%‡CI)	P
FV Leiden	0,337 (0,069-1,630)	0,176
FII G20210A	0,187 (0,020-1,712)	0,138
MTHFR C677T	2,482 (1,006-6,124)	0,049*
PAI-1 4G/5G	0,361 (0.134-0.973)	0,044*

*statistically significant $P < 0.05$; † odds ratio; ‡ confidence interval;

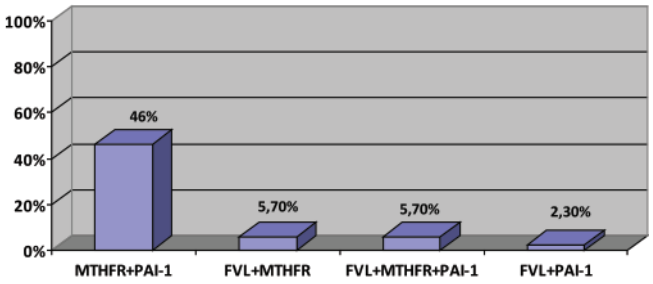

Figure 2. The most frequent compound genotypes (homozygous and/or heterozygous) in the analyzed group of patients from Central Serbia

DISCUSSION

Based on the estimated allelic frequencies of the four most commonly analysed variants worldwide (FV Leiden G1691A, FII G20210A, MTHFR C677T and PAI-1 4G/5G) in a population of women from Central Serbia with inherited thrombophilia who experienced a pregnancy loss, it was determined that the tested population was in genetic equilibrium. The most prevalent compound genotype in this study group was a combination of PAI-1 4G/5G + MTHFR C677T (46.15%), which was found to be the only significant predictor of common previous miscarriages.

Pregnancy is characterized by hypercoagulable states and microalbuminuria (1). In pregnancy, numerous changes in the haemostatic system, including an increased concentration of certain coagulation factors, a reduction in the concentration of natural inhibitors of coagulation, lower fibrinolytic activity, and slow flow of blood in the venous system of the lower limbs due to compression of the enlarged uterus, all contribute to the increased susceptibility to blood clotting (1, 10).

Successful pregnancy outcomes depend on adequate placental vascularization, and its damage is part of the various pathophysiologies of pregnancy complications. Physiological changes in coagulation and fibrinolysis during pregnancy may react with inherited and acquired thrombophilia states, placing these patients at a higher risk of pregnancy complications (9, 11).

The incidence of recurrent abortions is estimated to be 15-20%, and in 50% of the cases, the causes remain unexplained (12). According to data in the literature, pregnant women with thrombophilia, both congenital and acquired, have a higher incidence of RPL (5, 13). Inherited or acquired thrombophilia has been diagnosed in 50% to 65% of women with a history of unexplained foetal loss (14).

The single mutation in the gene for FV Leiden is a substitution of glutamine with arginine at position 506, which is known to be a cause of resistance to APC. This mutation results in increased production of thrombin and leads to hypercoagulability (15). Resistance to APC caused by this mutation is found in 20-40% of patients with venous thrombosis and in 60% of women with thrombosis, which occurs during pregnancy (15). Based on the conducted studies, the risk of thromboembolism was found to be increased 5-10 times in heterozygous carriers of the mutation, whereas the risk in homozygous carriers was increased 80-100 times (16). The mutation

in FVL is present in 5-12% of the general population. In our studied population, the allelic frequency of the FV G1691A variant was 9.77%, which was significantly higher than that in the healthy population in Serbia (2.9%) (17), consistent with the findings among women with recurrent foetal loss (RFL) in northern Serbia (9.5%) (18). Madjunkova et al. (19) reported the frequency of the mutated allele A in women with miscarriages in Slovenia (2.6%), Macedonia (3.8%) and Albania (2.7%), as well as the overall prevalence in all three groups (2.8%); these values were lower than those in our study. In our study, the presence of homozygous carriers of the FVL mutation was not detected, whereas heterozygous carriers were present in 19.54% of the cases, almost 6 times more often than in the recorded data (5.8%) for the healthy Serbian population (17). The observed presence of the mutated allele in our study is consistent with the results of other studies; for example, in Turkey, Isaoglu et al. did not find any homozygous carriers of this mutation, whereas there were 21.7% heterozygous carriers among women with RPL (20).

The variant G20210A is located in the 3' noncoding region of the FII gene, and it is associated with elevated levels of prothrombin in the blood (4, 16). Its presence increases the risk of deep vein thrombosis by approximately three fold (4, 15). Madjunkova et al. reported that the frequency of the FII G20210A gene variant among women with pregnancy loss was 1.5% in Slovenia, 3.8% in Macedonia and 3.6% in Albania. The overall prevalence in these three groups was 2.4% (19), which is similar to the observed prevalence of the mutated allele FII G20210A in the group of women with pregnancy loss in this study (2.87%). Moreover, the prevalence of the mutated allele FII G20210A in our subjects was not different from that in the healthy population in Serbia and the general worldwide population (2.87 vs. 2.1% and 2-3%, respectively) (17, 20). The same observation was made concerning the frequency of heterozygous carriers, which was not significantly higher than that in the healthy Serbian population and the general worldwide population (5.75% vs. 5.75% and 4.2%, respectively) (17, 20), and it was similar to that among women with recurrent foetal loss in the northern regions of Serbia (7%) (18). The results are in agreement with the data from other studies done in Turkey (21, 22).

The variant C677T in the MTHFR gene leads to the synthesis of the thermolabile form of the enzyme methylenetetrahydrofolate reductase (t-MTHFR), resulting in reduced synthesis of derivatives of folate for the remethylation of homocysteine to methionine and reduced folate intake. This process leads to hyperhomocysteinemia and increases the risk of venous thrombosis (4, 20). The estimated frequency of the mutated allele in our study population was 47.13%, which was significantly higher than that in the healthy group (31.2%) in the study by Djordjevic et al. (17). The observed presence of the mutated T allele in the population of women with pregnancy loss in Slovenia (38.3%), Macedonia (46.2%) and Albania (44.5%), as well as the overall prevalence in these three populations (41.2%), was similar or lower than the observed allelic frequencies in our study. Heterozygous and homozygous carrier genotypes were also more frequent in the analysed group of women with pregnancy loss than in the data for the healthy Serbian population provided by Djordjevic et al. (17) (43.68% vs. 39.2% and 25.3% vs. 11.2%, respectively). A similar or lower frequency was found in other populations (20, 21).

Mutated allelic variant 4 guanosine (4G) in the promoter of the PAI-1 gene at position 675 bp contains binding sites for only activators of transcription, in contrast to the normal allele, 5G, leading to elevated levels of PAI-1 in the blood and reduced fibrinolysis (5). The PAI-1 4G/5G genetic variant could be a risk factor for deep vein thrombosis, spontaneous abortions and preeclampsia (5). The frequency of the mutated PAI-1 4G allele (60.92%) was higher in our study than in the data for the healthy population in Serbia (55.49%) provided by Djordjevic et al. (5). In addition, the frequency of the mutated PAI-1 4G allele in the observed population was higher than that in the study by Madjunkova et al. (19), which included women with pregnancy loss in Slovenia (43.8%), Macedonia (42.5%), and Albania (43.6%), as well as the overall prevalence in all three groups (56.5%). The frequency of PAI-1 4G/4G genetic variant carriers in the population of women with pregnancy loss in the present study (41.38%) was higher than that in the study by Djordjevic et al. (5) ainvolving a healthy Serbian population (34.76%). Additionally, the frequency of PAI-1 4G/4G genotype carriers was higher in the present study than in a group of women with habitual abortion and miscarriages in different populations, for example, Polish and Iranian populations (23-25). The frequency of 39.08% heterozygous and 41.38% homozygous for the PAI-1 4G/5G variant in the population of women with PL in the present study was even higher than that in the study of foetal loss among women from other Serbian regions (26).

In the studies of habitual abortions carried out in different populations, Isaoglu et al. (21) found a statistically significant correlation between factor V Leiden and early and late RPL and between prothrombin G20210A and early RPL. Although the results of various analyses are often in conflict, the most commonly observed associations refer to the relationship between RPL and FV Leiden and FII G20210A gene variants (27). The results of Li et al.'s (28) meta-analysis showed a significant connection between the gene variant PAI-1 4G/5G and RPL. Li et al. (28) found that in the subgroup analysis by race, the PAI-1 4G/5G gene variant was associated with an increased risk of RPL in a Caucasian population, but the association was not observed in Asians. Shakar et al. (24) found that compared to the control group, carriers of 4G homozygous mutations were significantly more prone to RPL. The lack of a connection between C677T MTHFR and PL was reported by Dutra et al. (29). In the meta-

analysis by Wu et al. (30) , the results among Caucasians did not suggest an association between C677T MTHFR and RPL. This meta-analysis supports the idea that the MTHFR C677T genotype is associated with an increased risk of RPL, except in Caucasians (30). Li Luo et al. (31) found that the MTHFR C677T allele and the C677T haplotype were risk factors for RPL among a Han Chinese population. Ivanov et al. (32) found a weak association between T allele carrier status (both in the homozygous and heterozygous states) in Bulgarian women and recurrent embryonic lossT. The MTHFR C677T genetic variant is frequently present in the homozygous and heterozygous forms in the general Caucasian population (33). It is considered that the MTHFR polymorphism does not predispose to HHC when the folate status is sufficient (33). In combination with vitamin deficiency, heterozygotes for MTHFR have mildly increased homocysteine concentrations (33). In accordance with previous knowledge, the MTHFR C677T variant could be considered an agent for RPL in only combination with other risk factors influencing foetal development (33). In this research, we found a weak positive correlation between MTHFR C677T and RPL, which indicates that this variant should be investigated along with other thrombophilic mutations and accordingly interpreted.

Ozdemir et al. (34) found that homozygosity of 4G in PAI-1 and MTHFR C677T in Turkish women with RPL plays a crucial role and should be considered a risk factor for RPL. In the analysed group of patients with pregnancy complications in our study, 52 had recurrent spontaneous abortions. The most prevalent compound genotype was a combination of PAI-1 4G/5G + MTHFR C677T (46.15%), which was a significant predictor of common previous miscarriages.

CONCLUSION

In a group of women with spontaneous abortions with or without IUFD in the region of Central Serbia, there is an increased frequency of mutated FVL alleles and MTHFR C677T and an increased frequency of homozygous/heterozygous carriers of FV Leiden G1691A, MTHFR C677T and PAI-1 4G/5G variants. The finding of a weak positive correlation between MTHFR C677T and RPL in this study indicates that this variant is significant, along with the other thrombophilic mutations, and highlights certain dietary habits in this region, for example, low folate intake, as well as the importance of folate supplementation prior to pregnancy, particularly in women who are carriers of the MTHFR C677T variant.

In this research, we found dominance of the compound genotype PAI-1 4G/5G + MTHFR C677T in a group of women with pregnancy loss in Central Serbia, which was found to be the only significant predictor of common previous miscarriages, and showed their synergistic effect on the loss of pregnancy complicated by thrombophilia.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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SCREENING FOR ANXIETY DISORDERS AMONG SCHOOLCHILDREN WITH ASTHMA

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PROCENA ANKSIZNOZNIH POREMEĆAJA KOD ŠKOLSKE DECE SA ASTMOM

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ABSTRACT

The aim of this study was to perform screening for anxiety disorders among children with asthma and to reveal factors associated with general anxiety disorder and its specific forms.

This was a cross-sectional study conducted among outpatients with asthma during routine visits to pediatricians. They were screened for anxiety disorders using SCARED self-reported questionnaire. Additional data were collected using specially designed questionnaire as well as the patient files. Statistical analysis was performed by the SPPS software using descriptive statistics and logistic regression.

Study population consisted mostly of schoolchildren (n=58), 8-12 years old, and adolescents (13-17 years) (n=13). Approximately 33.8% respondents were positive for general anxiety disorder. The most common were separation anxiety and social anxiety, recorded among 49.3% and 32.4% of patients, respectively. Generalized anxiety and panic/somatic disorder were recorded in the same percentage of patients (21.3%), while avoiding school was the least frequent (14.08%). Influence of numerous factors was tested, but only the following showed significant effects: peak expiratory flow test was associated with general anxiety disorder, patient's age and gender with PD, and living place, asthma control according to GINA and age on GAD. Parent's smoking was associated with SAD, age and patient's weight status with SPH, and GINA asthma control with SA.

These findings suggest that anxiety disorders are common among children and adolescents with asthma. Various factors can be associated with general anxiety disorder and its specific forms, but some of them being preventable as avoiding smoking in the family.

Keywords: *asthma, children, adolescents, anxiety disorder, SCARED*

SAŽETAK

Cilj ove studije je bio da izvrši procenu anksioznih poremećaja kod dece sa astmom kao i da otkrije faktore povezane sa opštom anksioznošću i njenim specifičnim formama.

Izvedena je studija preseka kod vanbolničkih pacijenata sa astmom tokom rutinske posete pedijatru, a ispitivanje anksioznih poremećaja je izvršeno upotrebom SCARED upitnika. Dodatni podaci su prikupljeni na osnovu posebno dizajniranog upitnika i podataka iz medicinske dokumentacije. Statistička analiza je izvedena korišćenjem SPPS softvera i upotrebom deskriptivne statistike i logističke regresije.

Studijska populacija sastojala se većinom od školske dece (n=58), starosti od 8-12 godina i adolescenata (13-17 godina) (n=13). Približno 33.8% ispitanika je bilo pozitivno na anksiozni poremećaj. Najčešće su bile prisutne separaciona i socijalna anksioznost kod 49.3% i 32.4% pacijenata. Generalizovana anksioznost i panični poremećaj su zabeleženi u istom procentu (21.3%), dok je izbegavanje škole najmanje frekventno (14.08%). Ispitivan je uticaj brojnih faktora, ali su samo sledeći pokazali značajan efekat: test za vršni ekspirijumski protok bio je povezan sa opštom anksioznošću, godine i pol pacijenata sa paničnim poremećajem, mesto stanovanja, kontrola astme u skladu sa GINA klasifikacijom i godine pacijenata sa generalizovanom anksioznošću. Upotreba cigareta od strane roditelja je povezana sa separacionom anksioznošću, godine i stepen uhranjenosti pacijenata sa socijalnom fobijom, i GINA kontrola astme sa izbegavanjem škole.

Ovi rezultati sugerišu da su anksiozni poremećaji često prisutni kod dece i adolescenata sa astmom. Različiti faktori mogu biti povezani sa opštom anksioznošću i njenim specifičnim formama, ali neki od njih mogu biti preventabilni kao što je izbegavanje pušenja u porodici.

Ključne reči: *astma, deca, adolescenti, anksioznost, SCARED*

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ABBREVIATIONS

BMI - body mass index
FEV 1 - forced expiratory volume in the first second
GAD - generalized anxiety
GINA - Global Initiative for Asthma
PD - panic/somatic disorder
PEF - peak expiratory flow
SA - school avoidance
SAD - separation anxiety
SCARED - Screen for Child Anxiety Related Disorders
SPH - social phobia

INTRODUCTION

Anxiety is one of the most common psychiatric disorders with high prevalence in childhood (6-20%) and adolescence (8%) (1). Also, more than 10% of this vulnerable population worldwide has some chronic illness including asthma (2). Due to occasional hospitalization, early onset, long term medication usage and limited social life, certain psychological problems can occur among children with bronchial asthma (3). Taken together, prevalence of psychiatric illness is significantly increased and its estimated value ranged from 28% to 34% in children with asthma (4).

In 20th century, numerous studies have shown higher incidence of psychiatric problems among children with severe asthma in comparison to children without chronic illness (4,5). The review published in 2008 reported that one third of children and adolescents with asthma had anxiety disorder as comorbidity (6). Researchers suggested that the same neurotransmitters involved in anxiety and depression also are included in bronchoconstriction and inflammation. Furthermore, symptoms of anxiety can decrease ability to receive optimal treatment of asthma. On the other hand, asthma with its symptoms may interfere with individual functioning and precipitate anxiety and depression (7).

Most of children develop symptoms of both asthma and anxiety before the age of 6. It is important to treat anxiety early, in order to avoid serious consequences including social and additional mental problems (2). Moderate level of anxiety in children with asthma is necessary because indifference may cause ignoring symptoms and lack of adherence. In turn, excessive anxiety can cause medication overdose or inefficient asthma management due to paralyzing fear (8). Social anxiety is also common among children and adolescents with asthma (9,10).

Until now, there were 28 scales developed for identifying anxiety in children with long term physical conditions. Screen for Child Anxiety Related Disorders (SCARED) is one of the most satisfactory scales for identification of anxiety in children and adolescents (1,4). Psychometric properties of the SCARED have been examined in several cultural backgrounds including German, Chinese, South Africa, Italian, Brazilian, Persian and Arabian (11-13). Numerous studies indicated that the SCARED has good internal consistency, discriminatory and concurrent validity, and its scores correlated well with Spence Children's Anxiety Scale, the Youth Self Report and the Columbia Impairment Scale scores (13).

The aim of this study was perform screening for anxiety disorder among children with asthma and also to reveal factors associated with general anxiety disorder and its specific forms such as panic/somatic disorder (PD), generalized (GAD) and separation anxiety (SAD), social phobia (SPH) and school avoidance (SA).

METHODS

Study population

This was a cross-sectional study conducted among outpatients with asthma during their routine visits to pediatricians at Pediatric Clinic, Clinical Center Kragujevac, Serbia. The study was approved by the Ethics Committee of the Clinical Center (N_0 01-8325, 10.08.2015.) and the investigation was conducted in accordance to the principles of the Helsinki Declaration from January to March 2015.

The study population consisted of schoolchildren and adolescents (8-17years) with previously physician-diagnosed asthma. Before seeing a physician, the participants and their parents were informed about the purpose of the study and the children were included only if they themselves agreed to participate and their parents gave written informed consent. In total, seventy-four SCARED and demographic questionnaires were completed and returned to the researchers.

Questionnaires

The SCARED is frequently used, self-reported questionnaire consisting of 41 items that were validated and culturally adapted to Serbian language (14). It is used to assess symptoms of childhood anxiety disorders during previous three months in children 8 to 18 years old. The original SCARED scale was developed by Birmaher et al. in 1999 (15). The scale has five subscales where each item is scored on a 3-point scale (0 not true or hardly ever true, 1 somewhat true or sometimes true, and 2 very true or often true): panic/somatic disorder (PD, 13 items), generalized anxiety disorder (GAD, 8 items), separation anxiety disorder (SAD, 8 items), social phobia (SPH, 7 items) and school avoidance (SA or school anxiety symptoms, 4 items) subscale. The total SCARED score is the sum of all

41 items with possible range from 0 to 82, where higher scores indicate presence of higher anxiety in children. The cut-off values of otal scale and its subscales are: ≥ 25 for total SCARED, ≥7 for PD, ≥9 for GAD, ≥5 for SAD, ≥8 for SPH, and ≥3 for SA scale, respectively.

The data about total body weight, height, sex and age of patients, its living place and school success, hereditary predisposition (present or absent asthma in the family), parental smoking status (none, one or both) and a mean daily exposure to sunshine (cut-off value was one hour) were collected using special questionnaire made by the study investigators. The following data were collected from the patient files: values of biochemical tests (serum calcium, phosphate, concentrations of 25-hydroxy vitamin D3 or 25,OH vitamin D3 and a total serum immunoglobulin E), results of spirometry (forced expiratory volume in the first second or FEV 1 and p*eak expiratory flow* or PEF), medication, exacerbation rate, allergy skin prick tests according to the European Academy of Allergy and Clinical Immunology: inhalant allergens (ten: mildew, tree pollen, house dust mite, cat and dog's hair, pollen ambrosia, Festuca preatense, Dermatophagoides, feathers and cockroach) and food allergens (fourteen: spinach, tomato, chicken yolk and white, sea fish, orange, pork meat, wheat flour, carrot, soya, peanut, peas, cow's milk and cocoa), and a degree of asthma control according to the Global Initiative for Asthma (GINA) classification (controlled, partially controlled and uncontrolled) as rated by the attending physicians (16,17).

The data were described statistically at first, using measures of central tendency and dispersion. Univariate and multivariate logistic regression were used to identify factors significantly associated with anxiety. The Hosmer-Lemeshow test was used to assess the performance of the logistic regression model. The SPPS software (version 18.0) was used to perform all calculations.

Table 1. Characteristics of the study population

Patients characteristics	Mean ± standard deviation	Range
Number of patients	71	
Age (years)	10.49 ±2.33	8-17
Total body weight (kg)	42.38±15.94	20-92
Body mass index (kg/m^2)	18.94±4.11	13.64-30
Weigh status (BMI percentiles): - underweight - healthy weight - overweight - obesity	 - 4/71 - 46/71 - 9/71 - 12/71	
Gender (male/female)	39/32	
Living place (urban/village)	48/23	
School success	4.57±0.7	1-5
25,OH vit D3 serum concentration (ng/mL)	16.56±6.0	4.6-31.37
Serum calcium levels (mmol/L)	2.46±0.08	2.25-2.61
Serum phosphate levels (mmol/L)	1.45±0.19	0.96-2.14
Total serum Ig E (IU/mL)	422.66±629.76	2.69-3000
Sun exposure (<1h/ >1h)	51/20	
Allergy skin tests (+/-)	55/16	
Hereditary predisposition (yes/no)	45/26	
Parents smoking (yes/no)	32/39	
FEV1 (%)	97.36±11.13	74.9-125.6
PEF (%)	91.38±13.69	60.3-116.1
Exacerbation rate per patient	0.9±1.08	0-3
Montelukast (yes/no)	32/39	
H1 antihistamines (yes/no)	10/61	
Inhalation corticosteroids (yes/no)	50/21	
Azelastine/fluticasone (yes/no)	5/66	
GINA asthma control (No of patients): - controlled - partially controlled - uncontrolled	 - 24 - 43 - 4	

RESULTS

Total number of participants was seventy-four but both questionnaires were completed by seventy-one participants (97.26% response rate). The study population consisted mostly from schoolchildren (n=58), 8-12 years old, and adolescents (13-17 years) (n=13), with mean age of the total population being 10.5±2.33 years. All children had previously physician-diagnosed asthma and a slightly higher percentage was in favor of males in relation to females (55% vs. 45%). Both, body mass index (BMI) (kg/m^2) and BMI percentiles were calculated in the target population. Weigh status of children and adolescents was estimated using BMI percentiles for age and sex according to the Center for disease control and prevention growth recommendations (underweight: BMI <5th percentile, healthy weight: BMI ≥5th to <85th percentile, overweight: BMI ≥85th to <95th percentile and obesity: ≥95th percentile). Our study population consisted from 5.63% underweight patients (n=4), 64.79% healthy weight (n=46), 12.68% overweight (n=9) and 16.9% obesity patients (n=12). Table 1 provides baseline characteristics of the patients.

Mean value of total SCARED score was 22.31 (range 4 -54) and presence of childhood anxiety disorders symptoms was found in twenty-four children (33.80%). Mean scores of the subscales were: 4.57 for panic/somatic disorder (PD range 0-16), 5.27 for generalized anxiety disorder (GA range 0-17), 4.95 for separation anxiety disorder (SAD range 0-15), 6.38 for social phobia (SPH range 0-14) and 1.28 for school avoidance (SA range 0-7), respectively. According to the cut-off values of the subscales, separation anxiety (49.3%, n=35) and social anxiety (32.4%, n=23) were the most frequent. Generalized anxiety and panic/somatic disorders (21.3% both, n=15) were moderately frequent, while avoiding school trait was the least frequent (14.08%, n=10). Results of this analysis are summarized in the Table 2.

Table 2. Total scores of SCARED scale in the study population

Scale	Mean ± standard deviation	Present/ not present
SCARED total	22.31±12.03	24/47
Panic/somatic disorder (PD)	4.57±3.81	15/56
Generalized anxiety disorder (GAD)	5.27±4.18	15/56
Separation anxiety disorder (SAD)	4.95±3.82	35/36
Social phobia (SPH)	6.38±3.13	23/48
School avoidance (SA)	1.28±1.54	10/61

Using logistic regression factors associated with values above the cut-offs on the SCARED scale and its five subscales were identified. The following independent variables for both the full scale and subscales were accounted for the logistic regression: age, gender, weight status (using BMI percentiles: underweight, healthy weight, overweight and obesity), school success, living place, hereditary predisposition, parental smoking, sun exposure, allergy skin prick test, serum levels of calcium, phosphate, total IgE and 25,OH vitamin D3, rate of exacerbations and test of lung function (FEV1 and PEF), use of medication against asthma (montelukast, H1 antihistaminics, inhalation corticosteroids and nasal spray with azelastine and fluticasone) and asthma control status according to the GINA classification. The results of the logistic regressions for both complete scale and its subscales are presented in the Table 3.

DISCUSSION

The results of the study demonstrated that anxiety disorder was present in one-third of out-patients with asthma (children and adolescents) using the self-reported SCARED questionnaire. In regard to different types of anxiety, the results showed that the most common were separation anxiety (in almost half of the respondents) and social anxiety (approximately 33%). Both generalized anxiety and panic/somatic disorder were noted in a significantly smaller number of respondents (21%), while avoiding school trait was observed only in ten children.

In accordance with our results, Busing et al. reported that separation anxiety was the most common diagnosis in pediatric patients with asthma compared to healthy subjects (p=0.059) (18). On the other hand, the study conducted on 74 children with asthma found presence of separation anxiety disorder in only 8.1%, while panic disorder was the most frequently encountered (14.9%) (19). Earlier studies showed that social anxiety was frequent among adolescents. Presence of asthma led to reduction of their social interactions due to impairment in physical or social activities (19-24- 20-25). Moreover, they often feared from rejection by peers or felt different and isolated. It is known that peers have very important role in development of identity and social interactions (9, 25-26).

Reverse correlation between peak expiratory flow and symptoms of anxiety found in our study was supported by

Table 3. Results of the logistic regression models

Scale and its subscales	Logistic regression model quality	Significant factors and its adjusted odd ratios ($OR_{adjusted}$) with 95% confidence interval
SCARED total	CSRS= 0.350 NKRS= 0.485 HLT= 7.196	PEF = 0.924 (0.857-0.996) p= 0.04
Panic/somatic disorder (PD)	CSRS= 0.357 NKRS= 0.555 HLT= 3.462	Age = 0.565 (0.325-0.983) p= 0.043
		Gender = 22.835 (1.608-324.379) p= 0.021
Generalized anxiety disorder (GAD)	CSRS= 0.392 NKRS= 0.610 HLT= 3.578	Age = 0.384 (0.156-0.941) p= 0.036
		Living place = 52.065 (1.690-1604.072) p= 0.024
		GINA = 75.328 (1.060-5351.942) p= 0.047
Separation anxiety disorder (SAD)	CSRS= 0.351 NKRS= 0.468 HLT= 5.007	Parents smoking = 4.542(1.172-17.598) p= 0.029
Social phobia (SPH)	CSRS= 0.230 NKRS= 0.306 HLT= 10.444	Age = 0.675 (0.534-0.853) p= 0.001
		Weight status = 0.493 (0.251-0.968) p= 0.04
School avoidance (SA)	CSRS= 0.281 NKRS= 0.506 HLT= 1.001	GINA= 209.365 (1.693-25886.887) p= 0.03

CSRS: Cox& Snell R square, NKRS: Nagelkerke R square, HLT: Hosmer and Lemeshow test, PEF: p*eak expiratory flow*, p: statistical significant.

results of the study on adolescents with severe persistent asthma (10). In this study significant linear relationship between adolescents' asthma-related anxiety and their management of existing symptoms (β=0.03, p=0.021) was observed, as higher level of anxiety was associated with more steps necessary to control existing symptoms (10).

Factors associated with the GAD in our study were: age (older children expressed less of general anxiety disorder); living place (children from rural areas had fewer symptoms of generalized anxiety disorder), and GINA classification concerning asthma control (better asthma control led to less symptoms of generalized anxiety disorder). Gentile in his study connected exposure of inner-city violence to parental mental health conditions and subsequent nicotine addiction, as environmental tobacco smoke could lead to development of childhood asthma (27). Also, children and its family living in the city are more exposed to stress. In a study among pediatric patients with mild, moderate and severe asthma, emotional factors and family dynamics were triggers for the disease attacks in 16% of children with mild, 38% with moderate and 68% with severe asthma. Between severity of asthma and maternity anxiety no significant correlation was found. Disease duration was not associated to depression or anxiety (3). Literature data showed that frequency of anxiety disorder increased with age: 4.1% in children with asthma and 8.9% in adolescents with asthma (7,19).

