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Alternative therapeutic possibilities in schizophrenia

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ABSTRACT

The current tendency in the study of schizophrenia is to consider the evolution of schizophrenia as closely related to the person's psychodynamic development and the etiopathogenic bio-psychosocial conditions, schizophrenia representing a chronic psychosis that alters personality in depth. Currently, the multidisciplinary complex approach is the way to respond both to the challenges posed by the need to decipher the etiopathogenesis of major psychiatric disorders and the therapeutic and control possibilities of symptoms.Schizophrenia represents a major psychiatric condition with a well-contoured neurobiology support that affects the entire personality of the individual, manifested by a combination of anomalies of thought, perception, and alterations of social behavior.

Key words: schizophrenia, etiopathogenesis, therapeutic management, alternative therapies.Key words: Schizophrenia, alternative treatment, ethiopathogeic conditions.

Introduction

An important issue for public health, the disease has a clinical symptomssimilar throughout the world, regardless of the level of social, cultural, social andeconomic development. [10]

The etiopathogenesis of schizophrenia is extremely complex and undeciphered until now, the genetic, neurobiological and neurobiochemical components being added, with an important role, the psycho-social one. This level of vulnerability for the development of schizophrenia has led to the emergence of a descriptive model of correlation stress-vulnerability, where the most important roles belong to the genetic and environmental factors, as triggers of the disease and the evolution of the disorder itself, including the period of antipsychotic therapy, relapses and negative effects of social stress, substance abuse, decreased coping capacity and loss of socio-economic support. [19]

The action of psychosocial factors in the evolution of schizophrenia was also confirmed by neurobiological and neuroimagistic studies, the action of stress factors leading to increased release of glucorticoids through hyperactivity of hypothalamic-pituitary-cortico-adrenal axis, hyperactivity, which leads in long term to reducing of the hippocampus volume being considered one of the major biological markers of unfavorable evolution of schizophrenia. [16] On the other hand, one of the characteristic elements of schizophrenia, the lack of social functionality manifested by social withdrawal, lack of insight and initiative, altered reasoning and deficiencies in emotional expression, is caused by lesions of the prefrontal cortex and reducing of the volume of structures involved inthe process of social cognition (cerebral amygdala, upper temporal cortex). [1]

Regarding the therapeutic management of schizophrenia, at the moment the discussion is about a complex of goals aimed to reduce positive, negative, cognitive and affective symptoms, to achieve remission, to prevent relapse, to obtain safety and tolerability avoiding adverse effects and therapeutic risks, to provide neuroprotection preserving synaptic efficiency and to avoid cerebral structural changes [29].

These goals can be achieved using an extremely wide range of therapeutic methods, the psychopharmacological therapy occupying, without doubt, the central place, but without excluding other methods of treatment that involve other categories of mental health professionals, such as cognitive therapies [28], behavioral therapies [27], family and group therapies [13], supportive psychotherapy [24], training of social skills [22], ergotherapy [2].

However, obtaining the therapeutic goals is not enough, the patient's remission of the disease must also be doubled by maintaining this state for as long as possible, in order to achieve a good social functionality and patient reinsertion in the social environment of provenance. So, it becomes necessary to ensure a protective psychic climate, a careful follow - up of the patient for early notification of the symptoms of a possible recurrence, early therapeutic intervention preventing the aggravation of these evolutionary stages, evaluation of medical condition with psychogenic potential, a good communication with the patient, family, or his/ her companions in order to obtain the necessary information for mental status assessment, increase treatment adherence and compliance.

We will then review the main therapeutic alternatives of psychopharmacological treatment, these methods providing not only an augmenting effect of psychotropic medication, but also providing the conditions for a better control of remission periods and symptoms severity.

Cognitive- Behavioral Therapy.

Although it has been established as an effective psychotherapeutic method in depressive disorder, cognitive-behavioral therapy has found utility and effectiveness in the treatment of schizophrenia, following the failures recorded by other psychotherapeutic methods, especially psychoanalytic ones, with many studies related to this subject. [26, 6]

Cognitive impairment is one of the particular aspects of schizophrenia that favors cognitive-behavioral psychotherapeutic intervention, the techniques being individualized, both for the specificity of this psychiatric disorder (the methods used in schizophrenia are different from those used in depression anxiety disorders therapy) and for the patient's cognitive level. The key stages of Cognitive-Behavioral Therapy in schizophrenia include [24]:

- developing a therapeutic alliance based on the patient's perspective;
- developing alternative explanations for the symptoms of schizophrenia;
- reducing the impact of positive and negative symptoms;
- providing an alternative to medical model in order to maintain treatment adherence.