Control of asthma according to GINA was associated with avoidance of school (as the control of asthma gets better the school avoidance is less frequent). The study on high school students with symptoms of asthma reported significantly greater total social anxiety scores than in peers with no symptoms. In this study Social Anxiety Scale for Adolescents (SAS-A) and the Social Phobia and Anxiety Inventory for Children (SPAI-C) were used to diagnose anxiety (9). Additionally, children with well controlled asthma do not have increased risk of anxiety disorders, as it was shown in a study on 70 well-controlled asthmatics and 70 matched healthy controls (28).

Patient's age has proved to be protective against social phobia together with its weight status in our population. As previously mentioned, frequency of anxiety disorder increased with age and social anxiety is commonly reported disorder in adolescents (7,19,20). Nowadays, literature data show that childhood obesity is global, epidemic and multi factorial health condition. Researchers also suggested that obese children and adolescent have a higher risk for many physical and psychological consequences (29,30). Childhood obesity has complex etiology and it is associated with complex behaviors and outcomes, but the causal relationship as well as the mediated factors between obesity and children mental health, are not yet well established. Psychological consequences often included problems linked with quality of life, self-esteem, depression or anxiety in children and adolescent. Although literature data show a moderate level of evidence for a positive association between obesity and anxiety disorders, but a negative relationship has been also reported (31). Vila and co-authors suggested that was no correlation between severity of obesity and frequency of psychiatric disorders in population of 155 children, age 5-17 years (32). On the other hand, some studies indicated that obesity is associated with higher prevalence of asthma and wheezing symptom in adolescents or with an increased risk of having a dry night cough in children (33-35). Moreover, a large cross-sectional study using data from 43.297 children, aged 10 to 17 years, and BMI percentiles for gender and age, indicated that asthma and allergies were more common in obese children (36). Study conducted in the Netherlands among children ages 11 to 16.3 showed the relationship between self-reported social anxiety and the severity of respiratory symptoms (37).

In our study children with a parent-smoker showed higher level of separation anxiety disorder than children of non-smokers. This is supported by a review about the relationship between environmental tobacco smoke exposure (ETS) and childhood respiratory disease. Key findings were that up to 70% of children are exposed to environmental tobacco smoking globally. Maternal smoking and ETS exposure has adverse influence on lung development of infants and are associated with upper and lower respiratory tract infection in childhood, wheezing or asthma. Environmental tobacco smoke exposure reduces lung function early in life, establishing an increased lifelong risk of respiratory diseases (38).

Girls in our study showed correlation to panic/somatic disorder more than boys, and age also was a significant factor in logistical regression model (older children had less panic/somatic disorder). This is proven in our small sample. Katon et al. reported that a prevalence of panic disorder or agoraphobia has been varied between 0.6% and 4.7% among asthmatic children (39). Female gender was also one of the factors that significantly increased risk of several disorders concerning anxiety that was in accordance to our results (40).

Results of the current study indicated no association between serum concentrations 25,OH vitamin D3 and anxiety in asthmatic children. Previous studies also showed that vitamin D supplementation or deficiency status were not related to anxiety in young adults (41,42). Our findings did not found correlation between any allergy skin prick test and anxiety disorders using SCARED scale. However, the study on 80 children (mean age 8.1 years) showed that food allergies in children with asthma were associated with anxiety, using Multidimensional Anxiety Scale for Children (MASC) Total (p=0.007), MASC Humiliation Rejection, (p=0.02) and MASC Social Anxiety scales (p=0.02) (43).

The main limitation of our study was small number of respondents (a total sample of 71 children and adolescents with asthma). Attitudes, worries and behaviors of parents were not taken into account in our study, although these could be important confounders hidden in the background of some of the identified risk factors.

In conclusion, anxiety disorders, especially separation anxiety and social anxiety, are common among children and adolescents with asthma. Various factors can be associated with general anxiety disorder and its specific forms, but some of them being preventable as avoiding smoking in the family.

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EFFECT OF BEHAVIOURAL INTERVENTIONS FOR OBESITY PREVENTION IN PREGNANCY ON THE ADEQUACY OF GESTATIONAL WEIGHT GAIN AND RETENTION: METABOLIC HEALTH OF INDIAN WOMEN

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EFEKTI BIHEVIORALNIH INTERVENCIJA ZA PREVENCIJU GOJAZNOSTI U TRUDNOĆI NA PRAVILAN RAST I ODRŽAVANJE GESTACIJSKE TEŽINE: METABOLIČKO ZDRAVLJE INDIJSKIH ŽENA

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ABSTRACT

The aim of this study was to measure the adequacy of gestational weight gain (GWG) in Indian women using various behavioural interventions during pregnancy, which primarily aim to observe the effects on obesity markers and weight retention.

In this experimental study, one hundred and forty pregnant women underwent interventions in 5 groups, control (C), diet (D), home exercise (HE), supervised exercise (SE) and supervised exercise with diet (SED), from pregnancy through delivery with 2 months follow-up post-delivery. The outcome measures were GWG and baby birth weight.

A one-way ANOVA indicated no differences in the mean GWG between groups (12.39±4.71 kg, p=0.947). The control group had the most (50%) and both the supervised exercise groups had the fewest (32%) women who gained above the recommended GWG, followed by the diet group (33.3%). The D and HE groups had the most women who gained within the GWG range, while both the SE and SED groups had the most women who gained below the GWG range. However, these results did not affect the birth weight between the groups (mean 2.96 kg±0.40, p=0.203). In women with normal BMIs, (18.5-22.9 kg/m2), the diet group had the most effective maintenance of adequate GWG, with 15%, 55%, and 30% of the women gaining above, within, and below the recommended GWG, respectively. The SE and SED groups had the least postpartum weight retention (PPWR) at 2 months, followed by the HM, D and C groups; i.e., the results showed a trend in the desired direction clinically, although they were not statistically significant (p=0.12).

Supervised exercise can be effectively used as a pregnancy intervention to prevent excess GWG in Indian women. Diet counselling was found to be the next best intervention in combination with exercise, as well as for women with normal BMI.

Keywords: *Gestational weight gain, pregnancy, behavioural intervention, birth weight, metabolic health*

SAŽETAK

Cilj ove studije bio je da proceni adekvatan rast gestacijske težine (GTR) kod žena u Indiji upotrebom različitih biohevioralnih intervencija tokom trudnoće, sa primarnim ciljem da utvrdi efekte ovih intervencija na markere gojaznosti i zadržavanje težine.

U ovoj eksperimentalnoj studiji, 140 trudnica je podvrgnuto intervencijama i podeljeno u 5 grupa, kontrolna grupa (C), grupa na dijeti (D), grupa koja trenira (HE), grupa koja trenira pod kontrolisanim uslovima (SE) i grupa koja trenira i sprovodi dijetu (SED) od početka trudnoće do 2 meseca nakon porodjaja. Ishodi koji se prate su GTR i telesna težina novorođenčeta.

Jednofaktorska ANOVA utvrdila je da nema razlike u GTR između grupa (12,39±4,71 kg, p=0,947). Kontrolna je imala najviši GTR (50%), zatim obe grupe na treninzima (32%) a onda i grupa na dijeti (33,3%). D i HE grupa su imale najviše ispitanica koje su imale GTR u okviru preporučenog opsega, dok su grupe SE i SED imale najviše ispitanica sa GTR-om ispod preporučenog opsega. Ipak, ovi rezultati nisu uticali na telesnu težinu novorođenčeta ni u jednoj grupi (18,5-22,9 kg/m2), pri čemu je D grupa imala najefektnije održavanje GT sa 15%, 55% i 30% žena koje dobijaju na težini, ne dobijaju i gube na težini, respektivno. U SE i SED grupama najviše žena je imalo najnižu stopu održavanja telesne težine 2 meseca nakon porođaja, a zatim u grupama HM, D i C; tj., rezultati pokazuju očekivani trend kliničke promene iako razlike nisu bile statističke značajne (p=0,12).

Trening pod stručnim nadzorom može biti efikasna metoda u trudnoći koja sprečava GTR kod žena u Indiji. Odmah posle treninga, dijetalni režim je intervencija koja u kombinaciji sa treningom ima dobre rezultate i za žene sa normalnim BMI.

Ključne reči: *rast gestacijske težine, trudnoća, biohevioralne intervencije, težina na rođenju, metaboličko zdravlje*

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INTRODUCTION

Pregnancy is associated with normal weight gain, which is termed as gestational weight gain (GWG). This weight gain is essential for the normal physiological growth and survival of the foetus until delivery (1), and it is also an indicator of the health status of the expecting woman along with her child. Owing to the importance of GWG in pregnancy, in 2009, the Institute of Medicine (IOM) issued certain weight gain recommendations for expecting women according to their prepregnancy body mass index (BMI) (2). The GWG recommendation ranges per the prepregnancy BMI classification are 12-18 kg for underweight (i.e., BMI <18.5 kg/m^2), 11.5-16 kg for normal range (i.e., BMI 18.5-22.9 kg/m^2), 7-11.5 kg for overweight (i.e., BMI 23-25 kg/m^2) and 5-9 kg for obese (i.e., BMI >25 kg/m^2). It is recommended that women gain weight within these GWG ranges. Gaining above and below these ranges has been reported to result in various pregnancy complications in both western and Indian populations (3,4,5). Excess GWG may result in excess postpartum weight retention (PPWR), leading to obesity in the future, which may in turn increase the risk of various metabolic diseases such as diabetes, hypertension and other cardiovascular conditions in women (6). However, there are no such GWG recommendations specific to Indian women. Researchers have shown the effects of various pregnancy interventions for achieving adequate weight gain or preventing excess GWG in women with different BMI classifications (7,8,9). A majority of these studies are focused on overweight or obese women, whereas women with normal BMI are equally prone to the adverse effects of excess GWG (10). Owing to the same, as well as a rise in obesity among Indian women in recent years, we planned to investigate the trend of GWG in relation to the effects of different behavioural interventions during pregnancy, i.e., control group, diet, home exercise, supervised exercise and supervised exercise with diet, on GWG in pregnant Indian women.

MATERIALS AND METHODS

This paper presents secondary data on GWG from an experimental study with a different subject design (parallel group with pre- and post-comparisons) that was conducted from June 2016 to May 2017. The main trial had the primary aim to assess the effectiveness of 5 different behavioural interventions during pregnancy, i.e., control group, diet, home exercise, supervised exercise and supervised exercise with diet, on central obesity, weight retention and pregnancy complications in pregnant Indian women. The complete details about the study have been published elsewhere(11). The main trial is registered with the clinical trial registry of India with CTRI No. CTRI/2017/04/008322. The ethical approval for the same study was obtained from the university's ethical committee vide letter no. PTY/2016/555 in accordance with the Helsinki Declaration. All subjects signed the consent forms. This paper is a perspective from the main trial, aimed to assess the gestational weight gain patterns in pregnant Indian women as per the 5 different interventional arms from the main trial. The inclusion criteria were women with a singleton live pregnancy, BMI ≥18.5 kg/m^2, age ≥18 years and access to a mobile phone. One hundred forty pregnant women were studied for a duration of 10 months, i.e., from recruitment at 12-16 weeks of gestation until 2 months after delivery, from the outpatient department of a maternity hospital on the panel of GJUS&T (11). The exclusion criteria were multiple gestations, prior pregnancy complications such as diabetes, hypertension, and vascular disease, and being declared unfit by a gynaecologist. Moreover, all women had a similar socioeconomic status and were not physically active before being included in the trial. Initially, we intended to recruit 30 participants in each group, for a total of 150 participants. Owing to the demands of the study and the novelty of the antenatal exercise intervention in this region, recruitment was a challenge. Indian women are not inclined towards exercise during pregnancy. Therefore, women were recruited as per their availability to attend supervised classes at the hospital as a modification from the initial trial, which was originally planned as randomized trial. Those who were able to come for these sessions were randomized into two groups (exercise, exercise with diet), while those who expressed their inability to attend these sessions at the study hospital due to any reason were randomized into three groups (control, diet, home exercise). Previous studies conducted in western countries on diet and exercise interventions during pregnancy were conducted with even fewer participants in each group (12,13). Of these, one study had 68 women recruited in 4 groups, while the other had 113 women recruited in 4 groups (12, 13). Though from developed countries, these studies also highlighted the difficulties regarding recruitment for a pregnancy trial. We attempted the same in India, where women are not encouraged to be physically active during pregnancy owing to various social and cultural beliefs.

Intervention in all groups started at 20 weeks and continued until 36 weeks of gestation. Briefly, the interventions in the 5 groups were as follows: Women in the control group were advised once at initial recruitment to follow a healthy lifestyle during pregnancy, focusing on a balanced diet and including daily physical activity. Those in the diet group received a twice-weekly mobile SMS advising that a healthy diet be followed during pregnancy. Participants in the home exercise group were taught prenatal exercises for the home and advised at recruitment to regularly walk for 30 minutes per day. If they forgot any of the exercises, they were free to ask again during any antenatal visit. Women in the exercise group received weekly supervised prenatal exercise sessions with a trained female physiotherapist from 20-36 weeks of gestation, while those in the exercise + diet group received supervised exercise sessions along with dietary messages during pregnancy(11). Data were collected at recruitment (12-16 weeks), term (36-38 weeks) and 2

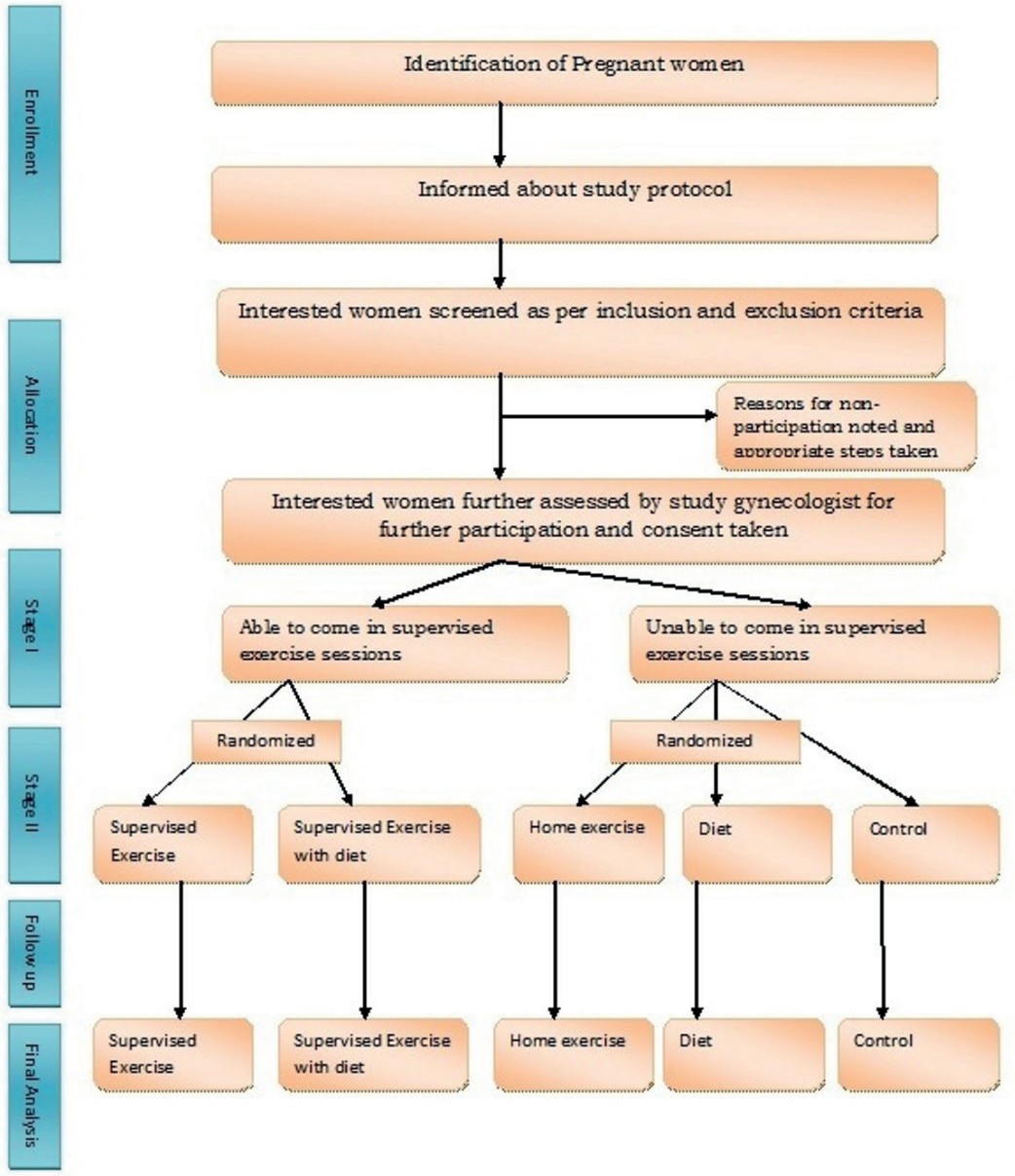

Figure 1. Shows the flow chart for participant recruitment and participation in the study

months post-delivery. The prepregnancy data were self-reported and cross-checked from the earliest available hospital records of each woman. Various studies have shown good validity of the self-reported prepregnancy weight if collected early in pregnancy (14,15,16). Women were prescreened for similar socioeconomic status (Kuppuswamy's SES) and physical activity levels (IPAQ-short version) using standard questionnaires before inclusion in the study. All were minimally active and had a similar socioeconomic status. Figure 1 shows a flow chart of participant recruitment and the final groups for the study.

OUTCOME VARIABLES

The main outcomes for this secondary analysis were GWG, PPWR and infant birth weight. GWG was calculated as the difference between the weight at delivery and the prepregnancy weight. PPWR was calculated as the difference between the final weight measured at the 2-month postpartum visit and the prepregnancy weight. Baby birth weight was recorded from the hospital records.

STATISTICAL ANALYSIS

Baseline readings and mean differences among the 5 groups were compared using one-way ANOVA (mean± SD with 95% CI), while Kruskal–Wallis ANOVA on ranks (median, 25th and 75th percentiles) was used for non-normally distributed variables. An intention-to-treat analysis was not preferred, as we excluded women who dropped out of the study or did not complete the follow-up data. The reasons for these exclusions were not the worsening

Table1. The baseline data of study participants

Demographic Characteristics	Control [30]	Diet [30]	Home Exercise [30]	Exercise [25]	Exercise +Diet [25]	F/H	P
Age [years]# Mean ± SD[P], 95% CI	26.23±3.62 [24.87-27.58]	26.46±4.15 [24.91-28.02]	25.86±3.45 [24.57-27.15]	25.92±3.62 [24.42-27.42]	26.84±2.99 [25.60-28.07]	0.33	0.86
Median [25%,75%] [NP]	26 [24,30]	25.5 [23,29]	26 [24,28]	25 [22.75,29.25]	27 [24,29.25]	1.27	0.866
Weight [Kg]# Mean ± SD[P], 95% CI	59.03±8.04 [56.03-62.04]	56.06±7.69 [53.19-58.94]	56.20±9.12 [52.79-59.61]	57.16±9.08 [53.41-60.91]	56.84±8.47 [53.34-60.34]	0.59	0.67
Median [25%,75%] [NP]	57.5 [53,67]	54 [50,60]	54.5 [50,60]	55 [50,64]	55 [50.75,62]	2.606	0.626
BMI [Kg/m^2]# Mean ± SD[P], 95% CI	23.12±3.62 [21.77-24.47]	21.82±2.75 [20.79-22.85]	22.42±3.30 [21.19-23.66]	22.10±2.93 [20.89-23.32]	22.15±2.63 [21.06-23.24]	0.90	0.460
Median [25%,75%] [NP]	22.2 [20.1,26.2]	21.25 [19.5,23.4]	21.4 [19.8,24.5]	21.5 [19.5,24.07]	21.60 [19.9,23.57]	1.882	0.757

P- parametric, #- Normality failed, NP- Non Parametric

Table2. Shows the between group comparisons for GWG and PPWR along with birth weight in 5 groups by one-way ANOVA

Study variables	Control [30]	Diet [30]	Home Exercise [30]	Exercise [25]	Exercise+ Diet [25]	F	p
GWG [Kg] Mean ± SD, 95% CI	**12.63±4.88 [10.81-14.45]**	**12.93±4.28 [11.33-14.53]**	**12.16±5.01 [10.29-14.04]**	**12.24±4.97 [10.18-14.29]**	**12.00±4.42 [10.17-13.82]**	**0.18**	**0.947**
Median [25%,75%] [NP]	**12 [10,16]**	**13 [10,16]**	**12.5 [8,16]**	**13 [8,15.25]**	**12 [9.5,15]**	**0.511**	**0.972**
PPWR [Kg]# Mean ± SD, 95% CI	**8.13± 4.66 [6.39-9.87]**	**7.46± 5.06 [5.57-9.36]**	**6.20± 3.98 [4.71-7.68]**	**5.88± 4.03 [4.21-7.54]**	**5.44± 3.92 [3.81-7.06]**	**1.86**	**0.12**
Median [25%,75%] [NP]	**7.5 [5,12]**	**7 [5.9]**	**7 [3,9]**	**5 [3,8.25]**	**5 [3,9]**	**6.391**	**0.172**
Birth weight [Kg] Mean ± SD, 95% CI	**3.07± 0.34 [2.94-3.20]**	**2.89± 0.49 [2.70-3.07]**	**2.91±0.40 [2.75-3.06]**	**2.87± 0.38 [2.72-3.03]**	**3.05± 0.40 [2.88-3.21]**	**1.509**	**0.203**

P- parametric, #- Normality failed, NP- Non Parametric

Table 3. Adequacy of GWG in different groups according to their pre BMI classification (Above, within, and below the recommended GWG range)

Groups	GWG Range	Normal BMI [11.5-16 Kg]	Overweight [7-11.5 Kg]	Obese [5-9 Kg]
Control [30]	**Above [15]**	**6 [35.2%]**	**2 [66.6%]**	**7 [70%]**
	Within [6]	**4 [23.5%]**	**1 [33.3%]**	**1 [10%]**
	Below [9]	**7 [41.1%]**	**0**	**2 [20%]**
Diet [30]	**Above [10]**	**3 [15%]**	**2 [50%]**	**5 [83.3%]**
	Within [14]	**11 [55%]**	**2 [50%]**	**1 [16.6%]**
	Below [6]	**6 [30%]**	**0**	**0**
Home Ex. [30]	**Above [13]**	**6 [31.5%]**	**2 [50%]**	**5 [71.4%]**
	Within [7]	**6 [31.5%]**	**0**	**1 [14.2%]**
	Below [10]	**7 [36.5%]**	**2 [50%]**	**1 [14.2%]**
Exercise [25]	**Above [8]**	**3 [18.7%]**	**3 [60%]**	**2 [50%]**
	Within [8]	**7 [43.7]**	**1 [20%]**	**0**
	Below [9]	**6 [37.5%]**	**1 [20%]**	**2 [50%]**
Exercise±Diet[25]	**Above [8]**	**4 [23.5%]**	**2 [50%]**	**2 [50%]**
	Within [9]	**6 [35.5%]**	**2 [50%]**	**1 [25%]**
	Below [8]	**7 [41.1%]**	**0**	**1 [25%]**

*GWG-Gestational weight gain, BMI- Body mass index

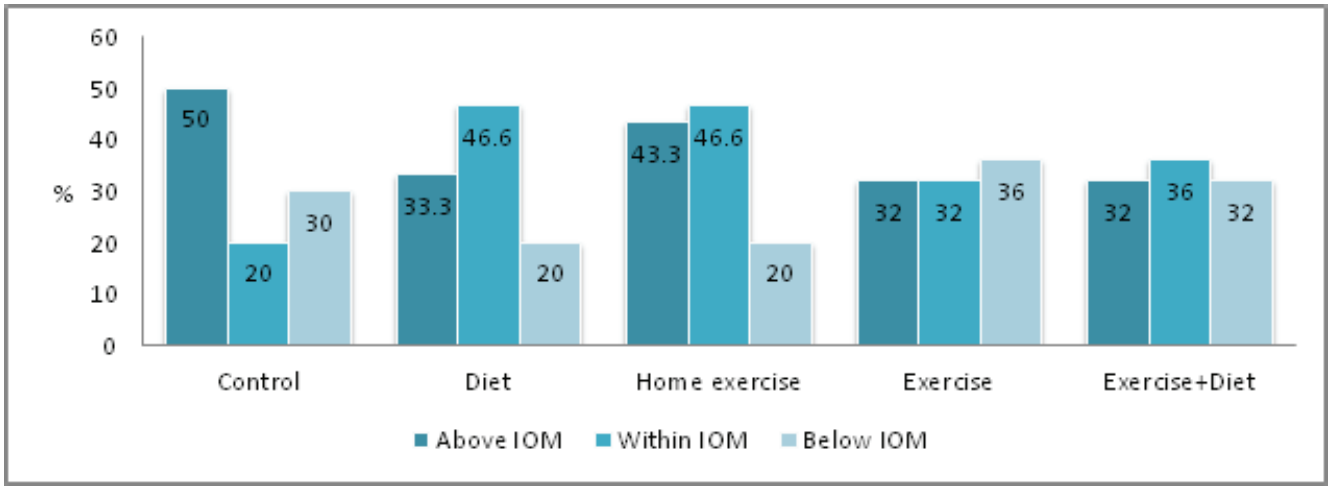

Figure 2. Adequacy of GWG in different groups according to their pre prgnancy BMI classification (Above, within and below the recommended GWG range)

of health conditions, but other unrelated personal reasons, such as weather conditions and dependence for transportation. We also had 3 test points, i.e., prepregnancy, full term and 2 months postpartum. If it were not done this way, while adjusting for missing data by the last observation carried forward method, assuming the last or baseline reading for the respective study variables would be inappropriate due to the physiological gain in weight and other parameters during the study and due to the special case of 'pregnancy'. All statistical analyses were conducted using SPSS version 21, and $p \leq 0.05$ was considered to be statistically significant.

RESULTS

One hundred and forty women were recruited in this trial: 30 women each in the control, diet and home exercise groups and 25 women each in the supervised exercise group and the supervised exercise with diet group. The average GWG for the study participants was 12.39±4.71 kg. All women were of the middle class and were not active before being included in the study. Table 1 shows the baseline data of the study participants.

All the groups had clinically comparable GWG. The interventions did not have a significant difference on total GWG between the groups. However, the trend for PPWR was in the desired direction, with least to maximum weight retention in the exercise + diet > exercise > home exercise > diet > control groups, although the results were not statistically significant (p=0.172). Further subgroup analyses for women in each intervention arm who gained above, within and below the recommended IOM GWG guidelines in each group are shown in Table 3.

Although all the groups had comparable GWG, the subgroup analysis showed that both of the supervised exercise groups had the least number of women who gained above the recommended levels of GWG (32% each), which was followed by the diet group (33.3%), home exercise group (43.3%) and control group (50%). These results show the effectiveness of supervised exercise over unsupervised home-based exercise. Diet intervention alone was more effective than home-based exercise in preventing weight gain above the IOM recommended guidelines in combined group comparisons.

However, the diet and home exercise groups (46.6% each) had the most women who gained within the IOM recommended GWG guidelines. This was followed by the supervised exercise groups (32% and 36%) and the control group (20%).

Both of the supervised exercise groups had the greatest number of women who gained less than the IOM recommended GWG guidelines (36% and 32%), indicating that there were no harmful effects of exercise on weight gain in pregnant women (Figure 2).

When considering only women with normal BMI, the diet group was found to be the most effective in preventing excess weight again above the recommended range (15%), with the most women staying within the weight gain ranges (55%) and the fewest women gaining below the recommended ranges (30%) (Figure 3). Such findings could not be extracted for overweight and obese women in each intervention arm due to missing data from certain groups for these BMI categories, making it difficult to draw conclusions about them (Table 3).

DISCUSSION

The results of the present study show that the different lifestyle interventions in pregnancy did not have any significant effects on GWG in Indian women. However, the 2-month PPWR was reduced in the desired direction, though not statistically significant. When comparing the adequacy of GWG between the groups, both of the supervised exercise groups had least number (32% each) of women who gained above the recommended GWG guidelines, followed by the diet (33.3%), home exercise (43.3%) and control (50%) groups. In 2009, the IOM issued new GWG guidelines, and a 2010 review reported to have no

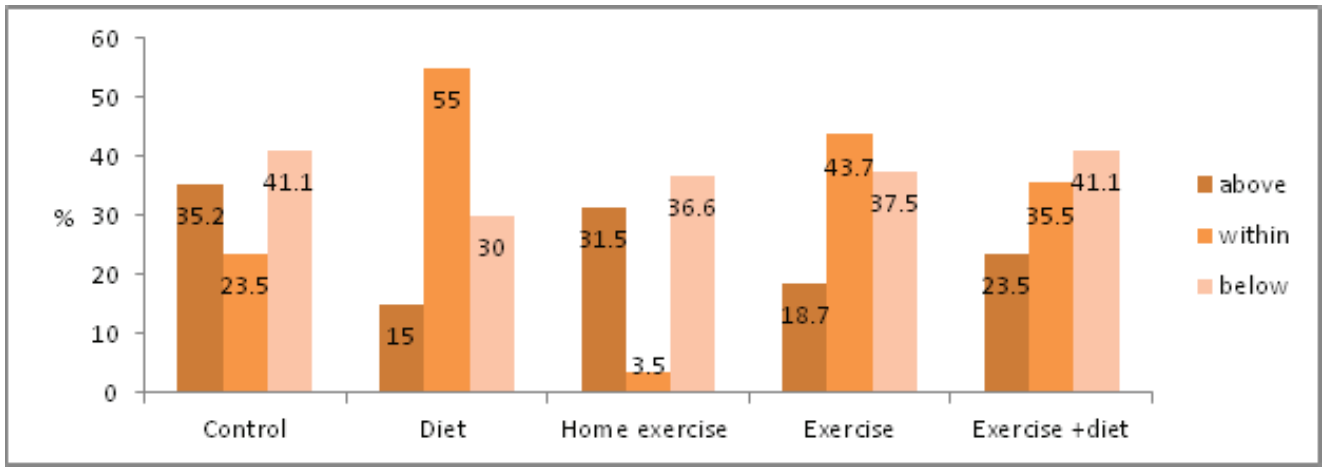

Figure 3. Adequacy of GWG in different groups (Normal BMI group) according to their pre pregnancy BMI classification (Above, within and below the recommended GWG range)

conclusive pregnancy interventions for preventing GWG (17). After that, studies showed the effectiveness of exercise and diet interventions alone and in combination for preventing GWG (18,19,20). Our results showed similar findings, as both the exercise group and the diet group were effective in preventing excess GWG in pregnant Indian women. Some previous interventions also included group supervised exercise combined with home-based exercise, as well as dietary counselling alone or with behavioural intervention (21,22,23). A recent 2018 review also showed the effectiveness of combined supervised exercises and regular dietary counselling in preventing excess GWG and PPWR (24). Our study tried to view the effect of the above interventions individually and combined in regard to supervised exercise and diet, with an additional home exercise intervention, and showed that diet proved to be better than home exercise alone in preventing excess GWG in women. The present study presented the various interventions separately in 5 groups, i.e., control, diet, home exercise, supervised exercise and supervised exercise with diet, to assess the effect of each intervention individually and when combined. Although the primary aim was to find the most effective intervention for reducing weight retention and obesity prevention in pregnant Indian women, the present findings could help to identify the next most suitable intervention for women who are unable to exercise during pregnancy due to any reason.

The diet and home exercise groups (46.6% each) had the most women who gained within the IOM recommended GWG guidelines. This was followed by the supervised exercise groups (32% and 36%) and the control group (20%). When comparing the number of women who gained below the recommended GWG guidelines, the supervised exercise + diet and the supervised exercise groups had the most, with 36% and 32%, respectively. There were no reports of adverse events due to any of the behavioural interventions, including the exercise groups. This indicates that all the planned interventions were safe for pregnant women. Women gaining weight below the recommended level is still viewed as a positive finding, as the IOM weight gain guidelines are set by referring to the BMI cut-offs for American women. By this approach, Indian women could be directed to gain more weight than required as per their Asian BMI cut-offs. However, there are studies from Asia that have found these IOM guidelines suitable (25,26) as well as unsuitable for Asian women (27,28). A 2017 Indian study reported that normal and overweight women who gain less than the recommended range have a low risk of caesarean section and macrosomia and an increased risk of preterm birth (PTB) (3). No such cases of PTB were reported in any of the groups in the present study, including the supervised exercise groups, which had the most women gaining below the IOM guidelines. There were also no significant differences in birth weight between the groups; it was comparable for all groups, with a mean birth weight of 2.95± 0.99 kg. Therefore, none of the study interventions had adverse effects on the average birth weight of the foetus.

When comparing women with normal BMI (18.5-22.9 kg/m$^{2)}$ between different interventions for GWG, the diet group showed the least number of women who gained above the recommended GWG range, followed by the supervised exercise groups. The diet group also had the greatest number of women who gained weight within the recommended range, and the least number of women who gained weight below the recommended range. Studies have shown diet alone to be effective in managing excess GWG (29), while a 2017 review reported inconclusive results for dietary interventions in pregnancy owing to the inconsistencies in the intervention content and sources imparting them, making it difficult to draw a definitive conclusion (30). This could be explained because dietary habits of a population differ considerably between countries, and within a country by ethnicity and race, influencing the outcomes to draw a conclusion.

Per our findings, this is the first interventional study conducted on pregnant women in India to assess the effect of 5 different prenatal behavioural approaches on pregnancy weight changes and obesity development. In

tions in pregnancy with different aims. The majority of these studies compared 2 groups, i.e., a control group and an intervention group. The maximum number of groups we came across was 4 (control, diet, exercise and exercise with diet), which included overweight and obese women (31). We also included women with normal BMI, as they are equally susceptible to the development of obesity during pregnancy (10). India is already the diabetic capital of the world, and the pace at which obesity is rising in women will deteriorate the future metabolic health of the nation, as pregnancy involves two individuals – a mother and a baby. None of the present study interventions had any adverse effects on baby birth weight. All the above points form the strength of this study, whereas the small sample size could be a study limitation. Since the data were collected from a single maternity centre in Hisar, Haryana, the generalization of results for the rest of India should be interpreted with caution owing to the vast diversity in the Indian population. Pregnant women and their families have apprehension in regard to exercise during pregnancy, and this becomes even more pronounced in an Indian society. Women are required to rest adequately and refrain from excess physical activity during and after delivery; i.e., a 2-month immediate postpartum resting period, commonly referred as *'japa'*, following child birth is culturally followed by a majority of the population in North India, where the study took place. Therefore, the concept of exercise during pregnancy was difficult to implement, leading to delays in recruitment and study retention. We also excluded underweight women, since the aim of the trial was to prevent the development of the rising problem of maternal obesity. However, India also constitutes a substantial number of underweight women, posing a double burden on the nation by the underweight and overweight populations. The effect of behavioural interventions during pregnancy could be assessed with a larger sample size and correlated with different regions within India. Unified national GWG guidelines specific to Indian women should be formulated for better interpretation of the adequacy of GWG.

CONCLUSION

Various behavioural interventions during pregnancy, in the form of diet and supervised exercise alone and in combination, aimed to prevent excess GWG could be implemented in Indian women without having any adverse outcomes. Supervised exercises are the most effective in preventing excessive GWG, followed by diet control. In women with normal BMI, reinforcement of a healthy diet was the most effective in preventing GWG above the IOM guidelines. There is a need for national GWG guidelines for Indian women to better understand the effects of various pregnancy interventions for a better-targeted approach.

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CONFLICT OF INTEREST

There is no conflict of interest.

SOURCE OF FUNDING

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DECISION TREE ANALYSIS FOR PROSTATE CANCER PREDICTION IN PATIENTS WITH SERUM PSA 10 NG/ML OR LESS

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ANALIZA STABLA ODLUČIVANJA U PREDVIĐANJU KARCINOMA PROSTATE KOD BOLESNIKA SA SERUMSKIM NIVOOM PSA 10 NG/ML ILI MANJIM

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ABSTRACT

Serum prostate-specific antigen (PSA) testing increases the number of persons who undergo prostate biopsy. However, the best possible strategy for selecting patients for prostate biopsy has not yet been defined. The aim of this study was to develop a classification and regression tree (CART) decision model that can be used to predict significant prostate cancer (PCa) in the course of prostate biopsy for patients with serum PSA levels of 10 ng/ml or less.

The following clinicopathological characteristics of patients who had undergone ultrasound-guided transrectal prostate biopsy were collected: age, PSA, digital rectal examination, volume of the prostate, and PSA density (PSAD). CART analysis was carried out by using all predictors. Different aspects of the predictive performances of the prediction model were assessed.

In this retrospective study, significant PCa values were detected in 26 (26.8%) of a total of 97 patients. The CART model had three branching levels based on PSAD as the most decisive variable and age. The model sensitivity was 73.1%, the specificity was 80.3% and the accuracy was 78.3%. Our model showed an area under the receiver operating characteristic curve of 82.6%. The model was well calibrated.

In conclusion, CART analysis determined that PSAD was the key parameter for the identification of patients with a minimal risk for positive biopsies. The model showed a good discrimination capacity that surpassed individual predictors. However, before recommending its use in clinical practice, an evaluation of a larger and more complete database is necessary for the prediction of significant PCa.

Keywords: *Prostatic neoplasms; prostate-specific antigen density; decision tree.*

SAŽETAK

Testiranje na prostata specifični antigen (PSA) povisilo je broj osoba kod kojih se izvodi biopsija prostate. Međutim, najoptimalnija strategija selekcije bolesnika za biopsiju prostate još nije definisana. Cilj ove studije je kreiranje modela klasifikacionog i regresionog stabla odlučivanja (CART) koji bi se mogao koristiti u predviđanju signifikantnih karcinoma prostate (PCa) tokom biopsije prostate, kod bolesnika sa serumskim nivoom PSA od 10 ng/ml ili manjim.

Prikupljane su sledeće kliničkopatološke karakteristike bolesnika kod kojih je učinjena ultrazvukom vođena transrektalna biopsija prostate: starost, PSA, digitrektalni pregled, volumen prostate i gustina PSA (PSAD). CART analiza je izvedena korišćenjem svih prediktora. Procenjeni su različiti aspekti prediktivnih performansi predikcionog modela.

U ovoj retrospektivnoj studiji signifikantni PCa su utvrđeni kod 26 (26.8%) od ukupno 97 bolesnika. CART model ima tri nivoa grananja, na osnovu vrednosti PSAD, kao najpresudnije varijable i starosti. Senzitivnost modela je 73.1%, specifičnost 80.3% a tačnost 78.3%. Naš model je pokazao površinu ispod krive od 82.6%. Model ima dobru kalibraciju.

U zaključku, CART analiza utvrdila je PSAD kao parametar identifikacije bolesnika sa minimalnim rizikom pozitivne biopsije. Model je pokazao dobru diskriminacionu sposobnost koja prevazilazi pojedinačne prediktore. Međutim, pre preporuke kliničke primene, neophodna je evaluacija veće i kompletnije baze podataka radi predviđanja signifikantnih PCa.

Ključne reči: *neoplazme prostate; gustina prostata specifičnog antigena; stablo odlučivanja*

ABBREVIATIONS

AUC - area under the receiver operating characteristic curve;
CART - classification and regression tree analysis;
DRE - digital rectal examination;
IQR - interquartile range;
NPV - negative predictive value;
PCa - prostate cancer;
PCA3 - prostate cancer gene 3;
PHI - Prostate Health Index;
PPV - positive predictive value;
PSA - prostate-specific antigen;
PSAD - PSA density;
SD - standard deviation;
TRUS - transrectal ultrasound;
TZ - transition-zone

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INTRODUCTION

Prostate cancer (PCa) is estimated to be the most common cancer among men in Europe (1). Prostate biopsy is the gold standard for diagnosing PCa in men with elevated total serum prostate-specific antigen (PSA) levels or abnormal digital rectal examination (DRE) findings. Usage of the PSA test has dramatically increased the number of men who have undergone prostate biopsy over the last decades. However, serum PSA level alone, in the intermediate range (4.1–10.0 ng/ml), lacks specificity, potentially causing unnecessary treatment complications with prostate biopsy. In addition, overdiagnosis and overtreatment of indolent PCa is a serious health issue in most developed countries (2).

Efforts have been made to decrease the number of unnecessary biopsies. Multiple PSA derivatives have been advanced as early detection biomarkers, including age-specific PSA reference ranges, percentage of free PSA (3), PSA velocity (4), PSA density (PSAD) (5), transition-zone (TZ) PSAD (6), or presence of hypoechoic lesions on transrectal ultrasound (TRUS) (7). The most advanced PCa biomarkers include (-2) proPSA, %p2PSA, Prostate Health Index (PHI) (8), 4-kallikrein panel (9) or urine-based biomarkers, such as prostate cancer gene 3 (PCA3) (10).

In the last two decades, there has been extensive development of predictive tools to aid clinicians in predicting PCa diagnosis. Numerous multivariate models based on the combination of various clinical and demographic variables expressed by nomograms (7, 11-13), artificial neural networks (6), and risk calculators (14-16) provide better clinical performance than the results obtained with individual predictors (6, 7, 15). Despite these major efforts, there is no agreement as to whether these predictive PCa models improve the predictive accuracy of PSA testing and whether one model performs better than another. Furthermore, only limited reductions in the rate of unnecessary biopsies are possible. Thus, the best possible strategies for selecting appropriate patients for prostate biopsy have yet to be defined.

Classification and regression tree analysis (CART) has been applied in urology, especially for prostate cancer in the prediction of aggressive prostate cancer on biopsy (17, 18) or bone scan positivity (19). The procedure is a graphic representation of a series of decision rules and selects a useful subset of predictors or classifies subjects into high- and low-risk groups. Furthermore, the results of CART analysis are presented as a decision tree, which is intuitive and easier to understand than the results of many other statistical methods.

Based on these considerations, the aim of this study was to develop and compare the predictive accuracy of classification trees with those of the most important individual predictors for predicting clinically significant PCa on biopsy in patients with serum PSA levels of 10 ng/ml or less.

PATIENTS AND METHOD

This is a retrospective study carried out using a database of 239 patients who had undergone ultrasound-guided prostate biopsies over a 1-year study period from September 2016 through September 2017. Patient referrals were obtained in the course of routine clinical care and not as part of a population-based screening trial. After obtaining institutional review board approval, the data were collected regarding clinicopathological characteristics for each patient regarding prebiopsy assessment and included the following: age, PSA, DRE, volume of prostate, PSAD, total number of cores taken, Gleason score, and number of positive core biopsies. Exclusion criteria were patients with incomplete data and medical therapy known to affect PSA levels. The study included only patients with serum PSA levels of 10 ng/ml or less. The primary outcome was the detection of clinically significant prostate cancer on biopsy. Clinically insignificant prostate cancer was defined histopathologically according to the PRIAS inclusion criteria for low-risk PCa: T1C/T2, PSA ≤10 ng/ml, PSAD<0.2 ng/ml/ml, one or two positive biopsy cores, and Gleason score (GS)≤6 (2).

A member of the urology team performed a DRE on all patients. The DRE was classified as normal or suspicious/positive. At presentation, the serum PSA measurement (UniCel DxI 600 Access Immunoassay System, Beckman Coulter, USA) was performed. Before the biopsy procedure, all patients received a cleansing enema and prophylactic broad-spectrum antibiotics. A Toshiba (Aplio 300) ultrasound device with a 5-10-MHz probe was used to obtain ultrasound data and prostate biopsy samples. All patients underwent ultrasound-guided prostate biopsies performed using an 18-gauge biopsy instrument (Md-Tech, Pro-Mag I 2.5, USA). A median of ten biopsy cores was obtained, which were evaluated per each hospital's standard procedure and by local pathologists. Prostate volumes were obtained by measuring the gland in three dimensions, and volume was estimated using the following formula: 0.52 (length (cm) × width (cm) × height (cm)). The PSAD was calculated by dividing the serum PSA by the calculated prostate volume.

Statistical Analyses

Descriptive statistics were used for demographic and baseline characteristics. We expressed continuous variables as the means and standard deviations (SDs) when normally distributed or as the medians and interquartile ranges (IQRs) if their distributions were skewed, and discrete variables as percentages. Categorical variables (frequencies) were compared using Fisher's exact or Chi-square test. Continuous numerical data were analysed using the t-test or the Mann-Whitney U test when the data were not normally distributed.

CART classification tree

We choose the CART growing method to attempt to maximize the within-node homogeneity. CART analysis was carried out on the whole sample using all the predic-

Table 1. Patients' baseline clinicopathological characteristics (N=97).

Characteristics		BPH/Insignificant Pca (n=71)	Significant Pca (n=26)	p
Age	mean ± SD, years	67.7 ± 7.1	68.6 ± 6.3	0.548
PSA	mean ± SD, ng/ml	6.7 ± 2.1	7.6 ± 1.6	0.059
Volume prostate	median (IQR), ml	52 (35)	37 (18.7)	0.002
PSAD	median (IQR), ng/ml/ml	0.12 (0.10)	0.21 (0.12)	0.000
DRE	abnormal n, (%)	6 (8.5)	5 (19.2)	0.158
Number of biopsy cores	median (IQR)	10 (0)	10 (0)	0.140
GS ≤ 6	n (%)	8 (8.2)	14 (14.4)	NA
GS = 7-10	n (%)	0 (0)	12 (12.3)	NA

PCa–prostate cancer; SD–standard deviation; PSA–prostate-specific antigen; PSAD–prostate-specific antigen density; IQR–interquartile range; DRE–digital rectal examination; GS–Gleason score; NA–not applicable

tors identified in the patient population. We selected the category of significant PCa as the category of primary interest in the analysis. We select the GINI impurity measure, which splits and maximizes the homogeneity of child nodes with respect to the value of the dependent variable. We controlled stopping rules with a maximum tree depth of 3 levels and the minimum numbers of cases for nodes by specifying that the parent node must have at least 10 cases and a child node at least 5 cases. The optimal number of leaves was determined by identifying the tree size that minimized the tree deviance when 10-fold cross-validation was used in the derivation sample.

For models derived from CART analysis, we calculated the sensitivity, the specificity, the positive predictive value (PPV), the negative predictive value (NPV), the accuracy, and the area under the receiver operating characteristic curve (AUC). Comparisons of AUCs between models and individual predictors were performed using the method proposed by DeLong et al. (20) The SPSS (version 23.0) software package was used for all analyses. Statistical significance was set at $p < 0.05$.

RESULTS

A total of 97 patients with serum PSA levels of 10 ng/ml or less were analysed. Cancer was detected in 34 (35.1%), and significant PCa was detected in 26 (26.8%) of patients. The majority of tumours (64.7%) were determined to be Gleason score 6 or less. Table 1 shows the clinical characteristics of patients with/without significant PCa included in the study. The mean age of the patients was 68 years. The mean PSA level in all patients was 6.9 ng/ml. The DRE was abnormal in 11.3% of patients. The median prostate volume was 47 ml. The median PSAD was 0.13 ng/ml/ml. There were no significant differences in age, PSA levels and DRE findings between patients with or without significant PCa. The most decisive variables at the moment of classification were PSAD and prostate volume (Table 1).

CART tree

A tree-based CART prediction model is shown in Fig. 1 and details the total number of patients (n) and the possible outcome of the class variables with high probability. There are 5 terminal and 4 non-terminal nodes, resulting from 3 *"if-then"* conditions. The most decisive variable at the moment of classification was the PSAD, which stratified patients into two classes in relation to the cut-off value of more or less than 0.16 for further work-up. The non-terminal nodes (node 1 and 2) represented patients in low- and high-risk groups (prevalence rates of 10.5% and 50%, respectively). Both nodes were further split on the basis of the patient's age: more or less than 70.5 years in low-risk group and more or less than 61.5 years in high-risk group. The incidence rates of cancer detection in the low-risk group after splitting were 15% and 0%, respectively (nodes 3 and 4). Younger patients in the high-risk group were associated with low prevalence of PCa (14.3%) compared to older patients (57.6%) (nodes 5 and 6). These nodes are also terminal

Table 2. Diagnostic performance of PSA density at diverse cut-off values and CART model.

PSAD cut-off value	TP	FN	TN	FP	Sensitivity (%)	Specificity (%)	Biopsy spread (%)	Missed (%)
0.07	26	0	6	65	100	8.45	6	0
0.10	24	2	19	52	92.31	26.76	22	8
0.15	20	6	47	24	76.92	66.20	55	23
0.18	17	9	53	18	65.38	74.65	64	35
0.21	15	11	57	14	57.69	80.28	70	42
0.24	8	18	62	9	30.77	87.32	82	69
CART model	19	7	57	14	73.1	80.3	66	27

TP–true positive; FN–false negative; TN–true negative; FP–false positive; CART–classification and regression tree analysis

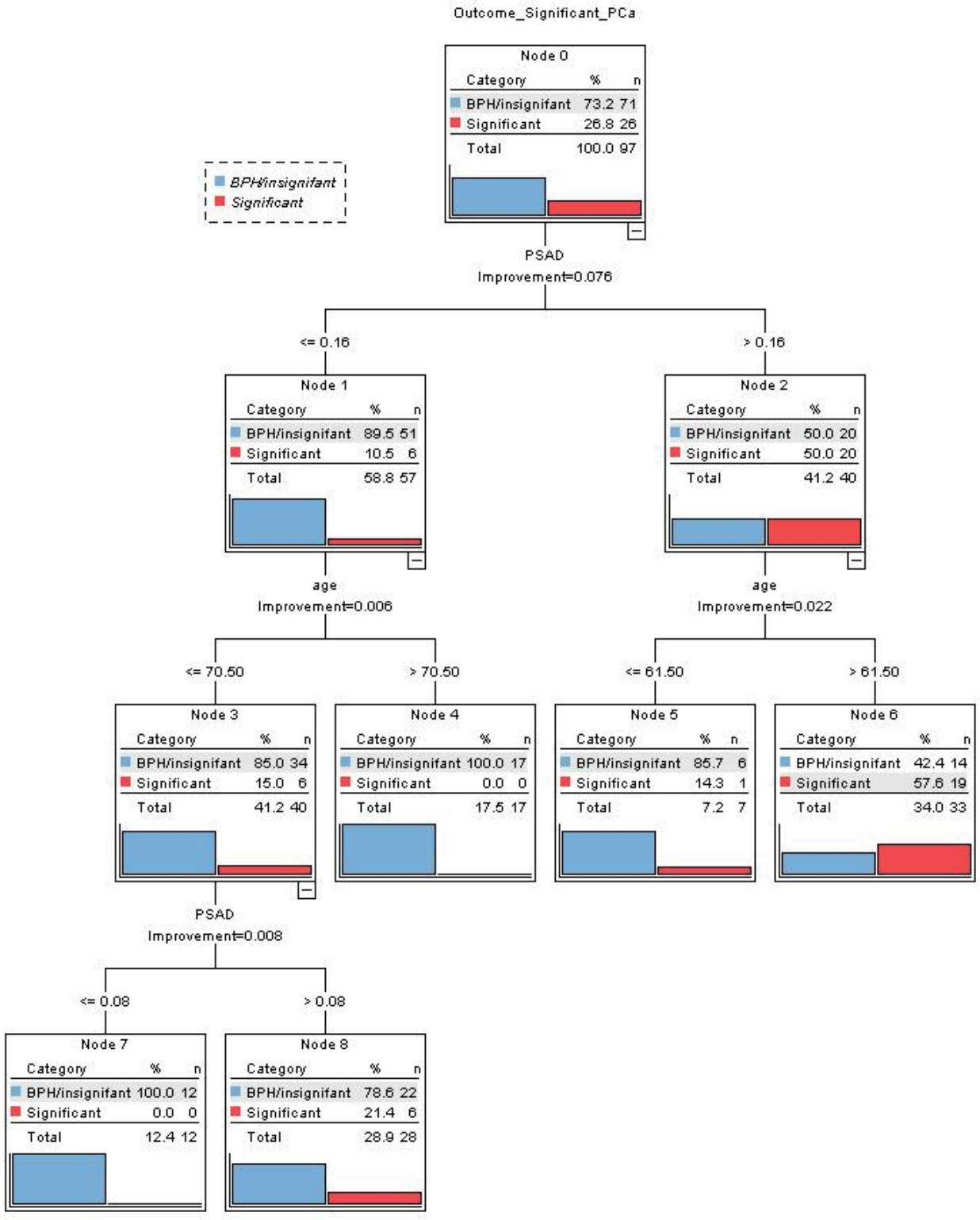

Figure 1. Tree-based CART prediction model.
The CART analysis was carried out on the whole sample using the all the predictors identified in the patients' population.

nodes. Finally, the non-terminal node 3 was further split on the basis of the PSAD of more or less than 0.08 (nodes 7 and 8). No patients had cancer if the PSAD was less than 0.08 (node 7). The misclassification rates of the entire sample and of the cross-validated estimate were 21.6% vs. 29.9%, respectively. The overall prediction accuracy of the CART model was 78.4%, and it was higher in the absence of significant PCa (80.3%) than in the significant PCa group (73.1%).

Diagnostic Performance of PSA Density at Various Cut-off Values

Since the CART analysis indicated that the PSAD was the most useful variable in predicting significant PCa, we next attempted to define the optimum cut-off value for PSAD. The diagnostic performances of different thresholds for PSAD are shown in Table 2. If the PSAD cut-off value was set at 0.15, which has been widely used for PCa detection, the sensitivity and specificity would be 76.6 and 66.2%, respectively; the number of patients requiring biopsies could have been reduced to 43 (55%) from 97, but 23% of the PCa patients would have been missed, with a PCa detection rate of 76.6% (20/26). Reducing the cut-off value to 0.07 (ng/ml/ml) resulted in a sensitivity of 100% and a specificity of 8.45%. Utilizing this parameter, the number of biopsies could have been reduced to 91 (6%) from 97, and none of the PCa patients would have been missed. However, according to our analysis, a PSA of ≤ 7.17 was considered optimum because it gave the highest sum of sensitivity and specificity.

Global metrics of test accuracy (AUC) for model and individual predictors are shown in Figure 2 and Table 3. The AUC for the model was shown to have moderate/good discriminatory capacity (82.6%), and in the pairwise comparison of ROC curves, the difference between the areas for CART and PSAD (5.4%) was not significant (P = 0.188), while that between the areas for CART and PSA (20.6%) was significant (P < 0.001). Graphical assessments of the CART model calibration are presented in Figure 3. The model was well calibrated (R^2=0.942). The CART model was found to have an overall sensitivity of 73.1% (95% confidence interval (CI) 52.2 – 88.4%) and a specificity of 80.3% (95% CI 69.1 – 88.8%). The positive predictive value was 57.6% (95% CI 39.2 – 74.5%), the negative predictive value was 89.1% (95% CI 78.5 – 95.5%), and the accuracy was 78.3% (95% CI 68.8 – 88.1%).

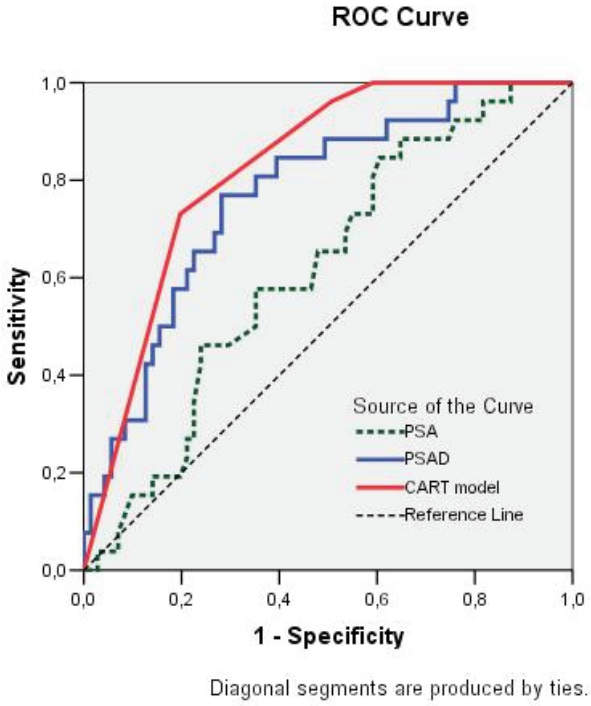

Figure 2. ROC curve analyses
A global metric of test accuracy (AUC) for the model and individual predictors (PSAD and PSA).

Table 3. Areas under the receiver operating characteristic curve of PSA, PSAD, and the CART model.