Extremely widespread in the UK and the United States, this psychotherapeutic method has proved effective in reducing psychotic symptoms, increasing medication adherence, improving response to chronic residual symptoms and as adjuvant therapy in admitted patients. [4]

Group Therapy.

Introduced as a therapeutic method in schizophrenia since the beginning of the 20th century, group therapy is supportive and social skills development (activities of everyday life) focused, with promising results in the improvement of anxiety symptoms, the ability to correctly perceive reality, self-esteem improvement, reducing hospital admission period and increasing compliance and adherence to the psychopharmacological tratment. [23] This psychotherapeutic method has also led to a noticeable improvement in the social functioning of schizophrenic patients and, consequently, to a positive attitude of the care team and the patients family, both in hospitalization conditions, as well as in ambulatory treatment. [8]

Family Therapy.

The psychosocial intervention for the schizophrenia patient's family is one of the most

used methods in the Anglo-Saxon cultural space (Great Britain, United States) as a therapeutic strategy in preventing relapse. In this regard, there is sufficient conclusive data on the effectiveness of these methods and even the necessity of using them in long - term therapeutic strategies in order to maintain remission in patients with schizophrenia. [15, 25] The specificity of these methods lies in their psycho-educational role, being provided additional information about the disease and therapeutic methods of schizophrenia to the family members, in order to lower the level of stress inside this micro-group, stress that can cause frequent relapse and worsening long-term prognosis of the disease. [17]

A limit of these non-pharmacological intervention methods is represented by cultural differences and variations in the educational level both of the patients and especially patient's family members or their belongings.

Occupational Therapy.

It ss another component of the psychosocial methods set in management of schizophrenia symptoms, proving its effectiveness in rehabilitation programs of patients with chronic psychiatric disorders, except for schizophrenia. Being included methodologically in group therapy categories, occupational methods have shown evidence of effectiveness in the improvement of positive symptoms of acute patients, being recommended for providing occupational therapies in a systematized manner in institutions that offer psychiatric care services for acute patients. [21, 14]

This type of therapy is based on accessible and attractive activities for patients, as a real starting point for the process of social reintegration of schizophrenic patients, by strengthening the remaining functional potential, increasing awareness on the ability to have socio-professional responsibilities and control dependence. [3]

Supportive Psychotherapy.

If usually classical psychotherapeutic methods do not have recommendations in the treatment of schizophrenia, the personality and the ego of the schizophrenic patient being profoundly affected by the illness, supportive psychotherapy, which may include advice, reassurance, education, providing models, setting boundaries, and testing reality is a valid option [7].

The first concepts of supportive psychotherapy were developed by psychoanalytic and psychodynamic schools [20, 9] for patients with structural defects of the ego or going through acute crises, with the objective of obtaining symptoms remission, strengthening the ego, improving social functioning, increasing self-esteem and functioning on following principles [18]:

- active, empathic efforts of the therapist to establish a positive therapeutic relationship;
- cognitive (re)orientation of the patient in order to improve perceptive and behavioral comprehension abilities;
- counseling and guidance in crisis situations and day-to-day issues;
- increasing self-esteem by strengthening and encouraging;
- orientation towards resources in order to rehabilitate the self-help capacity of the patient;
- avoiding conflicting situations and stimulate regression.

Meta-analyses of the studies conducted on supportive psychotherapy applied in schizophrenia and psychotic disorders have demonstrated an effectiveness of this method at least equal to that shown by cognitive-behavioral methods. [12]

Training of the Social Skills.

In family relationship and functioning area, there have been developed a series of training programs to improve social skills, both in patients with schizophrenia and members of their families, consisted of (re)learning some relatively simple actions performed in the social environment, such as the expression and recognition of emotions and feelings, communication techniques between family and patient, involvement in conversations and maintaining them, role-play games or homeworks.