Predictors	AUC (95% CI)
CART model	82.6% (73.5 – 89.5%)
PSAD	77.1% (67.5 – 85.1%)
PSA	61.9% (51.5 – 71.6%)

AUC–area under the receiver operating characteristic curve; PSA–prostate-specific antigen; PSAD–prostate-specific antigen density; CART–classification and regression tree analysis

DISCUSSION

In the current study, we used CART analysis to develop a prostate biopsy decision algorithm in patients with serum PSA levels of 10 ng/ml or less. CART analysis selected PSAD as an indication for low- and high-risk groups. Age as common predictor may serve in further risk stratification. The CART model was shown to have good discriminatory capacity and outperformed PSA and PSAD as individual predictors. Application of the model would lead to notably superior clinical outcomes than the current strategy of biopsying all men with elevated PSA, consequently resulting in a reduction of the number of unnecessary biopsies.

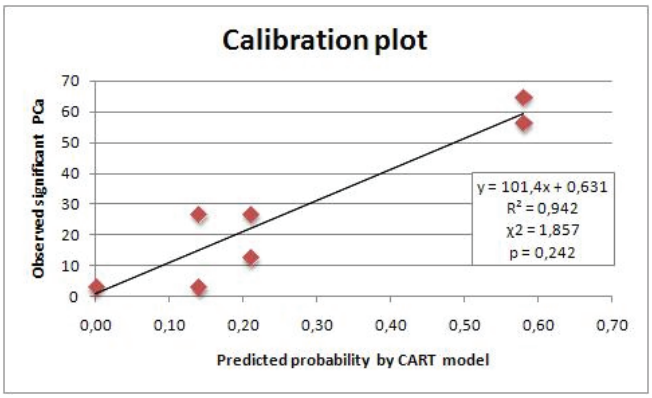

Figure 3. CART model calibration.
Graphical assessments of the CART model calibration.

Previous studies have established criteria associated with higher risk of significant PCa. They included age (7, 11-14, 17, 18), race (14), digital rectal examination (7, 11-16), total PSA (6, 12-16, 18), percentage of free PSA (6, 12, 13), PSAD (7, 17, 18), PHI (11), prostate volume (11, 12, 15-18), PSAD of the TZ (6), TZ volume (6), hypoechoic lesions on ultrasound (7, 16, 18), biopsy history (11, 14, 15) and family history (14). A wide variety of different

combinations of predictive factors has been identified. In line with previous studies, two of those predictors have reached statistical significance in the tree-based methods in our study. In our study ,, there were no significant differences in PSA levels between patients with or without significant PCa, which is in accordaence with many previous reports that serum PSA level alone, in the intermediate range, lacks proper the specificity. According to the analysis, PSAD was the most decisive variable at the moment of classification. The PSAD measurement is based upon the observation that PCa can produce an approximately 10-fold higher PSA concentration per volume of prostate tissue than in benign conditions. The PSAD has been suggested to differentiate benign from malignant prostate disease, especially in cases belonging in the grey zone (5). The PSAD asas an individual predictors outperformed PSA in our analysis,s as shown in thea global metric of test accuracy. Although there is controversy about cut-offs for PSAD, our result showed that the western reference (PSAD 0.15) (3) has moderate sensitivity (77%), and 23% of patients would have been missed, at the same time avoiding 55% of unnecessary biopsies. However, by reducing the value to 0.07 ng/ml/ml, a complete sensitivity similar to the value of 95% obtained by Catalona et al. (3) could be achieved. In studies that included patients with serum PSA levels of 10 ng/ml or less with similar design, a PSAD greater or less than 0.158–0.165 was the main splitting criterion (17, 21). These results support those of prior investigators, such as Catalona et al. (3), who reported that the commonly used PSAD cut-off of 0.15 detected only 59% of cancers in men with normal DREs and PSA levels between 4.0 and 10.0 ng/ml. Patients with cancer with lower PSAD values (0.15 or less) tended to have less aggressive disease (3), which is one of criteria for identifying very-low-risk prostate cancer. According to the findings of a recent study in our circumstances, patients with PSAD values above 0.17±0.06 should be included for biopsy (22). Furthermore, PSAD is an accurate predictor for adverse pathology prediction in patients with localized prostate cancer who undergo radical prostatectomy (23, 24). A previous study showed that PSAD could be a reliable clinical parameter for predicting prostate behaviour in cases of active surveillance. Patients with clinically localized prostate cancer and PSAD values <0.15 can be followed up safely on active surveillance, whereas cases with PSAD values >0.15 are at a higher risk of tumour progression and may be better managed by definitive therapy (25).

Our CART analysis identified two critical values for patient age, which is similar to critical values in other studies (younger than 60, 60 to 70 and older than 70 years) that examined survival after radical prostatectomy in relation to age, suggesting that men older than 70 years had a higher risk of disease and poorer survival (26). In addition, the detection rate of aggressiveness of PCa progressively increased with the age at diagnosis (27).

The accuracy levels of the present models were higher than the accuracy level of many earlier models. Our model resulted in an AUC of 82.6%, which is better than many other (73-82%) (7, 11-13, 15, 18) and similar to other reports (9, 16). In line with previous studies, our summary results suggest that the discriminative accuracy of the prediction model was better than PSAD and PSA testing (28). A systematic review that assesses the model's performance in the prediction of PCa suggested that none has clearly shown superiority over the others or can be considered as optimal (28). However, metrics of accuracy do not address the clinical value of a model.

The limitation of this study resides in its retrospective design, as the study was conducted in a single tertiary centre with a relatively small patient cohort that restricted generalization of the rules. Secondly, we included only those variables that were available to us. Because other advanced biomarkers were not available, we were unable to assess their utility in the current model. Furthermore, this analysis is limited by the bias introduced by false-negative biopsies. Recent studies have suggested that extended biopsy schemes and MR-targeted biopsies have demonstrated superiority over systematic biopsies for the detection of clinically significant disease (29). Next, criteria for insignificant PCa are not generally accepted. A recent study suggests that not all Gleason 3+4 cases will have aggressive disease (30). Furthermore, the prostate volumes of the study patients were measured by multiple operators. Therefore, inter-operator bias might interfere with our results. Finally, determination of prostate volume by TRUS may vary considerably (31). The lack of measurement precision for prostate volume has prevented the widespread clinical acceptance of PSAD. Nevertheless, using CART analysis, we could classify patients into a low-risk group (PSAD ≤ 0.16), which could avoid the biopsy procedure, and a high-risk group. Men in the low-risk group (positive biopsy result: 10.5%) could be selected with the CART model according to the following criteria: a PSAD of ≤ 0.16, age > 70.5 years or a PSAD of ≤ 0.08, age ≤ 70.5 years. Our study provides clear evidence that the statistical model could be used in everyday clinical practice to decrease unnecessary biopsies and had very small numbers of splits, unlike other models (7 splits) (18). The prediction model represents another step towards accurately estimating individualized risk of PCa in a patient population lacking optimal prediction procedures.

CONCLUSION

In summary, the CART analysis chose PSAD for the identification of patients at minimal risk for a positive biopsy. The model showed good discrimination and outperformed the most important individual predictors. However, before recommending its use in clinical practice, a larger and more complete database should be used to further clarify the magnitude of the model in terms of the prediction of significant PCa.

Conflicts of Interest

None.

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COSTS OF TREATMENT OF SEVERE COPD EXACERBATION IN SERBIA

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TROŠKOVI LEČENJA TEŠKOG POGORŠANJA HOBP-A U SRBIJI

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ABSTRACT

The main objective of this investigation was to determine and summarize the economic burden of severe COPD exacerbations that required hospitalization and the difference in the costs of treatment between patients with frequent (at least two exacerbations in one year) and infrequent exacerbation.

Our results suggested that significantly more resources had to be spent to treat patients with at least two hospitalizations during the study related to the use of medications primarily affecting the respiratory system (corticosteroids, p = 0.013, theophylline, p = 0.007) and total hospital stay (31336.68 ± 19140 RSD/517.53 ± 316.1 EUR versus 23650.15 ± 14956.0 RSD/390.59 ± 247 EUR, p=0.002) compared to patients who stayed in a semi-intensive care unit (12875.35 ± 20742.54 RSD versus 4310.62 ± 9779.78 RSD/ 212.64 ± 342.57 EUR versus 71.19 ± 161.51 EUR, p=0.006). Based on the total number of days in the hospital, the costs of the drugs, the materials used and services provided, patients from the frequent exacerbation group had significantly higher costs (80034.1 ± 36823.7 RSD/1321.78 ± 608.15 EUR versus 69425.5 ± 34083.1 RSD/1146.58 ± 562.89 EUR) comparedthan patients in the infrequent exacerbation group (p=0.039).

Our results indicate that significantly more funds will be spent treating the deterioration of patients who stay longer in the hospital or in the semi-intensive care unit. Their condition will require a significantly greater use of drugs that are primarily used to treat the respiratory system and, therefore, will utiliseutilize significantly more resources.

Keywords: *COPD, severe exacerbation, hospitalization, costs*

SAŽETAK

Glavni cilj ovog istraživanja je bio da se utvrdi i prikaže ekonomski aspekt ozbiljnih pogoršanja HOBP-a koje zahtevaju hospitalizaciju i razlike u troškovima lečenja između grupe pacijenata čestih egzacerbatora - sa najmanje dva pogoršanja tokom jedne godine i grupe pacijenata sa jednim pogoršanjem.

Naši rezultati sugerišu da značajno više resursa treba potrošiti za lečenje pacijenata sa najmanje dve hospitalizacije tokom jedne godine i to za upotrebu lekova koji prvenstveno utiču na respiratorni sistem (kortikosteroidi, p = 0.013, teofilin, p = 0.007), ukupan boravak u bolnici (31336.68 ± 19140 dinara u odnosu na 23650.15 ± 14956.0 dinara, ili izraženo u eurima 517.53 ± 316.1 EUR/ 390.59 ± 247 EUR, p = 0.002), boravak u poluintenzivnoj jedinici, 12875.35 ± 20742.54 dinara naspram 4310.62 ± 9779.78 dinara, odnosno 212.64 ± 342.57 EUR /71.19 ± 161.51 EUR (p = 0.006). Posmatrajući ukupan broj dana u bolnici, novac za lekove, korišćeni materijal i pružene usluge, značajno više sredstava je utrošeno za lečenje pacijenata iz grupe čestih egzacerbatora, 80034.1 ± 36823.7 RSD / 1321, 78 ± 608.15EUR, naspram 69425.5 ± 34083.1 RSD / 1146.58 ± 562.89 EUR za grupu pacijenata sa jednom egzacerbacijom (p = 0.039).

Može se zaključiti da će značajno više sredstava biti potrošeno u lečenju pogoršanja pacijenata koji pripadaju grupi čestih egzacerbatora. Ovi pacijenti će boraviti duže u bolnici ili u jedinici poluintenzivne nege, upotrebiće se značajno više lekova koji se prvenstveno koriste u lečenju respiratornog sistema, a samim tim će i troškovi biti značajno veći.

Ključne reči: *HOBP, teško pogoršanje, hospitalizacija, troškovi*

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INTRODUCTION

It is estimated that COPD is going to be the third major cause of mortality (1) and the seventh leading burden on the health system and economy (2, 3). The natural course and outcome of COPD are significantly influenced by exacerbations. These conditions present acute events leading to worsening of symptoms beyond the usual daily variations and are a major cause of morbidity and mortality (4). They accelerate the decline in lung function and thus promote the frequent use of medications, visits to the clinics and greater health care costs (5-7). The significant health and economic consequences of COPD exacerbation are still challenging for health care systems worldwide.

The total direct costs of COPD treatment within the EU are up to 38 bil EUR and up to 40 bil USD in America (8), while the average elderly COPD patient in Serbia costs the national health care budget approximately 138 000 RSD/2276.38 EUR according to the average official exchange rate in 2008 (9). Authors from Germany have indicated that the annual costs attributed to COPD amount up to 4 billion EUR (10). The suggested reasons for this amount are mainly the costs of drug use and the loss of work of the patients. The results of a study conducted in the Netherlands showed that a severe exacerbation of COPD that requires hospitalization costs the health system up to 1735 € (11), while in the southeastern part of Europe, the cost of exacerbation amounts to 1765$ (12). Interesting data have revealed the difference in the cost of treating patients with severe exacerbations and those without any exacerbations. In a study with over 200000 patients over a 3-year period, it was shown that the treatment costs of the group of patients with severe exacerbations may be increased up to 10-fold (13). Over one year, in almost half of the population of relatively well-treated patients, at least one exacerbation was associated with high costs, especially when there was severe disease deterioration requiring hospitalization (14).

The main objective of this investigation is to determine and summarize the economic burden of severe COPD exacerbations and the difference in costs of treatment between frequent (at least two exacerbations in one year) and infrequent exacerbations in groups of patients in the central part of Serbia. We only investigated severe COPD exacerbations that required hospitalization.

MATERIALS AND METHODS

Study subjects

This study was conducted as a cross-sectional study. We examined the economic impact of severe COPD exacerbations in patients who were hospitalized due to the index exacerbation of COPD in the tertiary care university hospital 'Clinical Centre of Kragujevac' during the three-year period (2010-2012). We reviewed a total of 512 individual medical records representing all hospitalized patients, female and male, with an established diagnosis of COPD who were older than 18 years during the study period. Out of this number, 174 patients fulfilled these criteria and were included for further analysis and subsequently divided into two groups - 64 subjects had frequent exacerbations, and 110 patients experienced only one exacerbation over the course of one year. The data from the remaining patients were excluded from further analysis due to incomplete medical records and conditions that caused the reduction in lung function, such as associated restrictive diseases (interstitial fibrosis, tuberculosis, etc.), lung carcinoma, previous surgical treatment of the lungs, recent myocardial infarction and pulmonary embolism, as described elsewhere (4). The study protocol was approved by the Ethical Board of the Clinical Centre of Kragujevac (authorisation number - 01-8644).

COPD exacerbations

For the purpose of this study, COPD exacerbation was defined by the criteria of the GOLD guidelines from 2014 (15). We analysed severe exacerbations that required hospitalization.

Statistical analysis

All costs of treatment were described by the mean values ± SD. To determine differences in the mean values of variables with a normal distribution of values, parametric Student's t-test was used, and its non-parametric alternative the Mann-Whitney test was used if the data did not follow a normal distribution. All data were analysed using the statistical program SPSS version 20, where a p value less than 0.05 was considered statistically significant.

RESULTS

One of the analyses conducted in this investigation is the assessment of the costs of the drugs used to treat exacerbations requiring patient hospitalization. All of the drugs used during the treatment of COPD deterioration were divided into two categories: drugs primarily affecting the respiratory system (corticosteroids, short-acting bronchodilators, oxygen, theophylline and prescribed preventive inhalation therapy - in this group of drugs, for the purpose of this investigation, we included fixed combinations of inhaled corticosteroids and long-acting bronchodilators (fluticasone/salmeterol and budesonide/formoterol) as well as a long-acting anticholinergic (tiotropium)) (Table 2) and drugs that affect associated illnesses (do not primarily act on the respiratory system, such as antibiotics (beta-lactams, quinolones and all antibiotics used), diuretics, antiarrhythmic agents, ACE inhibitors and calcium channel blockers) (Table 3). Data on the costs due to the use of medications primarily af-

Table 1. Medications costs

Variables	FE (n=64)	IE (n=110)	TEST VALUE AND SIGNIFICANCE OF NULL HYPOTHESIS
All medications costs	16791.67 ± 14147.22	13172.07 ± 9883.89	Z = - 1.359 p = 0.174
Costs of medications primarly affecting respiratory system	**6157.13 ± 3971.58**	**4785.340 ± 3534.2**	**Z = - 2.216** **p = 0.027**

FE - Frequent exacerbator group
IE - Infrequent exacerbator group

Table 2. Costs of medications primarly affecting respiratory system

Variables	FE (n=64)	IE (n=110)	TEST VALUE AND SIGNIFICANCE OF NULL HYPOTHESIS
Corticosteroids	945.07 ± 678.98	696.32 ± 428.31	**T = -2.533** **p = 0.013**
Short-acting bronchodilators	443.77 ± 534.07	365.93 ± 445.68	Z = - 0.429 p = 0.668
Oxygen	1259.39 ± 1603.5	981.98 ± 1521.81	Z = - 0.943 p = 0.346
Theophylline	439.51 ± 639.25	275.36 ± 169.31	**Z = -2.683** **P = 0.007**
Preventive inhalation therapy prescribed	3167.12 ± 2936.09	2509.64 ± 3055.03	Z = -1.663 p = 0.096

FE - Frequent exacerbator group
IE - Infrequent exacerbator group

Table 3. Costs of medications used for the treatment of exacerbation / comorbidity

Variables	FE (n=64)	IE (n=110)	TEST VALUE AND SIGNIFICANCE OF NULL HYPOTHESIS
β-lactam antibiotics	5415.74 ± 10034.92	4367.88 ± 7355.18	Z = - 0.191 p = 0.849
Quinolones	1843.65 ± 3193.71	1485.07 ± 2809.37	Z = - 0.112 p = 0.911
Total antibiotics	8623.54 ± 12899.92	6540.8 ± 8707.8	Z = - 0.167 p = 0.867
Diuretics	55.03 ± 100.1	36.41 ± 75.5	Z = - 0.505 p = 0.613
Antiarrhythmic drugs	44.89 ± 56.59	40.59 ± 5702	Z = - 0.639 p = 0.523
ACE inhibitors	153 ± 243.99	135.59 ± 209.15	Z = - 0.002 p = 0.999
Calcium channel blockers	54.39 ± 80.08	42.26 ± 65.49	Z = - 0,818 p = 0.413

FE - Frequent exacerbator group
IE - Infrequent exacerbator group

fecting the respiratory system suggested that significantly more resources were spent to treat the COPD exacerbations of patients who experienced at least two hospitalizations during the study period (Table 1). The main differences were related to the use of corticosteroids (systemic administration) and theophylline preparation (p = 0.013; p = 0.007) (Table 2). The maximum amount of money spent on the use of corticosteroids was 4004.1 RSD in the group with frequent exacerbations and 1819.0 RSD in the control group, and for the preparations of theophylline, 4828.9 RSD and 1200.9 RSD, respectively. Data on the duration of the use of this therapy were not fully available in the medical documentation we processed. There was no statistically significant difference in costs due to the use of antibiotics (beta-lactams, quinolines and all antibiotics used), diuretics, antiarrhythmic agents, ACE inhibitors and calcium channel blockers (Table 3).

Taking into account the hospital stays of the patients from both groups, we monitored the following variables: the time spent in the hospital, the number of hospital days in various organizational units, the material spent in this period and the services provided to each patient (Table 4). Patients who were in the group of cases stayed significantly longer in the hospital, with a total number

Table 4. Hospital treatment - costs

Variables	FE (n=64)	IE (n=110)	TEST VALUE AND SIGNIFICANCE OF NULL HYPOTHESIS
Material	3590.28 ± 2332.15	3176.43 ± 2417.41	Z = - 1.622 p = 0.105
Service	29007.56 ± 17526.32	29776.72 ± 20167.87	T = 0.291 p = 0.771
General care unit - number of days	6.25 ± 6.58	6.83 ± 6.15	Z = - 0.546 p = 0.585
General care unit - costs	10039.12 ± 10557.67	10971.59 ± 9884.54	Z = - 0.555 p = 0.579
Semintensive care unit – number of days	5.51 ± 8.87	1.84 ± 4.18	**Z = - 2.727** **P = 0.006**
Semintensive care unit – costs	12875.35 ± 20742.54	4310.62 ± 9779.78	**Z = -2.727** **p = 0.006**
Intensive care unit- number of days	1.92 ± 4.57	1.91 ± 4.4	Z = - 0.119 p = 0.905
Intensive care unit- costs	8422.21 ± 20053.45	8367.94 ± 19277	Z = - 0.122 p = 0.903
Total number of hospital days	13.68 ± 6.06	10.58 ± 4.36	**Z = - 3.573** **p < 0.001**
Total number of hospital days - costs	31336.68 ± 19140	23650.15 ± 14956.05	**Z = - 3.095** **p = 0.002**
Total costs	80034.1 ±36823.7	69425.5±34083.1	**Z = - 2.063** **p = 0.039**

FE - Frequent exacerbator group
IE - Infrequent exacerbator group

Table 5. Comparative review of costs during hospital treatment of COPD exacerbation expressed in different currencies

	FE (n=64)	IE (n=110)
RSD	31336.68 ± 19140	23650.15 ± 14956.05
$	466.74 ± 285.08	352.25 ± 222.76
EUR	517.53 ± 316.1	390.59 ± 247

FE - Frequent exacerbator group
IE - Infrequent exacerbator group

Table 6. Comparative review of treatment costs of COPD exacerbation in a semi-intensive care unit expressed in different currencies

	FE (n=64)	IE (n=110)
RSD	12875.35 ± 20742,54	20742.54
$	191.77 ± 308.94	64.2 ±145.66
EUR	212.64 ± 342.57	71.19 ± 161.51

FE - Frequent exacerbator group
IE - Infrequent exacerbator group

Table 7. Comparative review of total costs of COPD exacerbation treatment expressed in different currencies

	FE (n=64)	IE (n=110)
RSD	80034.1 ± 36823.7	69425.5 ± 34083
$	1192.05 ± 548.46	1034.04 ± 507.64
EUR	1321.78 ± 608.15	1146.58 ± 562.89

FE - Frequent exacerbator group
IE - Infrequent exacerbator group

Table 8. Differences in costs in the treatment of COPD exacerbations expressed in different currencies

	Intensive care unit – number of days	Semintensive care unit – number of days	Total costs
RSD	7686.53	8564.73	10608
$	114.48	127.56	158.01
EUR	126.94	141.45	175.2

FE - Frequent exacerbator group
IE - Infrequent exacerbator group

of hospital days of 13.68 ± 6.06 days compared to 10.58 ± 4.36 days in patients from the control group during the observed hospitalizations ($p < 0.001$). The number of days spent in the general care unit and the intensive care unit were similar, while a significant difference was shown when the number of days patients spent in the semi-intensive care unit was examined, where subjects from the group of cases spent 5.51 ± 8.87 days and subjects from the control group spent 1.84 ± 4.18 days ($p = 0.006$) (Table 4).

Resource utilization during the days in the hospital was also monitored and assessed as the amount of money spent on the materials and services provided to each patient. For the purpose of this study, the materials used in the patient's hospitalization included the following: X-ray films, syringes, needles, infusion and transfusion systems, patches, facial masks, medical alcohol, wadding, gloves, povidone iodide and other disinfectants, nasal and urinary catheters, urine bags, diapers, tubes and contrast agents. The services provided to the hospitalized patients included the determination of values such as sedimentation, CRP, fibrinogen, erythrocytes, leucocytes, platelets, haemoglobin, iron, sodium, potassium, calcium, chloride, pH, bicarbonate, gas analysis, total and direct bilirubin, AST, ALT, alkaline phosphatase, creatine kinase, total protein, albumin, urea, creatinine, uric acid, blood glucose, cholesterol, triglycerides, haematocrit, troponins, procalcitonin, microscopic and macroscopic urine assessment, urine culture, microbiological agentss, creatinine clearance, blood samples and urine samples for laboratory analysis, sputum for analysis, histological preparation, various examinations (physical, ultrasonic, X-ray), injection, infusion and inhalation.

Resources for the materials used during the observed hospitalizations were not significantly different among the patients from both groups, with a maximum of 12059.4 RSD in the group with frequent exacerbations and 14889.7 RSD in the control group. Additionally, the money spent for the provided services was similar in both observed groups, with a maximum value in the group with frequent exacerbations of 87767.6 RSD and 101937.5 RSD in the infrequent exacerbation group. Significantly more money was allocated to patients with more than one exacerbation during the total hospital stay (31336.68 ± 19140 RSD versus 23650.15 ± 14956.0 RSD, p=0.002) compared to the patients who stayed in a semi-intensive care unit (12875.35 ± 20742.54 RSD versus 4310.62 ± 9779.78 RSD, p=0.006), as average allocated money for the stay in the semi-intensive care unit (Table 4). Regarding the total number of days in the hospital, the costs of the drugs, materials used and provided services, significantly more amounts were allocated to patients from the group of cases (80034.1 ± 36823.7 RSD, in contrast to 69425.5 ± 34083.1 RSD for patients from the control group (p=0.039)). During the study, one patient's day in the general care unit cost 1605.24 RSD; in the semi-intensive care unit, 2337.6 RSD; and in the intensive care unit, 4385.12 RSD. All of the aforementioned results are shown in Table 4.

During the period under review, a total of 5122181.42 RSD was spent on all materials, services and medicines used for all patients in the group with frequent exacerbations, while for all patients in the control group, 7636810.05 RSD was allocated.

DISCUSSION

In spite of the numerous studies conducted worldwide and the efforts of health systems, the frequency of mortality in patients suffering from COPD is still on the rise, as opposed to the declining mortality due to other major causes of death such as carcinoma or cardiovascular diseases (16). COPD remains a major health challenge with a significant economic impact (17) and a disease with increasing direct and indirect costs of treatment (18). A significant upward trend from the 18 billion $ in 2002 to 30 billion $ in 2010 in direct costs of COPD treatment was observed in the United States (8, 19). The largest share in the cost of treatment for patients suffering from COPD is for exacerbations requiring hospitalization. It was suggested that up to 87% of all costs associated with the treatment of COPD are costs of hospital treatment (20). The total cost of COPD treatment on an annual basis was directly related to the presence of associated illnesses (21), while the correlation of the total cost with the degree of obstruction in the airways and quality of life indicators was not shown (22). Patients who have multiple associated diseases in addition to COPD have a higher risk of experiencing at least one exacerbation requiring hospital treatment (23), with significantly higher costs of such treatment (24). In addition to the increased costs incurred during hospitalization, it was shown that patients who have associated diseases in addition to COPD use approximately 50% more drugs for the treatment of cardiovascular disorders and almost two times as many antibiotics and psychotropic drugs, which additionally increase the overall costs of treatment (21). Overall, the cost of treating COPD patients with associated illnesses can be up to 4.7 times higher than the cost of treating COPD patients without associated illnesses (22). At the same time, the cost of treatment for these patients is 3.4 times higher than that of the control group of patients who do not have COPD (22). Since most of the resources for the treatment of COPD are used to treat exacerbations requiring hospitalization, direct medical costs can be representative in estimating the total amount of resources to be allocated for the treatment of these patients (9). An overview of the social component of each individual patient and an assessment of the indirect costs (absence from work, reduced working ability, early retirement, etc.) were not the goals of our research.

According to the available literature, studies comparing the costs of COPD exacerbations have not been carried out to date in Serbia.

We analysed data on costs due to the use of medicines primarily affecting the respiratory system, which suggested that significantly more resources have to be spent to treat COPD exacerbations in patients who experienced at least two hospitalizations in one year. These differences are mostly due the use of corticosteroids and theophylline preparations (p = 0.013; p = 0.007). This result could be predicted given that patients in the group with frequent exacerbations had more significant reductions in pulmonary function and a higher incidence of associated illnesses of the respiratory system as well asand they also stayed significantly longer in the hospital for the treatment of the observed exacerbation. Interestingly, there was no statistically significant difference in costs due to the use of all medications and total preventive inhalation therapy, although this therapy was significantly more often prescribed to the group of cases (data not shown). The reason is probably due to the difference in the price of the preparations that are classified in this group as well as the length of the use of these drugs. In previous investigations of the cost of treatment of patients suffering from COPD, it was revealed that in Serbia, one COPD patient of older age in 2008 cost the national health budget approximately 138 000 RSD (9). In 2008, the middle exchange rate of the National Bank of Serbia (NBS) for exchanging dollars was 50.01 RSD = 1$. At that time, the treatment of worsening COPD for the whole year cost the national health budget 9986.18 RSD or 199.7$ per patient. It should be noted that this amount does not include the costs of the treatment of associated metabolic disorders and cardiovascular diseases. Furthermore, the average number of exacerbations during the study year was 1.45 per patient, and the average length of the hos-

pital stay during the exacerbation was 12.84 days. Taking previous statements into account, we can conclude that the cost of the treatment of one exacerbation/hospitalization in 2008 in the same institution where our research was conducted amounted to 6887.02 RSD/137.72 $. Considering that the population in this study consisted of patients who experienced at least two exacerbations in 2008, it is expected that the costs would be similar to the costs of the treatment of exacerbations in the patients with frequent exacerbations in our examined population. When we compared the costs identified in previous research with our results, we obtained similar values. The duration of hospital treatment in our study of 13.68 ± 6.06 days did not significantly differ from 12.84 days, the average duration of hospitalization recorded in the previous study. According to the official middle exchange rate of the NBS from 2008 (50.01 RSD = 1$), the costs obtained in our study would amount to 123.12 $, similar to the previously established costs of 6887.02 RSD/137.72 $ (9), and according to the NBS middle exchange rate in 2012, 91.7$ (67.14 RSD = 1$). These differences in values expressed in foreign currency can be attributed to exchange differences in the observed years.

We noticed interesting data on the length of hospital stay during the exacerbation and the amount of money needed to be allocated. The average number of days spent in the hospital during the treatment of COPD exacerbations recorded in previous studies differs. It was 8.5 ± 8.2 days during 1993, 6.8 ± 6.6 days in 2001 (25), and up to 7 days in the period from 2006 to 2010 (26). The average length of stay in the hospital due to the treatment of COPD exacerbations during the period from 2010 to 2012 was 7 days, with patients being hospitalized for a minimum of 2 days and a maximum of 30 days (27). In relation to these data, the duration of hospital treatment in our study was prolonged. The total time patients spent in the hospital was 13.68 ± 6.06 days in the group of cases and 10.58 ± 4.36 days in the control group, showing a significant difference between the observed groups ($p < 0.001$). The shortest hospital stay was 5 days and the longest was 40 days in the group of cases, while the shortest hospital stay was 1 day and the longest 30 days in the control group. Time spent in general and intensive care units was similar, while a significant difference between the observed groups of patients was shown during the stay in the semi-intensive care unit: 5.51 ± 8.87 days and 1.84 ± 4.18 days ($p = 0.006$), respectively. Consequently, the costs of treatment during the course of isationhospitalization were different. In the three-year period (2010-2012), significantly more funds were allocated during the time spent in the hospital for the treatment of COPD exacerbations of patients who experienced at least two episodes in one year, namely, 31336.68 ± 19140 RSD in the group of cases and 23650.15 ± 14956.05 RSD in the control group ($p = 0.002$). We noted similar results comparing the period of stay in the semi-intensive care unit, with 12875.35 ± 20742.54 RSD in the group of cases and 4310.62 ± 9779.78 RSD in the control group ($p = 0.006$). The values of the amount of these costs recalculated in foreign currencies at the middle exchange rate of the NBS from 2012 (67.14 RSD = 1 $ and 60.55 RSD = 1 EUR) are shown in Table 6.

In assessing the overall cost of treatment of COPD exacerbations and in comparing them between the observed groups, we also found that significantly more funds needed to be allocated to treat COPD exacerbations requiring the hospitalization of patients who had undergone at least two exacerbations in a one-year period. Regarding the calculation of overall costs, our study included hospital expenses, all used medicines, materials and provided services. Table 8 shows the differences in the mean amount of funds to be allocated during the treatment of COPD exacerbations in the observed groups of subjects. However, the values of the resources differed significantly when considering the money that needed to be allocated during the total hospital stay as well as for the days spent in a semi-intensive care unit. It can be concluded that on average, it is necessary to allocate a total of 7686.53 RSD/114.48 $/126.94 EUR more during the hospital treatment of worsening COPD for one patient who has a higher chance of experiencing at least two exacerbations in one year. Similarly, for staying in a semi-intensive care unit, 8564.73 RSD/127.56$/141.45 EUR more needed to be allocated to patients with at least two exacerbations than patients who had a lower risk of experiencing additional exacerbations over a period of one year. The overall cost was also higher in the case group of patients, by values of 10608 RSD/158.01$/175.2 EUR. The amount of money spent on the materials used and the services provided at the observed hospitalizations was not significantly different between the patients of the observed groups.

Our results indicate that significantly more funds will be spent to treat the exacerbations of patients in the group of cases, which is a group of patients who have associated respiratory diseases. These patients will stay longer in the hospital or in the semi-intensive care unit, and their condition will also require a significantly higher use of drugs that are primarily used in the treatment of the respiratory system; , therefore, they will utiliseutilize significantly more resources.

In addition to the impact on patient quality of life, exacerbations torepresent the highest percentage of treatment costs. Consequently, decreasing the number of exacerbations is one of the most important goals of COPD treatment in order to improve patients' health status and quality of life and to reduce the costs of treating this disease (3, 28).

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CORRELATION BETWEEN BURNOUT SYNDROME AND ANXIETY IN MILITARY PERSONNEL

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KORELACIJA IZMEĐU SINDROMA SAGOREVANJA NA RADU I ANKSIOZNOSTI KOD PROFESIONALNIH VOJNIH LICA

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ABSTRACT

Professional military personnel are exposed to a large number of stressors every day at a higher rate than the civilian population, which can lead to psychological disturbances, primarily anxiety, as well as burnout syndrome. The aim of our investigation was to determine the correlation between burnout syndrome and anxiety in military personnel of the Serbian Armed Forces.

The cross-sectional study included a total of 311 professional military personnel (officers, non-commissioned officers and professional soldiers), between 23 to 53 years of age (35.3±7 years, on the average) without previous diagnosis of mental disorder. For purpose of this study we used Maslach Burnout Inventory (MBI) and Beck Anxiety Inventory (BAI). MBI contains three subscales, which measure three components of burnout: Emotional exhaustion (EE), Depersonalization (DP) and Personal accomplishment (PA). The statistical analysis included parametric and non-parametric descriptive statistics.

The highest level of burnout was measured on the subscales Emotional exhaustion (EE) in military personnel from 23 to 30 years old ($p<0.05$), while anxiety increased with age of military personnel ($p<0.001$). Total scores on the subscales Emotional exhaustion (EE) and Depersonalization (DP) increased, while on the subscale Personal accomplishment (PA) decreased with the increase of the total BAI score ($p<0.001$).

There was a correlation between burnout syndrome and anxiety in professional military personnel of Serbian Armed Forces. Improving the financial situation, paid recreational breaks and reduction of professional obligations could decrease anxiety and affect the prevention of the occurrence of burnout syndrome in the military environment.

Key words: *burnout syndrome, anxiety, military personnel, Serbian Armed Forces*

SAŽETAK

Profesionalna vojna lica su svakodnevno izložena velikom broju stresora, značajno u većoj meri u odnosu na civilno stanovništvo, što može dovesti do brojnih psiholoških poremećaja, pre svega anksioznosti, kao i sindroma sagorevanja na radu. Cilj našeg istraživanja bio je da se utvrdi korelacija između sindroma sagorevanja na radu i anksioznosti kod profesionalnih vojnih lica Vojske Srbije.

Studija preseka obuhvatila je ukupno 311 profesionalnih vojnih lica (oficiri, podoficiri i profesionalni vojnici) starosti od 23 do 53 godine (prosečno 35,3±7 godina), bez ranije dijagnostikovanog psihičkog poremećaja. Za potrebe ove studije koristili smo Maslahov upitnik sindroma sagorevanja (MBI) i Bekov inventar anksioznosti (BAI). MBI sadrži tri podskale, koje mere tri komponente sagorevanja: Emocionalnu iscrpljenost (EE), Depersonalizaciju (DP) i Lično postignuće (PA). Statistička analiza obuhvatila je parametarske i neparametarske deksriptivne statističke metode.

Najviši nivo sindroma sagorevanja je izmeren na subskali Emocionalne iscrpljenosti (EE) kod profesionalnih vojnih lica starosti od 23-30 godina života ($p<0.05$), dok se anksioznot povećava sa godinama života profesionalnih vojnih lica ($p<0.001$). Ukupni skor na subskalama Emocionalna iscrpljenost (EE) i Depersonalizacija (DP) se povećava, a na subskali Ličnog postignuća (PA) se smanjuje sa povećanjem ukupnog skora na skali anksioznosti ($p <0,001$).

Postoji korelacija izmedju sindroma sagorevanja na radu i anksioznosti profesionalnih vojnih lica Vojske Srbije. Poboljšanje finansijske situacije, plaćeni rekreativni odmori, smanjenje profesionalnih obaveza bi mogli da smanje anksioznost i da utiču na prevenciju pojave sindroma sagorevanja u vojnoj sredini.

Ključne reči: *sindrom sagorevanja, anksioznost, profesionalna vojna lica, Vojska Srbije*

ABBREVIATIONS

MBI - Maslach Burnout Inventory
EE - Emotional exhaustion
DP - Depersonalization
PA - Personal accomplishment
BAI - Beck Anxiety Inventory
SD – Standard devaition

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INTRODUCTION

Professional military personnel are exposed to a large number of stressors every day. Military personnel, considering the characteristics of their profession that include challenging working conditions, difficult and extreme training, specific military discipline, their obligation to be involved in the rules of armed commanding, principles of subordination, frequent changes, problems of adaptation to the military environment etc. may be exposed to stress at a higher rate than the civilian population, which can lead to psychological disturbances, primarily anxiety and depression, as well as burnout syndrome (1-3).

Burnout syndrome represents a combination of emotional exhaustion, depersonalization and negative feelings about oneself. Emotional exhaustion is an organism's reaction to stress. It represents a continuous "spending" of an individual's resourceful resource and is characterized by a disorganized mood (4). Depersonalisation is characterized by a cynical attitude and the perception of alienation from people in a random place, while the reduced personnel achievement refers to negative evaluations of personal competences and productivity, as well as in the experience of reduced self-efficacy (5). Thus, emotional exhaustion is a stressful, depersonalisation interpersonal, and a decrease achievement of the self-evaluative burnout component (6). Maslach defined the burnout syndrome as "the state of exhaustion in which a person is cynical and diminishes the value of his job, in doubting his own ability to do the job" (7).

Stressors that professional military personnel are exposed to, can cause some manifest or hidden disorders, especially anxiety, but also the development of burnout syndrome (8,9). Anxiety affects the quality of life of an individual, his professional readiness and combat readiness (10).

A recent study reported that anxiety is associated with burnout syndrome, sleeping problems, migraine and a lot of somatic and mental diseases (11,12). Therefore, the recognition of symptoms of anxiety is important for maintaining working ability and for preventing severe mental disorders (13,14).

There are some investigations where authors have examined the relationship between anxiety, emotional problems, job stress and work performance in civilian population (15) and in military environment both in war and in peacetime conditions (16-19). Burnout syndrome in military environment most often was investigated in medical personnel (20), while there were some investigations of burnout syndrome in police officers (21,22).

So far, in the Serbian Armed Forces no extensive research has been done on this topic. In our pilot project of burnout syndrome on relatively small patterns of military personnel (N = 55), it was shown that Emotional exhaustion (EE) and Depersonalization (DP) were present in 10.9% of subjects as well as 12.7% subjects in moderate levels, while lesser Personal achievement (PA) was present in 21.8% of subjects of high level (23).

Our investigation is a continuation of the mentioned pilot project, with the aim of determining the correlation between burnout syndrome and anxiety in military personnel.

METHODS

Cross-sectional study was conducted in the three barracks of infantry units of the Serbian Armed Forces, whose total number of professional military personnel met the required sample size of the respondents.

The study was conducted in September 2016, approved by the General Staff of the Serbian Armed Forces. A special permit for the research in the units of the Serbian Armed Forces was obtained from the Ministry of Defence too.

This study was conducted with approval by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac.

Study population

In this study were included a total of 311 professional military personnel (officers, non-commissioned officers and professional soldiers) between 23 to 53 years of age. All military personnel were exposed to approximately the same professional burden.

Participation in the study was offered to all members of the professional military unit who, during the study period, met the criteria for inclusion and exclusion.

Criterion for inclusion and exclusion were respected in this investigation. In investigation were included professional military personnel of the Serbian Armed Forces (officers, non-commissioned officers and professional soldiers); work under significant workload (guard, on-call, overtime, inability to use free days); age 23 to 53 years; at least three years of active professional military service, without current mental problem. Excluding criterion was professional military personnel with diagnosed psychiatric illnesses.

In our investigation were included only those who were volunteered to take part in it and they could drop out of the research if he/she felt that the questions in any way disturbed his/her mental well-being and disturbed him/her. Written informed consent was obtained from all participants prior to participation in the study. All participants were assured anonymity and that only group-level findings would be reported.

The size of the sample was determined based on the formula for determining sample size, because there were not a lot of researches of anxiety and burnout in military population. This number was added 10%, because of the possibility that the questionnaires will not be fully filled and in this way, we received a sample size of 311 respondents, with a previous decision that the alpha error level is 0.05, and the beta level at the limit of 0.01, which gives a 90% strength study (24).

Psychological instruments

Demographic questionaire included questions of age, gender, education, marital, proffesional and health status.

Psychometric assessments of the burnout and anxiety were made using: Maslach Burnout Inventory (MBI) (25) and *Beck Anxiety Inventory* (BAI) (26)

Maslach Burnout Inventory (MBI) is the most commonly used instrument to assess burnout. The MBI consists of 22 items. According to the MBI manual, it contains three subscales, which measure three components of burnout: Emotional exhaustion (EE), Depersonalization (DP) and Personal accomplishment (PA). The 9-item EE subscale assesses feelings of being emotionally overextended by one's work. The 5-item DP subscale measures having an unfeeling and impersonal response toward recipients of one's services. The 8-item PA subscale assesses feelings of competence and successful achievement. Each item could be answered on a 7-point Likert scale ranging from "never" (=0) to "daily" (=6). Burnout is indicated by high scores on Emotional exhaustion and Depersonalization and low scores on Personal accomplishment (25).

Beck Anxiety Inventory (BAI) is unspecific self-questionnaire. It served as the primary outcome for measuring the severity of anxiety in participants suffering from different primary anxiety disorders. The BAI assesses emotional, physiological and cognitive aspects of state anxiety. It consists of 21 items, rated on a 4-point Likert scale ranging from 0 = *not at all* to 3 = *severely*. The BAI scores are classified as low anxiety (0 to 21), moderate anxiety (22 to 36) and high anxiety (more than 36) (26).

Statistical analyses

Statistical analysis included parametric and non-parametric descriptive statistics, depending on the nature of data. Data analysis was carried out using SPSS (Statistical Package for the Social Sciences) software version 20.0.

For the normal distribution of all numerical parameters and scores Kolmogorov-Smirnov test was used.

Burnout (total scores of the three subscales MBI - Emotional exhaustion, Depersonalization and Personal accomplishment) and anxiety were analyzed according to the age of military personnel, divided into 3 age groups, (23-30, 31-39, 40-53 years old).

According to the levels of the total scores of the subscales of MBI (Emotional exhaustion, Depersonalization and Personal accomplishment) respondents were divided into 3 groups, with the high, mediate and low level, within which the total scores of anxiety were compared.

RESULTS

We got the results showing that in all monitored and calculated parameters and scores there was normal distribution (z was less than 1.96, and $p<0.05$), so that it was possible to apply parametric methods in further analysis.

MBI scale reliability analysis referring to Emotional exhaustion (EE) of subjects showed that the value of the Cronbach's coefficient was very high (0.827), while the MBI relating to Depersonalization (DP) of subjects was high (0.723) and MBI relating to Personal accomplishment of subjects (PA) was very high (0.868). The scale reliability analysis of our questionnaires was also high for the questionnaire BAI (anxiety) 0.883. Also, the values of the interclass correlation coefficient were significant, which confirms the compactness and high reliability of the BAI questionnaire.

The Cronbach's coefficient was obtained by using a program that covered the analysis of changes in the coefficient by eliminating individual issues that showed that these questionnaires were very consistent and reliable, and that there were no issues whose elimination would significantly increase the value of the reliability coefficient of the entire scale. Also, the values of interclass correlation coefficients were highly significant, which confirms their compactness and high reliability.

Demographic data of military personnel included in our investigation are shown on Table 1.

Comparisons of all numerical features and scores obtained from the questionnaires of our respondents showed that there were no statistically significant differences in relation to gender, whereby for the further analysis, the most important factor was that the respondents of both gender did not differ according to the average age, which further enables their comparison by gender and by other characteristics.

The comparison of all questionnaire parameters recalculated as the overall score of our respondents showed that there were no statistically significant differences in relation to gender in all scores.

Professional military personnel were aged from 23 to 53 years (35.3±7 years, on the average). Number of male

Table 1. Demographic data of military personnel

Variables		N	%	$\chi 2$	p
Gender	male	284	91.3	11.478	0.01*
	female	27	8.7		
Age (years)	23 - 30	98	31.5	5.598	0.01*
	31 - 39	140	45.0		
	40 - 53	73	23.5		
Education (years)	8	2	0.6	8.167	0.01*
	9-12	201	64.7		
	13-14	17	5.5		
	≥16	91	29.2		
Marital status	single	78	25.1	8.169	0.01*
	married	195	62.7		
	extramarital community	27	8.7		
	separated	3	0.9		
	divorced	8	2.6		
Total		311	100.00		

* $p<0.01$

Table 2. Total scores of the MBI and BAI

Total scores	min	max	X	SD
MBI-EE	0.00	39.00	8.77	7.47
MBI-DP	0.00	29.00	3.19	4.18
MBI-PA	12.00	48.00	40.49	7.82
BAI	0.00	39.00	4.83	5.66

MBI (Maslach Burnout Inventory)
EE (emotional exhaustion)
DP (depersonalization)
PA (personal accomplishment)
BAI (Beck Anxiety Inventory)

subjects was significantly higher than female ($\chi 2 = 11.478$; $p<0.01$). There were significantly more subjects with secondary school (64.7%) compared to other categories of education ($\chi 2 = 8.167$; $p<0.01$).

There were significantly more military personnel who were married (62.7%) compared to single (unmarried) and other (separated/divorced) categories of marital status ($\chi 2 = 8.228$; $p<0.01$).

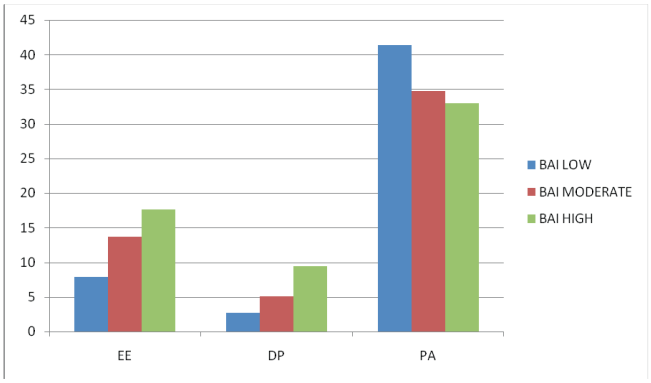
On Table 2 are shown the average total scores of the subscales of the Maslach burnout Inventory (MBI) and the average total score of the *Beck Anxiety Inventory* (BAI).

On Table 3 are shown correlation between burnout syndrome and anxiety and age of military personnel.

According to the age of military personnel, they were divided in three groups. The highest level of burnout was measured on the subscales EE and DP in youngest group of military personnel (23-30 years) and in military personnel from 31-39 years old on subscale PA. There was statistically significant difference only on subscale EE ($p<0.05$).

Anxiety measured on BAI questionnaire increased with age of military personnel. There was high statistically significant difference between total BAI score and age of military personnel ($p<0.001$).

Graph 1. Correlation between anxiety and levels of burnout syndrome of military personnel



BAI (Beck Anxiety Inventory)
MBI (Maslach Burnout Inventory)
EE (emotional exhaustion)
DP (depersonalization)

On Table 4 is shown the correlation between the total scores obtained on the BAI questionnaire and levels of MBI subscales.

Based on the results obtained on the MBI (Maslach burnout inventory) questionnaire, three groups of respondents were formed for each MBI subscale: a group of subjects with low, moderate and high levels of burnout.

The burnout level measured on the subscales EE and DP increased with the increase of BAI score. On the subscale PA, vice versa, scores decreased with the increase of BAI score. There were high statistically significant differences between total scores of all MBI subscales (EE, DP and PA) and total BAI score ($p<0.001$).

Table 3. Correlation between burnout syndrome and anxiety and age of military personnel

Scores	Age	N	X	SD	F	P
EE	23 - 30	98	9.37	7.16	4.204	0.016**
	31 - 39	140	8.26	6.94		
	40 - 53	73	8.97	8.78		
DP	23 - 30	98	3.60	4.53	0.662	0.516
	31 - 39	140	3.02	3.99		
	40 - 53	73	3.00	4.05		
PA	23 - 30	98	40.31	7.59	0.595	0.552
	31 - 39	140	40.98	7.32		
	40 - 53	73	39.78	9.03		
BAI	23 - 30	98	3.99	3.69	6.820	0.001***
	31 - 39	140	4.34	5.63		
	40 - 53	73	6.92	7.26		

BAI (Beck Anxiety Inventory)
MBI (Maslach Burnout Inventory)
EE (emotional exhaustion)
DP (depersonalization)
PA (personal accomplishment)
** p< 0.05
*** p< 0.001

Table 4. Correlation between anxiety and levels of burnout syndrome of military personnel

MBI	BAI	X	SD	F	p
EE	**Low**	7.89	6.64	20.167	0.001***
	Moderate	13.73	7.17		
	High	17.58	11.94		
	Total	8.77	7.47		
DP	**Low**	2.68	3.34	26.578	0.001***
	Moderate	5.10	4.56		
	High	9.41	8.57		
	Total	3.19	4.18		
PA	**Low**	41.35	7.24	16.162	0.001***
	Moderate	34.73	9.03		
	High	32.94	9.02		
	Total	40.48	7.82		

BAI (Beck Anxiety Inventory)
MBI (Maslach Burnout Inventory)
EE (emotional exhaustion)
DP (depersonalization)
PA (personal accomplishment)
*** p< 0.001

DISCUSSION

In our investigation of burnout syndrome and anxiety, 311 military personnel were included. Most respondents were healthy, married men, over 30 years old, with completed secondary school. The number of male subjects was significantly higher than female, which was expected, given the military environment in which the study was conducted.

Observed by age, Emotional exhaustion (EE) was highest in military personnel under 30 years old. Also our investigation showed that anxiety increased with age and that it was the highest in military personnel older than 40.

Professional military personnel belong to a group of workers who work in workplaces that are exposed to a large number of stress factors during their work. On one hand, there are complex work tasks in military environment that require special conditions of work. Stress at work is not the result of only one factor, but the sum of increased demands that include everyday military training, unpredictable working hours, constant need for overtime, impossibility of using free days, occasional guard, terrain, very often move to another units or city. Also, there is a demand for almost absolute respect to the principles of subordination that are standardized and related to greetings, absolute obedience of subordinates whereby any mistake can lead to severe consequences including different levels of punishing.

On the other hand, financial issues are at a high level as stress factors. Young and married professional military personnel have credits for apartments, or pay a monthly rent for rented apartments where they live alone or with their newly formed families.

Older professional military personnel have higher position in the units and less problems in obedience of subordinates. Besides the undoubted benefits for efficient and quality work that could represent another difficult requirement for some older employees, they have problems with accepting technology innovation and adopt them quickly, which is an additional job stress.

In military unit there is the collective feel of social and psychological connection with the unit when the collective interests regard as their own interests in operational and working groups to which they belong. Adaptation to the military environment have positive affects towards the military personnel, because they are "identified" with military units, characterized by cohesion and motivation with tasks and believe that there were no better alternatives in other working organizations that would meet their needs (3,10,27).

Problematic and disturbed relations with some colleagues and frequent interpersonal conflicts cause the inevitable stressful effects manifested by various symptoms which caused military personnel to become emotional exhausted or excessive overworked (28). But on the other hand more the peer support could decreased stressors related to the work role there are (3).

Emotional exhaustion is a stress component of burnout. The main cause of the burnout syndrome was the discrepancy between expectations at work and the individual's ability to satisfy it (29). Emotional exhaustion has severe health consequences and is often considered to be the core dimension of burnout (30).

The development of emotional exhaustion is a process. It started with physical and later on mental tiredness in military personnel, which led them to declining working capability. Finally they diminished desire to work anything (30).

In our investigation, we found statistically significant differences between anxiety, measured on the BAI questionnaire, and all three subscales of burnout. Correlation between anxiety and burnout has shown that the level of anxiety was in connection of increase the emotional exhaustion and depersonalization and decrease of personal accomplishment, which is consistent with the results of other studies (19).

Military personnel showed that burnout was present when job satisfaction decreased because of the financial and social effects of job dissatisfaction and the damaging physical/psychological impacts of burnout. Negative reaction involves emotional focus, slow or weak reaction or absence of any attempt at solving the problem (31).

Mismatch between high job requirements and individual potentials that are below or above individual potentials, frustrate them and make them dissatisfied, and that mismatch as such can be the source of stress (3). Sometimes, when emotional exhaustion could decline although when there is a good atmosphere at work, good interpersonal communications, but it increases in moment when military personnel become aware that they would be more successful in doing other better paid job.

During the time, they become tired of working because of the long-term pressure of working. Negative way of dealing with the stressor is associated with anxiety. Anxiety could be present at the thought of going to work. Often this can be exacerbated as individuals became frustrated or angry with themselves because he/she realized he/she could not give his/her best to military at the same kind of enthusiasm as in the past did.

However, despite numerous studies that have been carried out, relationship between burnout and anxiety is not clear. Anxiety is one of the symptoms of burnout (21), or it could exist individually. Anxiety disorder is characterised by excessive anxiety and worry accompanied by physical symptoms from the activation of the sympathetic nervous system. Anxiety is defined as the feeling of floating fear, embarrassment and uneasiness. It is a normal reaction to a stressful situation, however, if it lasts longer and if the person cannot control it, it goes into anxiety disorder (17-19).

Since, on the basis of our study, learning the adequate mechanisms of overcoming everyday stress significantly influenced on the reduction of the appearance of burnout, need further investigations in this area in order to become aware of mechanisms that act protectively (32,33). There is the importance of the quality of selection of the military personnel.

CONCLUSION

There is a correlation between burnout syndrome and anxiety in professional military personnel of Serbian Armed Forces. The emotional exhaustion has shown the highest levels in military personnel younger than 30, while anxiety was highest in military personnel older than 40.

Improving the financial situation, paid recreational breaks and reduction of professional obligations could affect the prevention of the occurrence of burnout syndrome in the military environment.

The results indicate further investigations in correlation of some socio-demographic variables and interpersonal sources of stress at work among professional military personnel.

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PERCEPTION OF HEALTHY LIFESTYLE AMONG STUDENTS OF MEDICAL SCHOOLS

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PERCEPCIJA ZDRAVOG NAČINA ŽIVOTA KOD STUDENATA MEDICINSKIH FAKULTETA

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ABSTRACT

Introduction/Aim: *The aim of the present study was to evaluate ideas and motivational attitudes of medical school students towards a healthy lifestyle and its components.* ***Methods:*** *The study was conducted from March until August 2017, at the Department of Medical Sociology, Healthcare Economics and Health Insurance and at N.A. Semashko Public Health and Healthcare Department, I.M. Sechenov First Moscow State Medical University. We ran a medical and sociological survey in 984 randomly selected students from eight Russian medical universities.* ***Results:*** *The article presents the results of the medical and sociological study aimed at assessing the ideas and motivational attitudes of medical students towards healthy lifestyle and its elements. Our research showed that students of medical schools, while recognizing the importance of preserving their own health, are still largely committed to an unhealthy lifestyle, secondly, they refuse sports activities even more often, and, thirdly, they prove not to be active enough when it comes to disease prevention and health preservation.* ***Conclusion:*** *Taking into consideration the obtained results, healthy lifestyle among students of medical schools is an exception rather than common practice.*

Keywords*: health, healthy lifestyle, medical students, motivation*

SAŽETAK

Uvod/cilj: *Cilj ove studije je da proceni ideje i motivacione stavove studenata medicinskih fakulteta prema zdravom načinu života i njegovim smernicama.* ***aaaq****Studija je izvedena u periodu od marta do avgusta 2017. godine na katedri za medicinsku sociologiju, ekonomiku zdravstva i zdravstveno osiguranje i na N.A. Semashko katedri za javno zdravlje i zdravstvenu zaštitu , I.M. Sechenov Prvog moskovskog državnog medicinskog univerziteta. Sproveli smo medicinsku i sociološku anketu na 984 slučajno odabranih studenata sa osam medicinskih univerziteta u Rusiji.* ***Rezultati:*** *Ovaj rad predstavlja rezultate medicinske i sociološke studije koja je imala za cilj da proceni ideje i motivacione stavove studentata medicine prema zdravom načinu života i njegovim principima. Naše istraživanje je pokazalo da studenti medicinskih fakulteta, bez obzira što priznaju važnost očuvanja svog zdravlja, još uvek vode nezdrav način života, odbijaju da se bave sportskim aktivnostima i nisu dovoljno aktivni što se tiče prevencije bolesti i očuvanja zdravlja.* ***Zaključak****: Uzimajući u obzir dobijene rezultate, zdrav način života među studentima medicinskih fakulteta je više izuzetak nego uobičajena praksa.*

Ključne reči: *zdravlje, zdrav način života , studenti medicine, motivacija*

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INTRODUCTION

The processes of globalization and integration in the modern world have a clear effect on the national educational system both at the macro-level and the level of particular institutions and individuals. As noted by Maslow in his major treatise, in a new, dynamically changing socio-historical situation: “We need a different type of person capable of living in a continuously changing environment”(1). At the moment, this requirement appears especially relevant to medical professionals.