The effects of these programs were also positive and beneficial for rehabilitation of social functioning of patients with schizophrenia and the improvement of the quality of their life. [11] In addition to these positive

outcomes, an easier social reinsertion was also achieved, supplemented by a job that provides schizophrenic patients not only a better quality of life or an improvement of their socio-economic status, but also a good prognosis in the evolution of the disorder and the relapse decrease. [5]

All these components of the contemporary therapeutic arsenal may lead through a judicious and individualized practice, taking into account the personal, biological and social characteristics of each patient with schizophrenia, to achieve the above mentioned therapeutic goals, but also to obtain recovery, the central issue of the present therapeutic paradigm.

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Роля на Витамин Д и Витамин $Д_3$ Рецептор - ВДР в процесът на ЕМТ и онкогенеза в кожа и други неспецифични за неговото действие тъкани

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Role of Vitamin D and Vitamin D_3 Receptor - VDR in EMT and oncogenesis in skin and other tissues nonspecific for its action

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РЕЗЮМЕ:

Всички геномни ефекти на 1α,25(OH)2D3 се медиират чрез свързването му с ВДР (Витамин Д Рецептор, VDR). ВДР регулира най-малко 229 гени, чрез свързването си към към поне 2,776 геномни ДНК свързващи места. Регулацията на ВДР експресията е все още ненапълно изяснена, предполагайки участието на $1,25(OH)_2D_3$, PKCα, PTH, глюкокортикоиди и 17β-естрадиол (E2). Добре известно е че слънчевата светлина (главно UVB) е необходима за ендогенният синтез на витамин Д. Пародоксално, същата соларна радиацция се смята за един от най-вредните фатори за кожата, свързан с развитието на кожни неоплазии.

$1α,25(OH)_2D_3$ увеличава експресията на

ABSTRACT

All the genomic actions of 1α,25(OH)2D3 are mediated through its binding to VDR (1,25-dihydroxyvitamin D_3 receptors). VDR regulates at least 229 genes through binding to at least 2,776 genomic DNA binding sites. The regulation of VDR expression is still incompletely understood, implicating $1,25(OH)_2D_3$, PKCα, PTH, glucocorticoids and 17β-estradiol (E2). It is well established that sunlight (mainly ultraviolet type B (UVB)) is required for the efficient production of vitamin D. Paradoxically, the same solar radiation is considered as one of the most harmful factors for the skin, thus contributes to the development of skin neoplasia.

$1α,25(OH)_2D_3$ up-regulated the expression

НАД+-зависимата 15-хидрокси-простагландин дехидрогеназа (15-PGDH) и намалява експресията на циклооксигеназа-2 (COX-2). Циклин-зависимият киназен инхибитор $p21^{waf1/cip1}$ е първият установен ВДР таргетен ген, активиран заедно с $p27^{kip1}$. Експресията на ВЕРФ (VEGF, главен туморен ангиогенетичен фактор) свързан протеин се понижава от EB1089, и по-слабо се повлиява от калцемичен аналог на 1α,25$(OH)_2D_3$, функциониращ като мощен анти-ангиогенетичен фактор. Наскоро бяха описани ефектите на 1,25$(OH)_2D_3$ върху експресията на няколко ЕМТ-ТФи. 1,25$(OH)_2D_3$ инхибира SNAIL1 и ZEB1 експресията в немалък клетъчен белодробен карцином, съпроводено с увеличена Е-кадхеринова експресия, Свръхекспресията на ВДР понижава експресията на стволовоклетъчните маркери, включително c-Met и CD44, в PDAC клетки.

Ключови думи: Витамин D_3, меланом, кожен рак

of NAD+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) gene and down-regulated cyclooxygenase-2 (COX-2) expression. The cyclin-dependent kinase inhibitor $p21^{waf1/cip1}$ was first suggested as a VDR target gene, up-regulated with $p27^{kip1.}$ The expression of VEGF (a major tumor angiogenesis factor) related protein has been shown to be down-regulated by EB1089, a less calcemic analog of 1α,25$(OH)_2D_3$, function as a potent anti-angiogenesis factor. Recently, effects of 1,25$(OH)_2D_3$ on the expression of several EMT-TFs have been described. Overexpression of VDR caused the downregulation of stem cell markers, including c-Met and CD44, in PDAC cells.