Reshetnikov AV pointed out the high dynamics of changes in health professions - related not only to the economic transformation of the health system, but also to social changes, as well as changes in the attitude to health and disease (2). Under these conditions, medical universities are increasingly focused on creating the systems aimed at training not merely knowledgeable and skilful professionals but also personalities capable of effectively adapting to the dynamically changing social environment (3-7).

Shchepin pointed out that personnel is the most significant and valuable component of the healthcare system, especially in the state and municipal instututions (8). That is why in medical universities, considerable attention is paid to the formation of health-preserving environment, promotion of healthy lifestyles and implementation of measures to improve health of future medical professionals. However, the effective implementation of these measures requires internal readiness of young people to lead a healthy lifestyle.

The objective of the present study was to evaluate ideas and motivational attitudes of medical school students towards a healthy lifestyle and its components.

PATIENTS AND METHODS

The study was conducted from March until August 2017, at the Department of Medical Sociology, Healthcare Economics and Health Insurance and N.A.Semashko Public Health and Healthcare Department, I.M. Sechenov First Moscow State Medical University (Sechenov University). We ran a medical and sociological survey in 984 randomly selected students from eight Russian medical universities.

For studying of representations and motivational attitudes of students of medical higher education institutions concerning a healthy lifestyle and its components in March-August 2017, at the Department of Medical Sociology, Healthcare Economics and Health Insurance (IPE) together with N.A.Semashko Public Health and Healthcare Department, Sechenov University conducted a medico-sociological survey, which was attended by 984 students of eight medical universities: V.I. Razumovsky Saratov State Medical University (SSMU – Saratov, Russia), Sechenov University (FMSMU – Moscow, Russia), Kazan State Medical University (KSMU – Kazan, Russia), N.N. Burdenko Voronezh State Medical Academy (VSMA – Voronezh, Russia), Rostov State Medical University (RostSMU – Rostov, Russia), Volgograd State Medical University (VolgSMU – Volgograd, Russia), Krasnoyarsk State Medical University named after V. F. Voino-Yasenetsky (Krasnoyarsk, Russia), I.P. Pavlov Ryazan State Medical University (RyazSMU – Ryazan, Russia).

An anonymous survey was chosen as a research method. The selection of participants was performed using a random sampling technique. The data processing was performed using SPSS Statistics 19 package.

Among the participants of the study, there were 68% of women and 32% of men. The age of respondents was expected to be in the range of 18-23 years. The age distribution of the survey participants was in line with the distribution of respondents' years of training, and was represented mainly by equivalent groups, with a slight decrease in the share of the senior course objectively explained by the movement of the population structure. The survey was attended by students of all faculties (in accordance with the proportions of the sample).

More than half of the respondents (59.5%) received higher education in medical universities on a state-funded basis, and 40.5% were self-funded on a contractual basis. It should be noted that among the surveyed university students, 11.3% had already had a degree from medical colleges (equivalent to nursing schools).

At the time of the survey, 7.1% and 19.6% of respondents were married and cohabited, respectively; 70.6% of respondents had no spouse (a partner); 3.3% had children; 2.7% refused to answer the question about the marital status.

According to the responses, 15.8% of participants had a substinence level of income, with a comparable variability of self-assessment of their own financial situation (the most frequent answers were "living beyond poverty, not enough money even for food", "only enough money for food", "normal income, but constantly have to save up"). One respondent out of five (19.6%) indicated the amount of monthly per capita income fell in the range from 11 to 20 thousand rubles (155-290 eur). Other 10.6% of students reported the income amounting to 21-30 thousand roubles per family member per month (around 300-430 euro). Only 10.3% of respondents whose income exceeded 30 thousand rubles (430 euro) per one family member per month could be attributed to the conditional middle class in terms of welfare. Other 22.0% refused to discuss the financial situation of their family, and 21.7% of respondents could not decide on the answer to this question.

According to the self-rating, 42.5% of respondents "live normally, but have to save up", another third of respondents (33.2%) say that their available income is sufficient for covering the needs, and 15% of respondents consider that they “have enough funds to live well”. However, 6.8% of respondents, according to their own estimates, live beyond the

poverty line and are forced to save even on food. In addition, 2.5% of students interviewed could not decide on the answer.

Most of the respondents (67.5%) did not have their own housing and lived in the apartment of relatives (29%), rented accommodation (28.9%) or university dormitory (9.6%); 32.5% of students owned a flat or a house.

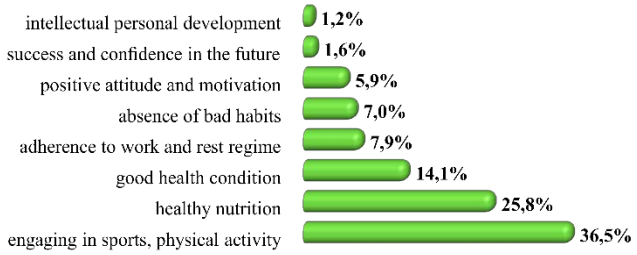
RESULTS

The conducted medical and sociological research allowed to obtain an idea of the basic motivational attitudes of students of medical schools towards healthy lifestyles and their components, as well as to obtain the data on the most common problems of youth related to preservation and promotion of their health.

Awareness of respondents about the principles of healthy lifestyle

The survey demonstrated that healthy lifestylein students, was associated predominantly with the physical activity and sports, and the organization of proper nutrition and good health ("a healthy body makes a healthy spirit") (Figure 1).

Figure 1. Respondents' associations with the term "Healthy lifestyle" (%, N=974)



Interestingly, among the most important components of a healthy lifestyle, students named the active lifestyle (88.1%), proper nutrition (84.7%), kicking bad habits (63.7%), compliance with the regime of work and rest (36.5%), compliance with the regime of the day (26.4%), emotion management, stress resilience (29.3%), acclimatization training (water quenching) (9.6%), intellectual development (17.1%), spiritual self-improvement (12.4%).

In addition, 89.1% of students in medical schools believe that a healthy lifestyle requires, first and foremost, willpower and patience. Also, to maintain a healthy lifestyle, in the opinion of students, the availability of free time (49.4%), financial opportunities (24.2%), motivation (22.1%) and understanding and support of close people (23.2%) are important.

In connection with the above, a logical question arises – which factors can contribute to the involvement of young people in healthy lifestyles? According to the majority of the survey participants (84.2%) who stated their opinion in the free fields of the questionnaire, the leading factor in the introduction to a healthy lifestyle was the presence of a person's internal motivation to preserve and strengthen their health. The view of 71.9% of the surveyed medical students is that family traditions of healthy lifestyles help promote the same in younger generations; while 31.4% of respondents suggested "the implementation of active state policy to increase the prestige and promote healthy lifestyle, as well as creating conditions for strengthening the health of citizens". More than a third of respondents (35.4%) believe that a positive example of an authoritative person is important for young people to lead a healthy lifestyle, and 17.2% believe that such an authority can be represented by famous people (actors, artists, media persons) who can inspire fans with their example.

As part of the survey, respondents were asked to assess whether their lifestyle complies with the basic principles of a healthy lifestyle. In accordance with the received data, only one third of students (35.4%) believe they lead a healthy lifestyle. More than half of respondents (53.0%) gave the answer "my way of life not entirely consistent with the principles of healthy lifestyle", and 11.6% admitted that their way of life was radically opposite to the principles of a healthy lifestyle.

The majority of participants (53.9%) who stick to a healthy lifestyle, are students of KSMU (Krasnoyarsk). They are followed by KSMU, where 42.9% of students adhere to the healthy lifestyle (HLS) principles. The third place belongs to RyazSMU (Ryazan), where one third of students (34.3%) who participated in the survey, considered themselves to be leading a healthy lifestyle.

Notably, three quarters of the surveyed students (75.4%) would like to lead a healthier lifestyle in the future, but for a number of reasons, they can not adhere to the principles of HLS currently, while 4.7% do not consider it necessary to adhere to a healthy lifestyle and do not plan to do so. Another 19.9% found it difficult to answer.

As shown by the survey data, the main reason for non-adherence among students is the high workload of education / job (73.2%). In addition, one in four respondents (23.1%) acknowledges that HLS cannot be managed simply due to the lack of finances to ensure a healthy diet. Nevertheless, a significant proportion of respondents (65% in total) frankly acknowledges that only their own inertia and internal reluctance (laziness, lack of willpower, motivation) prevent them from properly maintaining and strengthening their own health – and it must be recognized that the formation of motivation for a healthy lifestyle among students in this category of respondents will be one of the most difficult tasks. In addition, 11.6% of students admitted that their “busy personal life” hindered organising the proper nutrition, and 7.8% of respondents indicated that their circle of communication "would not understand the commitment to a healthy lifestyle." Other 3.2% are reluctant to give up their bad habits – which is certainly incompatible with the principles of a healthy lifestyle.

The involvement of medical students in sports

The most important component of a healthy lifestyle for people of any age category is the physical activity (9). Clearly, the health potential of exercise, is in the first place based on the properly organized and balanced physical activity. The majority of respondents (90.2%) agree with the statement that sports and physical education are essential for the promotion and preservation of human health.

At the same time, among the main results for sports, the respondents emphasised the positive impact of physical activity on the physical (88.4%) and mental development (31.1%), improvement in the appearance (80.9%), facilitating the stress relief (72.4%) and harmonization of the emotional sphere (38.9%), improving the performance (55.7%) and maintaining a high level of activity for a long time (48.3%), strengthening the reputation of young people involved in sports (16%), helping young professionals to achieve a higher qualification in their job (12.2%), enhancing the willpower and discipline (0.5%). However, 2.9% of young people consider sports to be a waste of time.

It is important to note that a lot is being done in Russia for the development of physical activity-based sports. Championships and international competitions, student Universiades, sports tournaments at the Federal and regional levels are held regularly. Professional and semi-professional sports clubs are actively reviving in the cities, and domestic sports are developing. It is of note that more than half of the respondents (57%) are interested in sports events in our country, and other 4.5% of students are interested in these activities "from time to time". However, 26.8% of the survey participants are indifferent to these events, and 11.7% found it difficult to answer.

Maintaining a healthy lifestyle requires a personal activity and attitude, which makes it important to boost interest among the university students in the opportunity to engage in sports. Among the surveyed students, 64.4% indicated that they had engaged in sports prior to entering the University, while the average duration was more than 5 years (5.1 years). Besides, 19.9% of respondents had a sports category and 24% took part in sports competitions.

Most of the medical students (61.2%) indicated that they had joined sports independently, 18.2% had been referred to sports classes by their parents, and one in ten (10.5%) had come to sport classes "along with a friend". A tenth (10.1%) of the students who participated in the survey were unable to decide on the answer.

The respondents' enthusiasm for sports was primarily facilitated by the motivation to improve their health (63.4%) and appearance (56.3%). In addition, 41% of respondents liked the aesthetics, culture, traditions adopted in the sport they were engaged in, and another 28.3% were attracted by the environment and community. A third (31.7%) of the survey participants indicated that they wanted to increase self-confidence through sports. For every fifth respondent, it was important to become a professional in this sport (20.4%), and 5.8% of respondents counted on receiving the additional income as a result of sports activities.

Thus, while obtaining a university degree, students appear to expect sports not only to improve their appearance (53.6%) and health (52.0%), but also to facilitate emotional relaxation, reduce stress, and enhance the mental activity (the total of 58.3% of opinions).

Being a university student, of course, requires the redistribution of time resources in favor of the academic disciplines and greater attention to obtaining the professional skills. In many respects, it is for this reason that only 40.7% of the total number of respondents were able to continue sports training after the start of a term (remember that 64.4% of respondents had been engaged in sports before the enrollment).

Interestingly, the structure of students'interests prior to medical school and after years of training was shown to undergo marked changes. Whereas before the beginning of student life, the major share of hobbies consisted of a highly active sports requiring regular practice (swimming, volleyball, dance, athletics, martial arts), after the admission to the university, sports allowing to schedule classes at a convenient time, such as training in the gym, came out on top of others.

To understand the motivation of students to lead a healthy lifestyle, it is important to clarify the reasons for refusal of youth to engage in sports. As part of the survey, the majority of students (70%) indicated that they could not continue to play sports due to the high workload in education / work. The lack of funding hinders young people from sport for 16.4 per cent of. In addition, 14.2 per cent of respondents indicated that they were not currently involved in sports because they had no desire. In other 9.7% of respondents, what interfered with sports activities was a busy personal life, and 5.3% reported that their family circumstances did not allow for this. Health problems limited sports opportunities for 2.7% of students who participated in the survey. Finally, 1.4% of the respondents admitted that they were “just too lazy”.

At the same time, according to the survey, one third of respondents (32.0%) was completely satisfied with the level of their physical activity at the moment, while other 53.5% felt "not completely satisfied", and 14.5% – were categorically dissatisfied with the values of their physical activity.

Education and health are closely interlinked. It is extremely important that the conditions of education and life of students contribute to the preservation and strengthening of their health, and in this regard, the administration of medical Universities pays considerable attention to the formation of health-preserving behavior in future doctors, strengthening of their health and promotion of sports. It should be noted that among those who strive to take care of their health, more than a half (53%) use the opportunities of their university –

for example, 46.6% of respondents indicated that in order to deal with the health issues, they use clinics of their university, 25.8% attend sports facilities at their medical schools, and 1.1% go to the swimming pool. However, almost half of the students (47%) do not use the opportunities provided by their universities for the health improvement.

Complying with the work-rest regime in medical students

An important role in maintaining health is the correct daily routine, which implies keeping up with the regime of work, rest, sleep, and nutrition. The high dynamics of modern life, stress, imbalance of the physical activity and mental stress lead to the chronification of fatigue and exacerbate the disease.

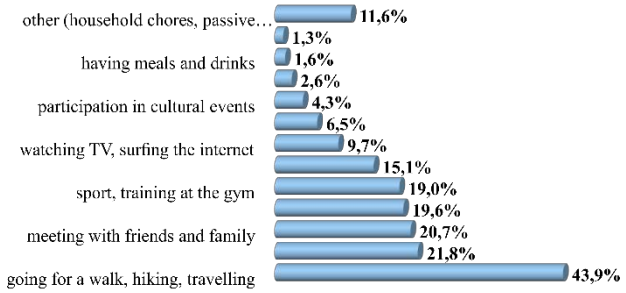
According to the survey, the majority of respondents fail to comply with the regime of the day and harmonize the ratio of work and rest. Thus, only 16.3% of respondents do fully comply with the regime of the day, while 54.2% of respondents admitted that they could not completely comply, and other 29.5% responded that they were absolutely unable to organize the proper regime and schedule. This means that the vast majority of respondents, despite their efforts to comply with the regime of the day, face certain obstacles that prevent them from living up to it.

According to the self-report, students associate their unbalanced regimes mainly with igh workload at universities/jobs (85/5%). Also, the "urge "to surf the net" (as reported by 31.6%) hinders the harmonious organization of work and rest and daily routine for almost a third of respondents. As one of the obstacles to complying with the regime of the day, a quarter of respondents (25.2%) named “being busy with their hobbies”. In addition, 22.6% explained violations of the regime of the day with a busy personal life, and 15.3% of respondents chose the answer "family circumstances", which indicated the fact that in some cases, the rules adopted in the family and the fulfillment of their obligations could cause students –not to comply with the regime of the day (for example, when due to the family circumstances students had to study and prepare for exams in the evenings or at night).

It is notable that the high pressure of workload is exacerbated by the lack of recreation among young people. Despite the different need for sleep in different people, on average, to ensure the normal functioning of the nervous system, it is recommended to sleep for at least eight hours. However, 79.5% of students reported chronic sleep failure – one in five respondents (20.4%) admitted that he/she slept less than five hours a day. Only 20.5% of the survey participants had the opportunity to sleep for at least eight hours a day. At the same time, even among those who sleep formally enough (8 hours or more), only 14.6% consider themselves satisfied with the number of hours of the night rest (i.e. consider the duration of their night sleep as optimal). It can be assumed that this is due to the high level of chronic fatigue and stress. Note that the systematic lack of a full night rest can have an adverse effect on the health outcomes, performance and progress of students.

Fundamentally important for any young person, including medical students, is the question of spending leasure time. According to the results of the study, 43.9% of the survey participants prefer to spend their leisure time actively (meetings, sports, walking, traveling), but a significant proportion of the rest of the students favours the passive rest (sleep, reading, watching TV, etc.) – which is primarily due to the accumulated fatigue as a result of the high workload (Figure 2).

Figure 2. Preferred ways to spend leisure time among medical students (%, N= 920)



Most often, the cultural leisure of medical students implies going to the cinema, cafes and restaurants – most of the respondents visit these places at least 1-2 times per month. In addition, once or twice a year, students go to the theater, museums and exhibitions. Unexpectedly, 63% of the interviewed medical students have never visited a night club.

At the same time, the best type of recreation according to most of the respondents is that involving the physical activity – 50.8% of the surveyed gave this answer. Supporters of the passive recreation are 37.6% of medical students, and 10.3% of the respondents prefer to alternate leisure with a passive “doing nothing”; 1.3% of respondents found it difficult to answer.

It is not surprising that students find travelling to the countryside as the most affordable kind of recreation: 58% of respondents prefer to spend their free time outside big cities, “in the nature”. At the same time, the average values for this type of rest in students amount to 29.6 days a year.

The second most popular option is staying within the territory of the Russian Federation (travelling around Russia): this answer was chosen by 33.3% of respondents. The average duration of this type of vacation for students is 18.7 days. In turn, travelling abroad is available to almost every fourth student (24%), while the average trip abroad is 14 days.

Keeping a healthy diet among medical students

Food is a source of vitality, and a balanced diet and quality of food have a decisive influence on one's health. The

study examined some characteristics of the organization of the diet in respondents.

The responses of medical students who took part in the survey demonstrated their understanding of the following notion: compliance with the diet is one of the most important conditions for a healthy lifestyle. Among the principles of healthy eating known to participants of the study, the majority of respondents (83.6%) pointed out "balanced diet in accordance with energy costs and needs". More than a half of the respondents (53.8%) believe that in order to ensure a healthy diet, it is necessary to include a large number of vegetables in the diet, and just less than a half of the survey participants (48.6%) are of the opinion that a healthy diet involves the inclusion of more fruit. For 45.1% of respondents, the main principle of healthy eating is the diet diversity. Other 39.7% of medical students consider it necessary to strictly follow the diet. The need to exclude fried food and food containing preservatives from the diet was indicated by the equal number of respondents – 39% respectively, and other 24.4% of respondents spoke in favor of excluding spicy and salty dishes from the diet. "Careful chewing of food" was named as the main condition of a healthy diet by 22.9% of the survey participants. Every fifth (19.5%) respondent considers the refusal to eat after 18.00 to be the fundamental principle of healthy eating. The important principle of healthy eating, in the opinion of 15.8% of the students interviewed, is the exclusion of snacks between the main meals. Those who frankly admitted that they did not know any principles of healthy eating and, accordingly, did not adhere to them, constituted 3.5% of respondents. Only 1.1% of respondents believe that in order to ensure a healthy diet, it is necessary to abandon the intake of sugar and sweets. Compliance with the water balance as the condition of healthy nutrition was mentioned by 0.6% respondents, while "split meals" was considered as the main principle of healthy nutrition by just 0.1% of the respondents.

The data obtained allow us to conclude that medical students who took part in the survey were familiar with the rules of healthy eating and had a notion of a healthy diet. However, only 21.4% of the survey participants did comply with a "proper" diet.

The majority of respondents (78.6%) indicated that they could not set certain meal hours and organized the proper diet due to the high workload at universities (and, in some cases, jobs).

In addition, one of the most significant obstacles to complying with a healthy eating regime was the "lack of financial resources" (35.7%), a much smaller number of respondents named "family circumstances" as the reasons for non-compliance with the diet (6.9%), as well as love for delicious food (1.8%) and "laziness" (1.6%). The curious factor was the "lack of willpower to abandon tasty but harmful products", which was indicated by 1.4% of respondents.

Only a quarter (25.5%) of students expressed confidence that they adhered to a healthy diet. Other 56.5% of respondents admitted that, despite the desire to comply with the principles of proper nutrition, they could not always resist the harmful food, and 18.0% considered their diet completely unhealthy.

As part of the survey, respondents were asked to describe their established diet. According to the obtained data, the daily grocery set of a medical university student includes: vegetables/herbs (57.4%), milk and dairy beverages/cottage cheese (54.3%), bakery products (52.8%), fruits (52.1%), cereals/cereals (40.8%), sweets/butter pastries (40.8%)and poultry meat (35.2%).

At the same time, the diet of the student of medical University includes a large number of bakery products and sweets, which is associated with the risk of extra weight, common disorders of the gastrointestinal tract, and can not be attributed to the category of products suitable for a healthy diet. Fish, for example, is only consumed by students 1-2 times per month at best, which can be linked to the lack of financial resources cited by respondents as one of the reasons that prevented adherence to a healthy diet. Note that 13.7% respondents deliberately refuse sausages, recognizing them as the "harmful" products.

However, 11.4% of students drink alcohol daily or regularly (1-2 times a week). In addition, more than half of respondents (51.4%) occasionally drink the alcoholic beverages. At the same time, more than a third of respondents (35.9%) have said that they never drink alcohol, which is clearly a good indicator.

In general, the obtained data allow to conclude that despite the presence of theoretical knowledge of respondents regarding compliance with the regime of proper nutrition, there is a compelling need to further improve the literacy of students in the field of healthy eating, as well as the organization of time to ensure the maintenance of a healthy regime and diet. It also appears necessary to intensify efforts to increase the personal motivation of medical students to lead a healthy lifestyle.

The prevalence of harmful habits and addictions of different kinds in respondents

One of the principles of a healthy lifestyle is quitting bad habits. According to the obtained data, 79.9% of the surveyed students, in their own opinion, do not have any bad habits. However, among the remaining fifth of the respondents (20.5%), most often, there is a trend of having several bad habits at the same time. So, 15.1% indicated that they are smokers, 7.3% – that they regularly drink the alcoholic beverages, and 2.9% of respondents find harmful their addiction to sweet dishes and fast food.

The debut of smoking occurred in high school (16-17 years) in over half of smokers (51.9%) and 26.7% – that is, one in four nicotine-dependent students – began smoking at

the legal age (18 years and older). In high school (11-15 years), the smoking habit was formed in 17% of smokers. Some students admitted that they started smoking at the age of 7-10 years (2.4%) and even earlier (1.9%).

On average, the expenses of smoking students on cigarettes do not exceed 50 rubles per day – and the financial accessibility contributes to the "preservation" of this abuse in students. The smoking respondents have on average 9-10 cigarettes per day and most often do it in the company of friends.

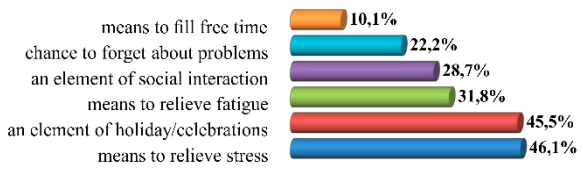
Note that 40.8% recognize that smoking is harmful to health, 34.3% of the smoking respondents consider smoking an unpleasant addiction, and one in four (26.3%) associates this habit with the need for constant financial expenses. In addition, 29.3% of smokers do not like the unpleasant smell of cigarettes. At the same time, for 42.9% of students, smoking a cigarette is a way to relieve pressure and stress, and 18,3% see it as a way to spend free time. Of the total number of smokers, 16.3% consider smoking as an attribute of communication in the company of friends.

At the same time, not everyone with this addiction wishes to give up smoking – only a third (31.5%) expressed a desire to do so, while 14% do not want to part with the habit of smoking. It is remarkable that more than a half of young smokers (54.5%) found it difficult to answer.

The involvement of young people in consumption of the alcoholic beverages is certainly a wake-up call. Note that the first alcohol sample of the overwhelming number of respondents of this group (78.6%) occurred between the ages of 14 and 18, and the first alcoholic beverage was beer. At the same time, we must admit that the use of alcohol by the majority of students of the medical university is sporadic.

According to the respondents' own estimates, the alcohol consumption is primarily an opportunity to relieve stress and fatigue, as well as an attribute of the holiday. In addition, it is alarming that for every tenth respondent (10.1%), drinking alcohol is a way to "fill their free time" (Fig. 3).

Figure 3. The motivation for the consumption of alcoholic beverages among respondents (%, the sum of percentages does not equal 100% as question allowed for multiple answers, N= 222)



At the same time, a quarter of respondents (24.8%) believes that alcohol brings undeniable harm to health. In addition, among other aspects of the negative impact of alcohol, students named the regular financial expenses (14.6%), the formation of an unpleasant addiction (13.5%), and odour (10.4%).

It is interesting that among those who, in principle, consume the alcoholic beverages, 40.7% do not consider it necessary to abandon this habit and only 7.3% would like not to drink alcohol in the future.

One of the most pressing addictions among young people is that to the Internet (social networks) and gadgets: 36.3% of respondents admit that they are seriously affected by the Internet addiction: another third (37.4%) do not consider themselves addicted to the gadgets and social networks, and 26.3% found it difficult to answer this question. Mainly, the addiction is manifested in the abuse of social networks by young people (67.1%), but students also spend a lot of time watching movies (33.7%) and reading online (20,4%).

The attention medical students pay to their health

The key to preserving and promoting health is a person's careful attitude to the state of their own health (10). According to the self-report, 62.3% of respondents considered themselves to be in good health; however, the third of respondents (34.6%) considered their health just "satisfactory", and 2.7% characterized the state of the health as "bad" or "very bad".

According to the survey, 29.9% of medical students suffer from chronic diseases, 26% are overweight, 11.9% of students have the genetic load of a number of diseases, and 2.7% of respondents have a disability. Frequent colds are present in 27.8% of students, 26.1% of them with the common allergic reactions. The presence of dental problems was noted by 23% of the survey participants. A third (35.1%) of the students indicated that they did not have health characteristics.

It is interesting that despite the presence of all kinds of diseases, 90.1% of students are at least partially satisfied with their own health – whereas 29.5% are fully satisfied, 61.0% of respondents consider themselves partially satisfied with their health.

Regarding the self-assessment of their own care of their health, 41% of respondents considered that they took enough care of it; while an approximately equal share of respondents (39.3%) reported a negative evaluation of the degree of care for their health.

That said, the question about measures of health preservation yielded the following response frequency: “having no bad habits” – 68.7%, engaging in sports and the physical activity – 46.8%. Next, 44.1% of the respondents believe that regular medical examination allows them to maintain good health. Other 28.1% of students responded that in order to maintain health, they adhered to a healthy diet, and one in five (21.4%) mentioned the work-rest regime; 12.5% of respondents found it difficult to respond. Finally, 11.7% medical students admitted that they did nothing to improve their health.

A positive indicator is the fact that 37.7% of medical students tried to tackle the health issues proactively. However, more than half (52%) of students who participated in the

survey noted that they were accustomed to solving the health problems "as they come on", and other 8% seek medical help only in the case of serious health problems. In addition, 3.3% of respondents "do not pay attention to health problems" – which indicates that the increase in students' motivation for a preventive approach in matters related to their health would be a useful resource for improving the health outcomes.

DISCUSSION

According to the results of the study, a healthy lifestyle is an immutable value for the majority of medical students. However, actual adherence to the principles of a healthy lifestyle in terms of nutrition, physical activity, daily routine, work and rest regime, was only characteristic of a little more than a third of the students. The main obstacle to this is the high workload at the university/job (73.2% of opinions), as well as internal reluctance (laziness, lack of willpower, motivation) of students to take care of the maintenance and strengthening of their own health. Moreover, every fourth respondent recognizes that they cannot commit to a properly healthy lifestyle because of the financial difficulties.

The high workload at the university is perhaps the most significant barrier to healthy lifestyles of students: for example, 83.7% of students reported that the university workload prevented them from complying with the daily routine, limited the possibility of decent night rest in 79.5% (every fifth student slept less than 5 hours a day), it did not allow them to organize the correct diet (78.4%), and reduced the opportunity to engage in sports.

At the same time, most of the surveyed medical students are satisfied with their own health, and believe they pay enough attention to the preservation and strengthening of their health. The survey data showed that the majority of students cared about their health, in that, they aimed to refrain from bad habits, regularly underwent the medical examinations, and tried to maintain sufficient levels of the physical activity. However, not all respondents found that it was possible to comply with the regime of day and nutrition, and one person out of nine admitted that they did nothing to preserve theirhealth. In addition, almost half of the students (47%) did not use the facilities of their Universities for the benefit of their health.

CONCLUSION

In general, while recognizing the importance of preserving their own health, medical students, are, first of all, committed to unhealthy lifestyles, secondly, they increasingly refuse to engage in sports, and, thirdly, they are insufficiently active in the prevention of diseases and health preservation. This is confirmed by the study, which suggests that at the moment, leading a healthy lifestyle among medical students is an exception rather than the common practice – which, of course, requires intensification of efforts to increase the motivation of future doctors to lead a healthy lifestyle.

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CONFLICT OF INTEREST

None.

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INTERLEUKIN-32 IN INFECTION, INFLAMMATION AND CANCER BIOLOGY

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INTERLEUKIN 32 U INFEKCIJI, INFLAMACIJI I BIOLOGIJI TUMORA

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ABSTRACT

Cytokines are small pleiotropic polypeptids secreted dominantly by the cells of the immune system. These polypeptids are main mediators of innate and acquired immunity, responsible for clonal expansion and differentiation of immune cells, initiation of immune response and enhancing of effector functions of leukocytes. Cytokine-related effects are most studied in the fields of inflammation, immunology, and cancer biology. In this review we discuss one of the most intriguing, recently discovered proinflammatory cytokine, interleukin 32.

Keywords: *Interleukin 32, infection, inflammation, tumor*

SAŽETAK

Citokini su mali polipeptidi koje luče dominantno ćelije imunskog sistema. Oni su glavni posrednici urođene i stečene imunosti, odgovorni za klonsku ekspanziju i diferencijaciju ćelija imunog sistema, pokretanje imunog odgovora i pojačavanje efektorske funkcije leukocita. Njihovi efekti se najviše izučavaju u oblasti inflamacije, imunologije, i biologije karcinoma. U ovom preglednom radu govorimo o jednom od najinteresantnijih, nedavno otkrivenih proinflamatornih citokina, interleukinu 32.

Ključne reči: *Interleukin 32, infekcija, inflamacija, tumor*

INTRODUCTION

Cytokines are small proteins secreted by cells of immune system and many others. They are principally involved in homeostatic mechanisms by mediating and regulating inflammatory/immune responses in various diseases and affect cellular interactions and cell communication system (1–4). They direct the development, maturation, localization, interactions, activation and life span of immune cells (2, 5). Cytokines are main mediators of innate and acquired immunity, responsible for clonal expansion and differentiation of immune cells, initiation of immune response and enhancement of effector functions of leukocytes. They are key factors in preventing and stopping an uneventful immune response (1, 3, 6, 7). They also have a role in important physiological processes such as wound repair (3, 5). Almost every biological discipline studies these factors, but cytokine-related effects are uppermost in the fields of inflammation, immunology, and cancer biology. These peptides act in autocrine, paracrine and endocrine manner, dependent on their site of activity (5). They are principally classified into various groups based on their biological roles. Their characteristic are pleiotropism (activation of numerous types of responses), redundancy (functionally overlapping), synergy (between cytokines to amplify the effect), antagonism (i.e. regulation of duration and potency of the response, important for avoiding autoimmunity), feedback and feedforward loops – for negative and positive (e.g., signal amplification) regulation (3, 5). Cytokines directly influence cancer growth or they indirectly contribute to antitumor activities of lymphocytes (8). In past two decades among great number of discovered cytokines and their functional and regulatory roles, interleukin-32 (IL-32) is one of the most intriguing. Since the

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discovery in 1992. it was pronounced as a powerful proinflammatory cytokine with ability to induce production of other proinflammatory cytokines and chemokines (9, 10). The aim of this review is to emphasize actual data and future perspective of interleukin-32 (IL-32).

History of the IL- 32

In 1992. Dahl et al. reported a gene that was highly expressed in activated T- cells and IL-2 activated NK-cells and therefore it was called NK4 (11). This NK4 gene was rapidly upregulated in human peripheral blood mononuclear cells (PBMCs) after stimulation and activation of T-cells. Authors demonstrated high polymorphism of the NK4 gene. Sequence analysis revealed that the NK4 - encoded protein had a predicted molecular mass of 27 kDa (12–14). In addition, it was suggested that the NK4 protein contained Arg–Gly–Asp (RGD) motif, important in cell adhesion (15). The NK4 transcript contains a potential signal sequence cleavage site between amino acid 31 and 32, indicating that NK4 can be secreted by the classical secretion pathway (12, 13). Nevertheless, for the next 13 years, the biological function of NK4 was not known. In 2005, Kim et al. showed for the first time that the NK4 protein had biological function and a recombinant form of the protein induced production of several proinflammatory cytokines (16). It was discovered accidently while studying the genes induced by IL-18 and was found to stimulate the production of various chemokines, pro-inflammatory cytokines including IL-1β, IL-6, IL-8, TNF-α and macrophage inflammatory protein-2 (MIP-2) (14, 16). Authors revealed that NK4 protein activates signal transduction pathways such as nuclear factor-kappa (NF-kb) and p38 mitogen activated protein kinase (MAPK). Since the NK4 protein possess significant proinflammatory properties, his name was changed to IL-32.

Isoforms

IL-32 gene was found to be located on human chromosome 16p13.3 and was reported to exist in nine different isoforms by mRNA alternative splicing including IL-32α, IL- 32β, IL-32γ, IL-32δ, IL-32ε, IL-32ζ, IL-32η, IL-32θ, and IL- 32s (small), and all of them have specific activities and properties (12, 17, 15). IL-32α is the most abundant while IL-32β is the most common transcript (12, 18, 19). The different isoforms of IL-32 originate by splicing of pre-mRNA of the isoform IL-32γ. It was not discovered why the IL-32γ mRNA transcripts are spliced or is this phenomenon similar in all cells. IL-32γ is the most potent isoform of IL-32, concerning his role in cell death and cell activation, and this may explain why IL-32γ is spliced to less harmful isoforms. In addition to promoting cytokine production, overexpression of endogenous IL-32γ caused cell death, which in contrast doesn`t occur with the IL-32α isoform (15, 20). The difference in the size of the isoforms, ranging from 14.9 kDa (IL-32α) to 26.7 kDa (IL-32γ), and the tertiary structure of the isoforms may be part of the explanation for their different potency (21, 22). The endogenous level of IL-32 can be modulated in immune cells by exposure to various stimuli. Pathogen-related agents, such as lipopolysaccharide (LPS), muramyl dipeptide (MDP), and double-stranded RNA and several cytokines such as TNF-α and IFN-γ can induce IL-32 expression (23–25). Namely, pathogen-associated molecular patterns (PAMP) and endogenous stress signals termed danger-associated molecular patterns (DAMP) binds to pattern-recognition receptors on cell membrane (26, 27). Exposure of monocytes, macrophages, or endothelial cells to these stimuli induce the expression of endogenous IL-32, both on mRNA and protein levels, via NF-κB/ activated protein-1 and phosphatidylinositol-3 kinase/Akt signaling pathways. Furhter IL-32 induces various proinflammatory cytokine production via NF-κB and p38 MAPK (Figure 1). One of the most noteworthy observations is that IL-32 is still not found in rodents, such as mice and rats. Recent discoveries in this area confirmed earlier hypothesis – alternative splicing can be a mighty regulator of isoform types which are produced in various conditions and tissue types (18).

IL-32 signaling and role in cell biology

Earlier investigations concluded that IL-32 downstream signaling involved multiple pathways - NF-κB and p38 MAPK pathways, Erk1/2 and PI3 K/Akt pathways, and IL-32-exposed human macrophage-like THP-1 cells resulted in the phosphorylation of p300 and DAPK-1 (23, 10, 28, 29). But until today it was not clear whether this cytokine acts on intra- or extracellular level. Further studies revealed high affinity of IL-32 to urinary and neutrophile proteinase 3 (PR3), which consequently (30) proposed as membrane binding protein for IL-32, and regulator of its activity by splicing into the various isoforms (22, 31, 30). IL-32 can also bind to the membrane integrins αVβ3 and αVβ6, but not to αVβ8 (32). Well known fact is that integrins are involved in cell signaling and are important for cell adhesion, survival, and cytokine production. It has been proposed that αVβ3 and αVβ6 could be the receptors for extracellular IL-32 (30). Many reports showed that IL-32 acts inside the cell (30). Releasing of this cytokine is possible after cell death (9, 25). Using special modeling software, some authors conclude that IL-32 has similarities with focal adhesion targeting region (FAT) of focal adhesion kinase (FAK-1) (15). FAT targets FAK-1 to bind with integrin via paxillin. FAK and paxillin are two focal adhesion–associated proteins with crucial function in integrins downstream signaling (33). These signals regulate important biological cell functions, such as migration, proliferation, and survival (34, 35, 33). So far, we can conclude that there are at least two membrane binding proteins for extracellular IL-32 – PR3 and integrins, and another yet unknown receptor (18). Intracellulary, for the activity of this cytokine are responsible FAK-paxillin proteins, which after binding with IL-32 regulate various cell functions, such as cell growth, metabolism, cytokine production, cell adhesion, migration, proliferation, differentiation, angiogenesis and apoptosis (9, 30, 36, 37).

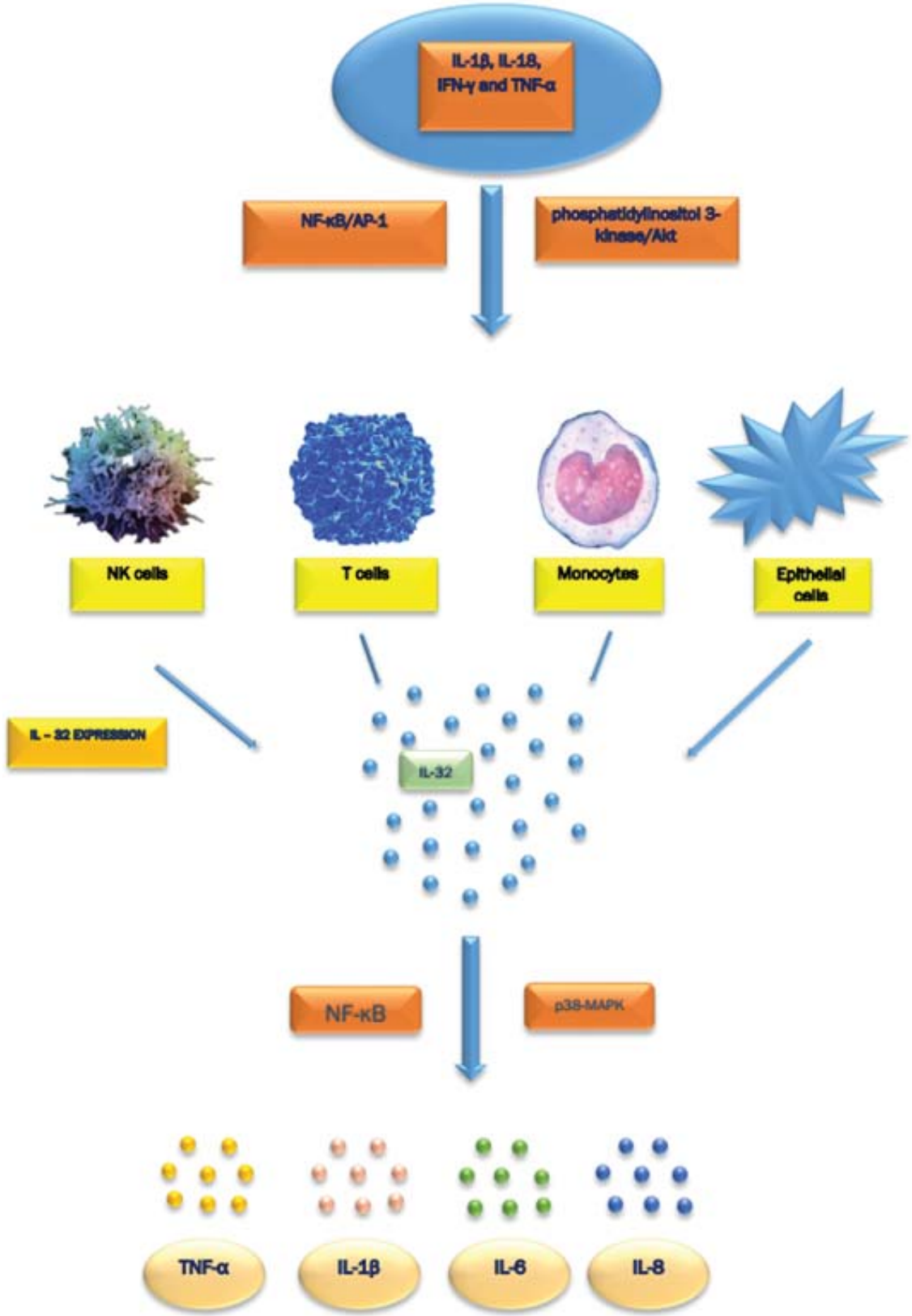

Figure 1. Expression of IL-32 in various cell types upon stimulation with IL-1β, IL-18, IFN-γ and TNF-α. Signaling pathways are NF-κB/activated protein-1 and phosphatidylinositol 3-kinase/Akt. IL-32 can induce various cytokines and chemokines - TNF-α, IL-1β, IL-6 and IL-8 via NF-κB and p38-MAPK signaling.

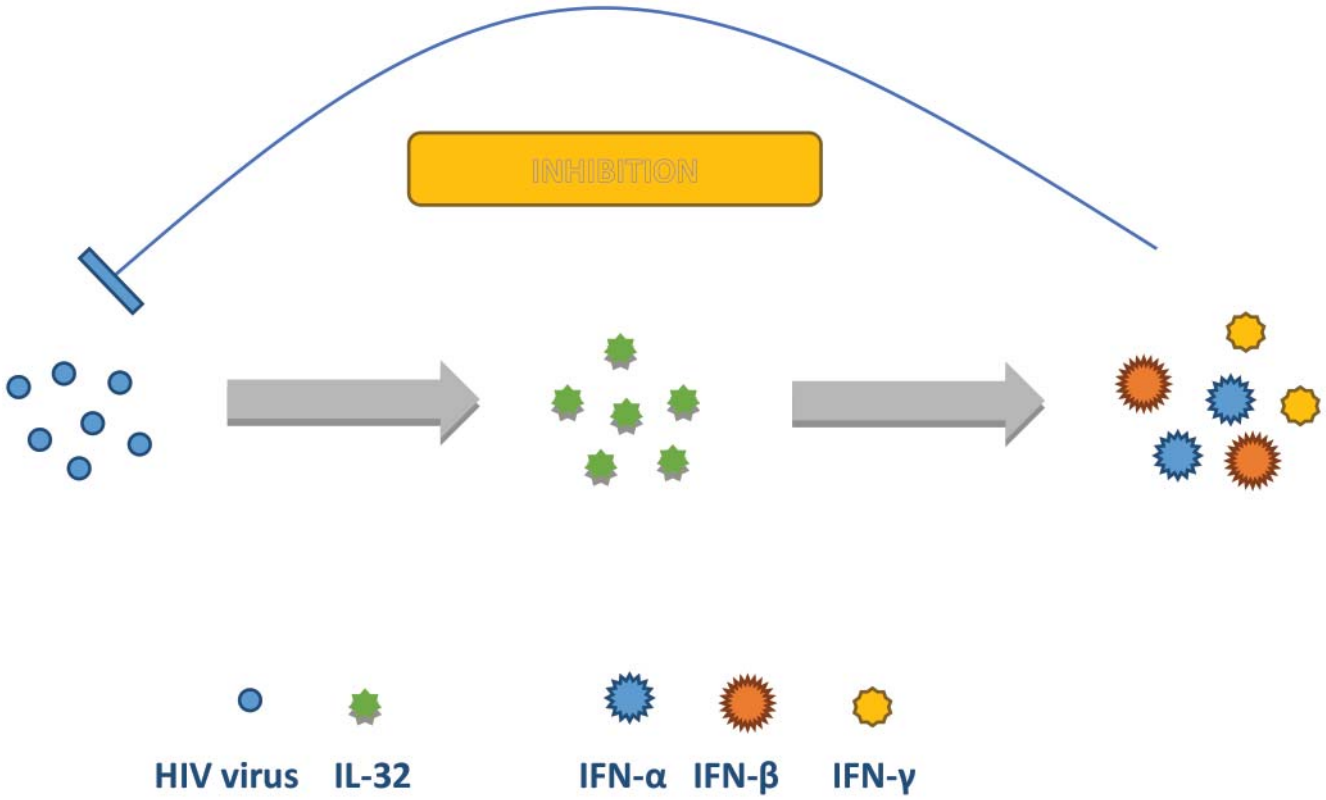

Figure 2. HIV virus induces IL-32 expression. IL-32 promotes induction of various proinflammatory cytokines, and among them most important are interferons - IFN-α, IFN-β, IFN-γ. They suppress further replication of this virus.

Role in viral infections

In last decade many studies published data revealing antiviral IL-32 properties, and increased expression and circulating levels of this cytokine in patients with viral infections. Reports analyzing IL-32 in patients with H1N1 influenza pointed that infected patients had an increased circulating level of IL-32 (38). Antiviral activity of recombinant IL-32γ was found in WISH (a human amnion cell line) infected with vesicular stomatitis virus (38, 39). Study conveyed earlier revealed that plethora of cytokines were increased in patients with influenza virus: IFN-β, interferons type III, IL 1α, IL-1β, IL-6, IL-23, IL-12, and IL-32γ (14). Influenza A virus induces IL-32 overexpression through NF-κB and cAMP response element-binding (CREB) pathways (40). Similar studies revealed that IL-32 expression depends on cyclooxygenase-2 (41, 42). Overexpression of IL-32γ inhibits further replication of this virus. Regarding the role of this cytokine in HIV-1 infections, results showed that interferons are crucial for the anti-HIV-1 effect of recombinant IL-32γ, and silencing of endogenous IL-32 reduced the levels of Th1 and proinflammatory cytokines, which confirm the anti-HIV-1 property of IL-32. Blockade of any of the interferons α, β or γ enhanced further HIV virus replication (17, 43–45). Based on link between IL-32 activity and production of IFNα, β or γ, various authors proposed that IL-32 exhibits its antiviral properties by all these interferons (39, 21) (Figure 2). IL-32 expression is induced by hepatitis C and Epstein-Barr viruses (EBV) also (23, 46, 47). Human papillomavirus (HPV) induces IL-32 expression via E7-mediated COX-2 stimulation (41). In hepatitis B virus infection, HBx protein encoded by HBV genome, plays an important role in the hepatic inflammatory processes. Study of Pan X et al. showed that HBx could induce IL-32 expression in Huh7 cell in a dose-dependent manner by NF-κB pathway (46), and it is correlated with the severity of liver inflammation/fibrosis in patients with chronic HBV infection (48). In vitro and in vivo results of different studies showed that IL-32 expression in human macrophages serves to protect the host by facilitating apoptosis of the host cell and thereby depriving Mycobacterium tuberculosis of a protected survival as an intracellular microorganism (49, 50). Similar results were obtained with Mycobacterium avium intracellulare. In M. leprae infections, form of the disease was dependent of differentiation of dendritic cells induced by IL-32 (51, 52).

Role in inflammatory and autoimmune diseases

Role of IL-32 in rheumatoid arthritis (RA) was intensively studying. Immunohistochemistry staining of synovial tissue specimens revealed expression of this cytokine in sy-

novial lining, sublining, and endothelial cells, especially in macrophage-like cells (53, 54). This expression correlated with inflammation, TNF-α, IL-1β, IL-18 levels and with acute phase protein CRP and erythrocyte sedimentation rate (ESR) (10, 55, 56). IL-32 was upregulated strongly after stimulation with TNF-α and further induced enhanced production of proiflammatory cytokines IL-6 and IL-8. That could be explanation for the TNF-α/IL-32/TNF-α-positive auto-inflammatory loop and success of the anti-TNF-α therapy in up to 50% of these patients. In another study that enroled patients with rheumatoid arthritis, osteoarthritis and ankylosing spondylitis serum levels and synovial tissue expression of IL-32 were measured (57). The elevated level of IL-32γ in ankylosing spondylitis joint positively correlate with osteoblast differentiation via DKK-1 suppression (58). In inflammatory bowel diseases, e.g. ulcerative colitis and Crohn's disease, IL-32 has a important role in pathophysiology and progression. Namely, bacterial peptidoglycan muramyl dipeptide through binding with nucleotide-binding oligomerization domain containing protein 1 (NOD1) and 2 (NOD2) via caspase-1-dependent mechanism leads to induction of IL-32 expression. Further activation of various factors leads to enhanced production of IL-1β and IL-6, well-known proinflammatory mediators (50, 59). Chronic obstructive pulmonary disease represents inflammatory response to toxic particles and gases. Pathogenesis of this disease involves IL-32, highly expressed in lung tissue specimens, and this expression correlates with degree of airflow obstruction (60, 61). Asthmatic patients had a higher level of systemic IL-32. Studies with this disease pointed that IL-32 inhibits angiogenesis by suppressing VEGF, being endogenous regulator of proangiogenic factors and controller of airway remodeling. It was well documented that IL-32 has an important role in pathogenesis of allergic rhinitis and chronic rhinosinusitis, by stimulating proinflammatory cytokines and chemokines (62, 63). Keratinocytes are a major source of this protein, which enhances their apoptosis and aggravates further worsening in atopic dermatitis. Patients with psoriasis have no significantly increased serum level of IL-32, compared to those with atopic dermatitis (64, 65). In atherosclerosis IL-32 amplifies local inflammation and attracts more circulating monocytes and other inflammatory cells to the subendothelial compartment. Together with activation of matrix metalloproteinases (MMP) 1, 9 and 13, this will contribute to the enhanced vascular inflammation, plaque instability, thickening of the fibrous cap and disruption, leading to acute coronary syndrome (66). Overexpression of IL-32γ in transgenic mice with provoked sepsis lead to more severe disease (67).

Role in cancer biology

Currently there are very actual reports about role of this cytokine in cancer and cancer therapy. One of the first that imposed possible role in cancer explained that in chronic myelomonocytic leukemia IL-32 expression was markedly reduced, while in myelodiplastic syndrome was elevated and associated with enhanced apoptosis of the bone marrow stem cells (68, 69). Several reports confirmed that IL-32 was higly expressed in tumor tissue, compared to adjancent tissue without cancer cells: brain, breast, lung and stomach cancer (30). In vitro studies showed that this protein induces migration and invasion of cancer cells. Overexpression of IL-32 contributes to invasion and metastasis in primary lung adenocarcinoma, induced by increased expression of MMPs 2 and 9 via NF-kappaB activation (70). IL-32α exhibits more migratory ability to melanoma cells, through downregulation of E-cadherin expression (71). In stomach cancer, this cytokine is involved in process of carcinogenesis since beginning. That was proved in patients with confirmed Helicobacter pylori inflammation, which is confirmed carcinogen (72, 73). Their gastric mucosa expresses higher levels of IL-32, as well as levels in sera of same patients. Opposite to these findings, there are several reports about inhibitory effects of IL-32 on cancer cell growth, especially via the NF-κB and STAT3 signaling (29). IL-32θ inhibited epithelial-mesenchymal transition (EMT), resulting in the suppression of migratory and invasive capabilities of HT29 colon cancer cells (74). Higher levels of IL-32 were reported in many other cancers: melanoma, thyroid, renal cell (18, 71, 75). Recent studies have revealed higher expression of IL-32 in human pancreas, liver, and esophagus cancer tissues, compared with normal tissue or serum (76–78). One of the hallmarks of tumor is angiogenesis. Data about role of IL-32 in this process are still controversial and insufficient. Examination of the in vitro and in vivo models on endothelial cells (EC) in pulmonary arterial hypertension- PAH and glioblastoma multiforme (GBM) showed that IL-32 requires cofactor (IFN-γ) to sensitize EC, i.e. to exert its biological activity (32). Also, examination of effects in neonatal HUVEC (human umbilical vein endothelial cells) and adult pulmonary microvascular EC, using an in vivo and an in vitro angiogenesis assay, showed that induction of IL-32 in ECs was related with activation and proliferation of these cells, as well as angiogenesis (32). Angiogenic effect in certain concentrations on HUVEC was even greater than with VEGF, but was not dependent on VEGF(18, 32). Authors conclude that IL-32 exerts its angiogenic properties in EC via integrins (at least in some part), requires second stimuli (with LPS, IFN-γ or unknown cofactor) for endothelial cell responsiveness. IL-32 utilize regulation of IL-8, MMP-9, activin A, and endostatin, but not VEGF or TGF- β1 to induce angiogenesis in EC (32). In other study with asthmatic patients, assays with normal human bronchial cells stimulated with TNF-α, IFN-γ, Th1 cells and rhinovirus infection were examined(79). Results showed that IL-32 inhibited angiogenesis by decrease of VEGF production in these cells. Authors conclude that the IL- 32–mediated decrease in VEGF secretion by NHBE cells during airway inflammation supports the anti-angiogenic effect of this cytokine (79). Interestingly, there are no literature data about possible role of IL-32 in lymphangiogenesis and consequently role in spread of cancer through the lymphatic system.

Conclusion and future perspectives

IL-32 and its modulatory role in innate and aquired immunity and other processes are still under intense investigation. From known facts it could be concluded that main biological functions of IL-32, acting predominantly intracellulary, include induction of the expression of various pro-inflammatory and some anti-inflammatory cytokines and, hence, contribution to the progression and pathogenesis of various pathological conditions and systemic infections. Important issue and role in tumor angiogenic processes and carcinogenesis should be explored. That could influence and modify further therapeutic strategies in various pathological conditions.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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A GIANT EXULCERATED PHYLLODES BREAST TUMOR - A CASE REPORT

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GIGANTSKI EGZULCERISANI FILODNI TUMOR DOJKE – PRIKAZ SLUČAJA

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ABSTRACT

Phyllodes tumors of the breast can be benign, malignant, or borderline. Benign and borderline tumors are rare tumor types that have a positive outlook and high survival rate, while the risk of recurrence is typical for malignant breast tumors. Giant phyllodes tumors are larger than 10 cm in diameter and demand a serious diagnostic and treatment approach.

In this study we present a case of a female patient treated for an exulcerated breast carcinoma- a giant borderline phyllodes tumor of the breast. The patient presented to the department for the right breast lump with ulcerated skin and nipple abnormalities. The core biopsy was performed and the patient was diagnosed with a benign tumor. Simple mastectomy was performed and final histopathological report revealed a borderline phyllodes tumor. Diagnosis and treatment of a giant phyllodes tumor remain a great challenge for the surgeons. Establishing the preoperative diagnosis based on histopathological findings is imperative to disease management. Surgery is the mainstay of treatment and mastectomy has been the traditional procedure; in cases where suspicious findings in the axilla are revealed, radical mastectomy is performed and the axilla is to be dissected.

Keywords: *phyllodes tumor, borderline tumor, breast, mastectomy*

SAŽETAK

Filodni tumori dojke mogu biti benigni, granični i maligni, to je retka vrsta tumora dojke sa dobrom prognozom i preživljavanjem kod benignih i graničnih tumora ali i sa značajnim procentom recidiva posebno kod malignih tumora. Tumori veći od 10 cm u prečniku su gigantski filodni tumori i zahtevaju ozbiljan pristup u dijagnostici i lečenju.

U ovom radu prikazujemo slučaj bolesnice koja je lečena zbog gigantskog egzulcerisanog graničnog filodnog tumora dojke. Bolesnica se javila hirurgu zbog tumorski izmenjene desne dojke sa prisutnim egzulceracijama na koži i deformisanom bradavicom. Urađena je CORE biopsija koja je ukazala da je tumor benigne prirode. Urađena je prosta mastektomija i definitivni patohistološki nalaz je ukazao da se radi o graničnom filodnom tumoru. Dijagnostika i lečenje gigantskih filodnih tumora predstavlja izazov za hirurga. Postavljanje preoperativne pato-histološke dijagnoze treba da bude imperativ. Lečenje gigantskih filodnih tumora podrazumeva prostu mastektomiju, osim kod klinički i dijagnostički sumnjive aksile kada je potrebno uraditi i radikalnu disekciju.

Ključne reči: *filodni tumor, granični tumor, dojka, mastektomija*

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INTRODUCTION

Phyllodes tumors of the breast are rare tumor types that contain two types of breast tissue: stromal - connective tissue and glandular tissue. (1) This tumor type was first described in 1838 by Johannes Muller. (2) Phyllodes tumors of the breast account for less than 1% of the breast tumors and they are mostly seen in women between 45 and 49 years old. (3) The World Health Organization classified them histologically as benign, borderline, or malignant. Benign tumors are more frequent having an incidence of 40-50%. (4) The phyllodes tumors can resemble fibroadenomas and the distinction between phyllodes tumor and fibroadenoma is clinically important, as these two tumor types require adequate treatment options. The median size of phyllodes tumors is around 4 cm. (5) Giant phyllodes tumors are those larger than 10 cm in diameter and account for about 20% of all phyllodes tumors; rarely they can reach sizes up to 40 cm in diameter. (6, 7) Management of the giant phyllodes tumor presents diagnostic and treatment challenges for the surgeon. Ulceration of the skin of the breast is not typical for phyllodes tumors and therefore it can present a diagnostic dilemma.

In this study we present a case of a female patient treated for an exulcerated breast tumor - a giant borderline phyllodes tumor of the breast.

CASE REPORT

A 59-year-old female patient G.S. presented to the department in our hospital with a giant exulcerated tumor of the right breast in July 2017. She reported that she had had an injury of the right breast in 2004 and a few months after the injury she noticed a firm palpable breast mass. The mass grew quickly and quite large every year, and a year before the surgery, the patient noticed wound-like skin changes in the breast. She got her first menstrual period at the age of 12, she had two deliveries and two miscarriages; the last period was at the age of 50. A clinical breast examination (inspection) showed a giant mass occupying the whole right breast with ulceration. The palpable breast mass was firm, with ill-defined borders, occupying the entire right breast. (figure 1) There were no palpable axillary lymph nodes. The left breast appeared normal. The ultrasound revealed a large heterogeneous mass with some cysts in the right breast. Axillary lymph nodes up to 6mm were present. Freehand core biopsy was performed and specimens were sent for pathological examination. The histopathological findings indicated a benign phyllodes tumor. A radical surgery was to be done based on the decision of the consilium of oncologists. The patient underwent a right simple mastectomy (figure 2), and the patient was discharged on the fourth postoperative day. Histopathological findings showed that tumor tissue was of moderate cellularity, with dual epithelial -mesenchymal differentiation, necrotic zone, with myxoid degenerative

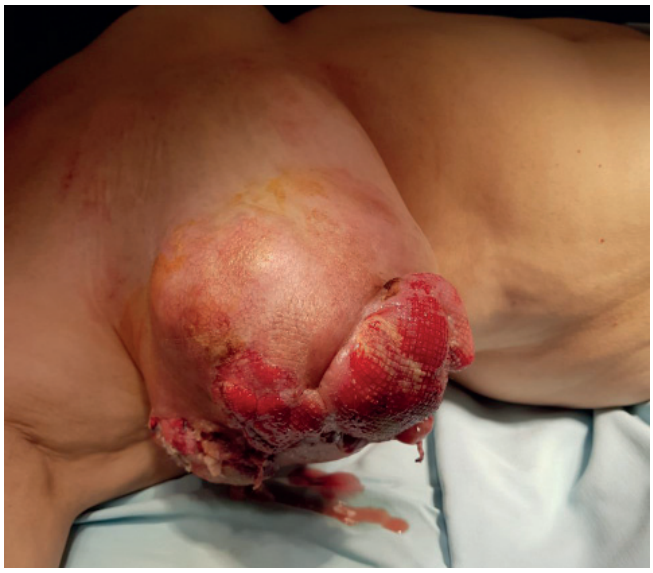

Figure 1: Right breast with deformation caused by a tumor

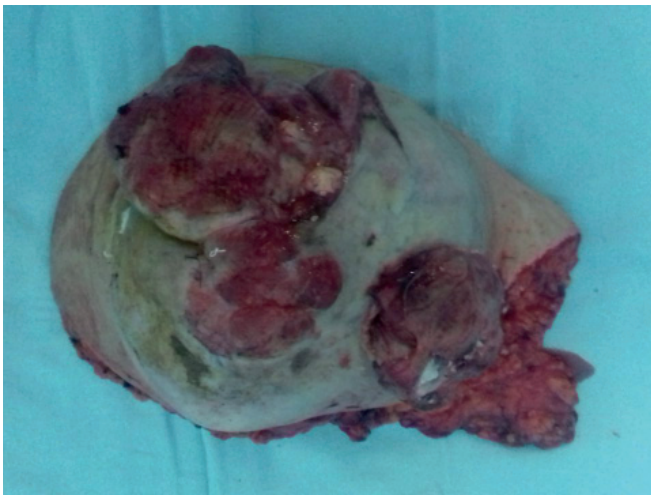

Figure 2: Right breast - simple mastectomy specimen

changes, a mild inflammatory infiltrate and light bleeding. The immunohistochemical analysis showed that tumorous cells were partly positive for vimentin, diffuse positive for SMA, and negative for p63 and p53. We concluded that it was a borderline phyllodes breast tumor. (Figure 3) The patient attends follow - up appointments and so far disease progression has not occurred.

DISCUSSION

Phyllodes tumors of the breast are a rare group of breast tumors that are typically large, fast-growing, and painless masses that stretch the overlying skin. (8) The peak incidence is between 40 and 50 years old, this is about 10 to 15 years later than fibroadenomas and 20 years earlier than most invasive ductal and lobular cancers. (3,9) The median size of phyllodes tumors are usually 4 cm though there has been reports of large tumor size up to 50 cm and these are mostly malignant. (10) The our patient had a 15cm large

phyllodes tumor and because of its size it was a giant breast tumor. Though ulceration and nipple retraction have been reported in some case reports, they remain uncommon; their presence indicates a malignant breast tumor. (11,12). In this case report, the patient had skin ulceration and a deformity of the nipple which made us believe it was a malignant breast tumor. Diagnosing phyllodes tumor includes ultrasound, mammography and additionally MRI and yet it is still difficult to differentiate between the two types of breast tumors - phyllodes tumors and fibroadenomas. (3) Our patient was believed to have a malignant phyllodes tumor because of the giant breast tumor mass with skin ulceration and the first ultrasound revealed a large mass occupying her whole right breast. The mammographic appearance of the left breast was normal. (BI-RADS 2) When there are indications of phyllodes tumor the diagnosis has to be made through core needle biopsy. This has only a sensitivity of 75% for differentiating phyllodes tumors from fibroadenomas so if clinical suspicion remains excisional biopsy is indicated for the correct differentiation. (3) Recurrence was observed in 21%, 46%, and 65% of patients with benign, borderline and malignant phyllodes tumor so some authors advocate simple mastectomy; (13) however, there are many studies showing no significant difference between breast conserving surgery and mastectomy taking into account overall survival and period without metastasis although the patients who underwent breast conserving surgery had higher recurrence rate. (14) Successful treatment of a phyllodes means that wide excision has to be used with intention of surgical margins of minimal 1 cm, but in some cases, like the one we present, partial mastectomy is necessary (15) - nevertheless the core biopsy revealed a benign tumor, it was the tumor size that defined the treatment method. Borderline and malignant phyllodes tumors rarely spread to the lymph nodes (<1% have pathological lymph nodes). (9, 16) Palpable axillary lymphadenopathy has been reported in up to 20% of cases but these are often reactive in nature. (17) Diagnostic procedures, as well as clinical examination of the patient, did not reveal lymphadenopathy so radical axillary dissection was not necessary; it was also taken into account that preoperative breast biopsy revealed a benign phyllodes tumor. Adjuvant chemotherapy and/or radiotherapy have not been proven to be useful in the treatment of phyllodes tumor (4,12) The patient was not recommended postoperative adjuvant therapy by the oncology consillium. She was recommended to attend follow-up appointments.

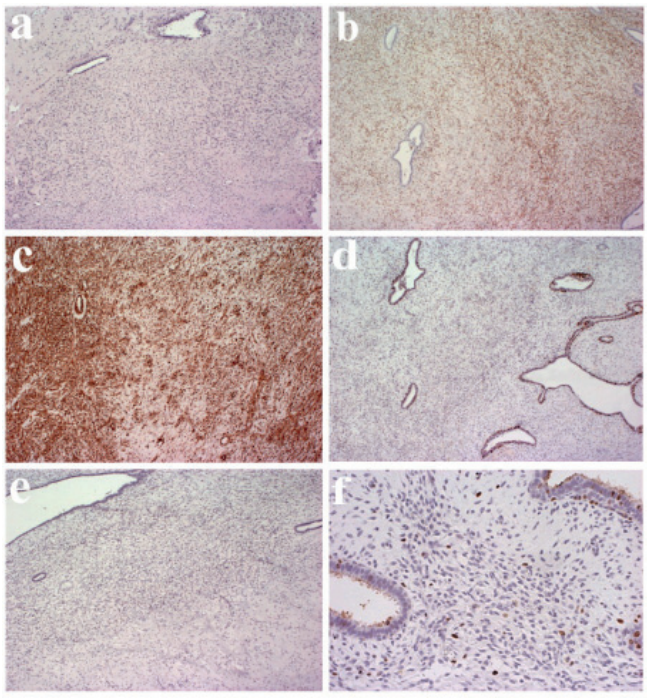

Figure 3: Borderline phyllodes tumor of breast - diagnosis was confirmed on routine H&E stained sections of the tumorous tissue (a) and immunohistochemical analysis (b-f). Tumorous cells were partly positive for vimentin (b), diffuse positive for SMA (c), and negative for p63 (e) and p53 (e). (original magnification, x100) Ki-67 proliferation index is low – 6% (f) (original magnification, x200)

CONCLUSION

Diagnosis and treatment of giant phyllodes tumors remain a great challenge for the surgeon. Establishing the preoperative diagnosis based on histopathological findings is imperative to disease management. Mastectomy has been the traditional procedure in treatment of giant phyllodes tumors; in cases where suspicious findings in the axilla are revealed, radical dissection should be performed. The prognosis for benign and borderline phyllodes tumors is very good. The patient with phyllodes tumors should not receive adjuvant therapy.

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VASCULAR ACCESS FAILURE - CAUSE OR COMPLICATION OF CENTRAL VENOUS CATHETERIZATION: CASE REPORT

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NEUSPEŠNOST VASKULARNE KATETERIZACIJE - UZROK KOMPLIKACIJE CENTRALNE VENSKE KATETERIZACIJE: PRIKAZ SLUČAJA

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ABSTRACT

The quality of life and patient survival rate in terminal chronic renal insufficiency depends on the duration of vascular approaches. Dialysis catheters are used to establish an adequate vascular approach when emergency hemodialysis is indicated and when all approaches are exhausted. Complications of CVC can be classified into three categories: mechanical (hematoma, arterial puncture, pneumothorax, hemothorax, catheter misplacement, and stenosis), infectious (insertion site infection, CVC colonization, and bloodstream infection) and thrombotic (deep vein thrombosis). Despite the increasing prevalence of haemodialysis patients with complex access issues, there remains no consensus on the definition of vascular access failure or end-stage vascular access. The dilema in these cases remains whether the generalized vascular insufficiency is the cause or a complication of exhausted vascular accesses. This case report is one of the examples of combined complications with generalized vascular access insufficiency. During the year and a half of the chronic dialysis program, the patient had several changes of vascular approaches, and each approach became dysfunctional in certain time due to various causes. After six months of successful hemodialysis, the patient was admitted with signs of infection and during hospitalization was again subjected to multiple changes of the vascular approach due to infection, thrombosis, and vascular access failure.

Keywords: *central venous catheter, infection, thrombosis, vascular failure*

SAŽETAK

Kvalitet života pacijenata sa terminalnom hroničnom bubrežnom insuficijencijom zavisi od trajanja vaskularnih pristupa. Dijalizni kateteri koriste se za uspostavljanje adekvatnog vaskularnog pristupa u slučajevima kada je indikovana hitna hemodijaliza i kada su svi drugi pristupi iscrpljeni. Komplikacije postavljanja centralnih venskih katetera se mogu grubo podeliti u tri kategorije: mehaničke (hematom, oštećenje arterije, pneumotoraks, hemotoraks, pogrešno postavljen kateter i stenoza), infektivne (infekcija mesta uboda, kolonizacija centralnog venskog katetera, sepsa) i trombotske (duboka venska tromboza, insuficijencija krvnih sudova, embolija). Jedna od ređih komplikacija je generalizovana slabost venskog sistema. Iako je učestalost pacijenata sa kompleksnim vaskularnim pristupima usled slabosti krvnih sudova u porastu ne postoji konsenzusna definicija ili podela insuficijencije vaskularnih pristupa (krajnji vaskularni pristup). Jedna od dilema u ovakvim slučajevima je utvrđivanje da li je generalizovana insuficijencija venskog sistema uzrok ili komplikacija iscrpljenih vaskularnih pristupa. Ovaj prikaz slučaja predstavlja jedan od primera kombinovanih komplikacija uz generalizovanu insuficijenciju vaskularnih pristupa. Kod opisane pacijentkinje je tokom godinu i po dana hroničnog dijaliznog programa promenjeno nekoliko vaskularnih pristupa za dijalizu, od kojih je svaki nakon izvesnog vremena postao disfunkcionalan usled različitih uzroka. Nakon šest meseci uspešne hemodijalize pacijentkinja je primljena zbog znakova infekcije i tokom hospitalizacije ponovo biva podvrgnuta višestrukim promenama vaskularnog pristupa zbog infekcije, tromboze, i insuficijencije vaskularnih pristupa.

Ključne reči: *centralni venski kateter, infekcija, tromboza, generalizovana vaskularna insuficijencija*

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INTRODUCTION

Dialysis patients are able to survive longer due to advances in nephrological care leaving those who are not fortunate enough to receive a transplant on long-term dialysis. In most cases of prolonged renal impairment, haemodialysis is the main treatment modality (1).

The quality of life and patient survival rate in terminal chronic renal insufficiency depends on the duration of vascular approaches. Since arteriovenous fistula (AVF) has the highest survival rate and the least complications, it should be a primary vascular approach whenever it is possible. Despite the priority of AVF, in almost 80% of patients with the indication for chronic dyalisis, a treatment starts with dyalisis catheter: temporary or permanent (2).

Dialysis catheters are used to establish an adequate vascular approach when emergency hemodialysis is indicated and when all approaches are exhausted. Although catheter placement provides a vascular approach, there is a possibility of reporting a number of complications: generalized infections, endocarditis, thrombophlebitis, blood vessel stenosis, vascular weakness, pneumothorax (3).

Catheter infections cause significant morbidity and increase patient mortality rate by more than 50% compared to patients with native AVF (3).

Central venous catheters are used as a permanent solution in patients with inability to make new vascular approaches or with contraindications for such solutions (AV fistula), as well as in elderly patients with poor prognosis. The most common CVC insertion sites are the right internal jugular vein or the right or left subclavian vein, while the left internal jugular vein is used less often because of the proximity of the ductus thoracicus and a possible damage to it (4).

CASE

A female patient aged 65 years was admitted to the Center for nephrology and Dialysis, Clinical Center Kragujevac due to general weaknes, fatigue, shivering and dysfunctional Hickmann catheter, with moderate bleeding in the area of catheter. Several latest hemodyalisis were difficult due to technical dysfunction of Hickmann catheter. At the admission the patient was aware, oriented in all three directions, afebrile, eupnoic, with the aspect characteristic for patients with renal dysfunction, turgor was weakened. On physical examination: Thorax was symmetrically respiratory mobile, with Hickmann catheter in the area of right subclavian artery; postoperative scar in the right lumbal area with hernia after nephrectomy. Respiratory function and heart beat were normal; BP: 100/60 mmHg.

The patient was on a chronic hemodyalisis programe (3x4 hours) for a year and a half as a treatment method for terminal renal dysfunction due to renal calculosis. She had a right-sided nephrectomy after prerenal absces a year ago. First temporary dyalisis was made through the placement of central venous catheter in right internal jugular vein. For the first three months the patient was on a chronic hemodyalisis through AV fistula, created on the distal part of the left forearm, which became thrombotic after few months. Following that, the patient had another dysfunctional AV fistula. Six months ago central venous catheter for hemodyalisis was placed in the left jugular vein and subsequently, due to catheter dysfunction, another catheter in left femoral vein. Due to exhausted vascular approaches, peritoneal dialysis was used, but the patient had recurrent peritonitis so the application of permanent catheter (Hickman) in the right subclavian vein was made.

After six months of successful hemodyalisis, the patient was addmited with lumbal pain and signs of inflamation: procalcitonin: 3.17 ng/mL (normal values 0.5-2 ng/mL), white blood cells (WBC): 15.9 x 10^9/L (normal values 4-10 x 10^9/L) and C-reactive protein (CRP): 178.7 mg/L (normal values <5 mg/L). Sympthomatic and empiric therapy with i.v Vankomycin 20 mg/kg was started during the last dyalisis for 5 days. Negative Staphylococcus coagulase was isolated from hemoculture and a clinical pharmacologist was consulted for the further therapeutic approach. Since the patient is allergic to Ceftriaxone and Amoxicillin with Clavulanic acid, a therapy with Vankomycin was continued, which reduced inflammatory markers after a few days: WBC: 5.6x10^9/L, CRP: 132 mg/L, PCT: 1.92 ng/mL.

Hematologist was also consulted, because the dialysis catheter (Hickman) was often obstructed by coagulum. After the placement of CVC both arms of catheter were heparinised according to the protocol several times, but obstructions were quickly re-established. The repositioning and purification of Hickman's hemodialysis catheter (Figure 1) was done under aseptic conditions, and the reposition was done because it was not functional. In the same act, the actilisation was applied to both arms in order to break the thrombus into the lumen of the catheter. After the action of the drug and the purification of the catheter, it became functional again.

After initial improvement, the patient developed repeated signs of infection and a new obstruction of Hickman's catheter after several days. Given that all possible approaches to placing CVC have become dysfunctional and difficult to obtain by regular placement techniques, presumeably due to general weakness of blood vessels, we have opted for ultrasound-guided CVC application. This method enabled us to locate the right jugular vein and open a new approach, after which the patient was stabilized (Figure 2A/B).

DISSCUSION

A critical factor in the outcome for haemodialysis patients is definitive vascular access either in the form of an arteriovenous fistula (AVF) or an arteriovenous graft (AVG). Autologous AVFs are a preferred choice for supe-

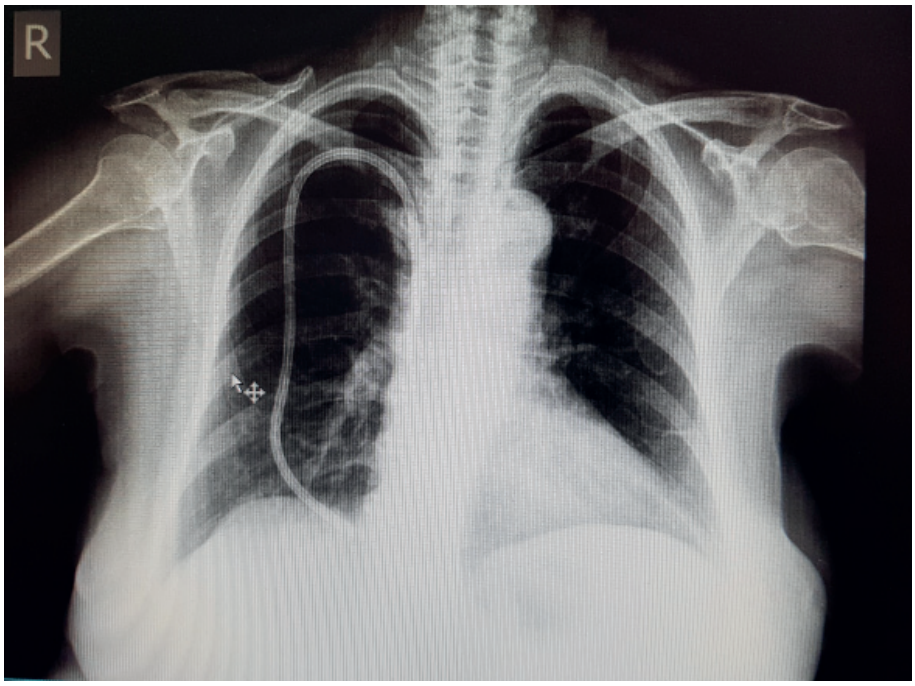

Figure 1.

rior long-term outcomes, better infection resistance and fewer interventions. Central venous catheters (CVC) in contrast have poor patency, higher infection rates and are associated with complications including central venous stenosis (5,6). With our patient, the protocol for dyalisis was followed since her first permanent vascular access was done through AVF, but since this approach was several times dysfunctional, patient was transfered onto CVC approach for dyalisis. This, however, was also followed by numerous complications: often thromboses, infections, vascular insufficiency, causing the exhaustion of vascular accesses.

Complications of CVC can be classified into three categories: mechanical (hematoma, arterial puncture, pneumothorax, hemothorax, catheter misplacement, and stenosis), infectious (insertion site infection, CVC colonization, and bloodstream infection) and thrombotic (deep vein thrombosis). These three categories occur in 5%–19%, 5%–26% and 2%–26% of patients, respectively (7). Our patient often had infectious and mechanical complications

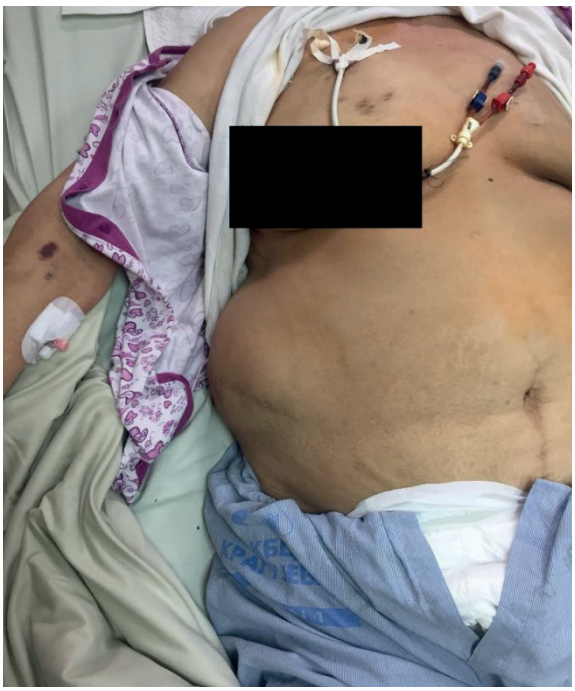

Figure 2A.

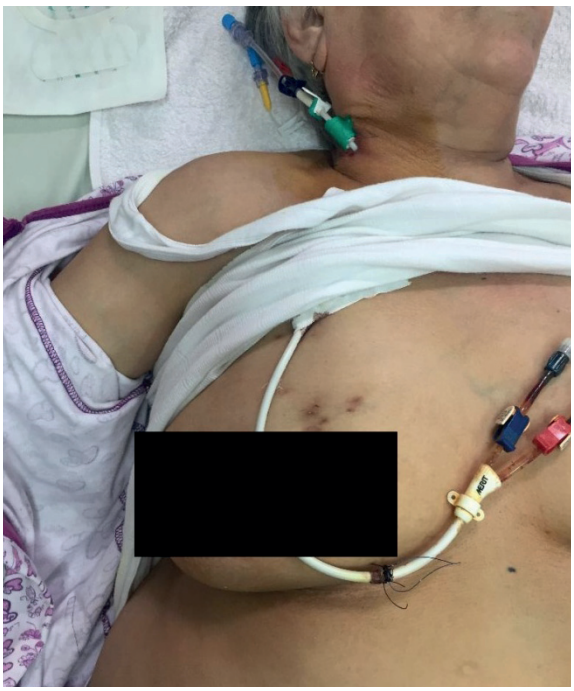

Figure 2B.

before the current hospitalisation. Complications associated with CVC insertion range from 5% to 19% (8). They can be distinguished as insertion and indwelling complications. The insertion complications are vascular injury (arterial puncture, pseudoaneurysm, arteriovenous fistula), hematoma, air embolism, pneumothorax and malposition. Indwelling complications are infection, thrombosis, catheter pinching/kinking and fracture with possible embolization (9).

Infection of CVC leads to increased morbidity and costs in health-care systems. Femoral access has been shown to be associated with an increased risk of infection, but some authors suggest that there is no difference among the three puncture sites when the strict sterile technique is followed (10). As for our patient, the occurence of infections was not associated with a specific CVC placement site, since she had prehosital infection associated with all three approaches: femoral, jugular and subclavian.

Microorganisms that most often colonize CVC are cogulase-negative Staphylococcus, Staphylococcus aureus, Gramm-negative microorganisms and Candida spp. (11). Hemoculture showed a presence of cogulase-negative Staphylococcus as a cause of inhospital infection in our patient, which confirmed above mentioned statement on incidence of antimicrobial agents.

For short-term use, the subclavian veins have been reported to be associated with lower incidence of associated infection than the internal jugular or femoral veins. However, according to a recent meta-analysis, there is no difference in the incidence of catheter-associated bloodborne infection between those three sites of vascular access, probably as a result of the implementation of new procedures and techniques for prevention (evidence level 1b) (12).

Fibrin sheaths, that cause catheter malfunctions, begin to format the catheter entry site into the vessel as an inflammatory response to the presence of a foreign body. In time, 100% of fibrin sheaths are colonized with bacteria (7). Both thrombosis and infection were often found in our patient, which was causing catheter malfunctions and need for a new approaches.

Thrombosis causing catheter malfunction can occur either within the catheter lumen or within the vessel lumen. A prevention of thrombosis is usually achieved by filling the lumen of the catheter with an anticoagulant (heparin or citrate) with or without antibiotic. Intravascular thrombosis is usually asymptomatic and only manifests itself with catheter malfunction (7). Our patient was treated with antibiotic therapy (Vankomycin) since the begining of hospitalization. Although she was treated with antibiotics and had an initial improvement, she had a reinfection and signs of sepsis, probably due to antibiotic resistence of infectious agent. In consultation with hematologist we made additional analyses for discovering the cause of reoccuring thrombosis which was causing the malfunction.

The preferred site for catheter placement is the right internal jugular vein, low in the neck and close to the jugular bulb so that there is little chance for catheter kink when tunneling to the chest wall. When the right vein is occluded, the right external jugular vein should be used before attempting access on the left side. The left internal jugular vein is the third choice, and is a technically challenging approach owing to the tortuous course from the left vein to the superior vena cava. Once the internal and external vein are exhausted in patients, other alternatives can be entertained, such as subclavian veins (13). In our patients all of the approaches were exhausted due to often infections, thrombotic occlusions and vascular insufficiency. Since there was a difficulty with replacing the CVC in new place we decided to use ultrasound-guided technique which helped us establish a new and secure approach.

Despite the increasing prevalence of haemodialysis patients with complex access issues, there remains no consensus on the definition of vascular access failure or end-stage vascular access. A group of authors tried to define a classification system-based anatomically to reflect the degree of severity of access failure. They have defined end-stage access failure as occurring when bilateral venous occlusion or severe stenosis that renders standard upper limb access options non-viable. In many dialysis programmes, there will be patients who are considered to have exhausted definitive access options and are maintaining dialysis on a CVC. These patients can be classified as end-stage vascular access. As this group is disparate and comparisons of outcomes are difficult, it is proposed that a classification system should be used (5). Using the aforementioned definition, it could be said that our patient may be considered to have this rare condition, since all of the approaches were exhausted.

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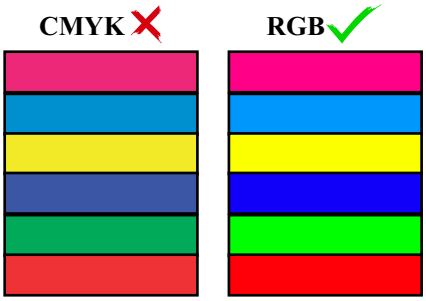

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