Keywords: Vitamin D_3, melanoma, skin cancer

Introduction

There are two main forms of vitamin D in nature: vitamin D_2 (ergocalciferol), which is photochemically synthesized in plants, and vitamin D_3 (cholecalciferol), which is synthesized in the skin of animals and humans in response to sunlight. Until the 1960s, either vitamin D_2 or vitamin D_3 was believed to be responsible for all the known vitamin D activity. Subsequently, an as-yet unknown compound was purified and identified as a steroid vitamin D, 25-hydroxyvitamin D_3 (25$(OH)D_3$-calcidiol), in 1968. In 1971, it was determined that the steroid vitamin D was a precursor of a new steroid hormone, 1,25$(OH)_2D_3$ (calcitriol). 1,25$(OH)_2D_3$ results from the hydroxylation of vitamin $D3_2$ by 25-hydroxylase and 1α hydroxylase in the liver and kidney, respectively [19].

The major source of vitamin D is the exposure of epidermis to solar irradiation. In the photochemical reaction 7-dehydrocholesterol (7-DHC) is converted to vita¬min D3 under UVB light (280–320 nm) in keratinocytes of the basal layer of epidermis. After its release to the extracellular space, vitamin D3 is captured in the capillary bed by vitamin D binding protein (DBP). Vitamin D_3 produced in the skin or obtained with food is biologically inactive and requires two subsequent hydroxylations to gain its full hormonal activity. Initially, vitamin D_3 is converted to 25-hydroxyvitamin D3 (25(OH) D3) in hepatocytes, by the key vitamin D 25-hydroxylase — CYP2R1. 25(OH)D3 is the major metabolite of vitamin D, thus its serum level is widely used in clinic as the repre¬sentation of vitamin D status. The second requisite hydroxylation occurs in the kidney, due to the action of another hydroxylase — CYP27B1 (25-hydroxyvitamin D_3 1-α-hydroxylase) which results in formation of 1,25$(OH)_2D_3$, calcitriol or vitamin D_3. The level of vitamin D is tightly regulated by CYP24A1, which is 24-hydroxylase. Its activity leads to deactivation of 1,25$(OH)_2D_3$ or 25$(OH)D_3$ and their subsequent removal with urine. Finally, recently revealed alternative metabolic pathway for 7-DHC and vitamin D with major contribution of CYP450scc (CYP11A1) broadens the spectrum of naturally occurring vitamin D derivatives [23].

Vitamin D is well known as being essential for bone health. It is a group of fat-soluble prohormones, which are converted in the

body into a number of biologically active metabolites that function as true hormones, circulating in the blood and regulating the activities of various cell types - both calcemic and noncalcemic. Their known important action is the maintenance of plasma Ca2+ concentration by increasing Ca2+ absorption in the intestine, mobilizing Ca2+ from bone and lowering its renal excretion [1].

Vitamin D is produced endogenously in the skin.

It was also demonstrated that keratinocytes produce abundant quantities of 1α,25(OH)$_2$D$_3$ from 25-OH D$_3$ under the regulation of exogenous 1α,25(OH)2D$_3$. Importantly, biologically active vitamin D$_3$ production varies with the degree of the keratinocytes differentiation. What is more, since keratinocytes express VDR, they are therefore able to respond to the fully active form of vitamin D$_3$ — 1α,25(OH)$_2$D$_3$, which, together with calcium, is one of the most potent regulators of the epidermal differentiation. *In vivo* calcium forms gradient in the epidermis **(Fig. 1),** with the lowest concentration in *stratum basale* and the highest in the stratum granulosum. 1,25(OH)$_2$D$_3$ increases the expression of involucrin, transglutaminase, loricrin and filaggrin and potentiates calcium-induced differentiation of the keratinocytes at the level of both gene expression and mRNA stability. It also enhances formation of the cornified envelope. This phenomenon occurs, at least partially, due to the ability of the hormonal form of vitamin D$_3$ to increase intracellular calcium levels via induction of the calcium receptor (CaR) and phospholipase C (PLC). On the other hand, 1α,25(OH)$_2$D$_3$ inhibits proliferation of keratinocytes. During the differentiation process of epidermal cells specific genes are sequentially turned on and off, due to the collective work of 1α,25(OH)$_2$D$_3$ and calcium, to fulfil the inherent specialization of keratinocytes. For instance, keratinocytes of the basal layer express cytokeratins 5 and 14, which are replaced by cytokeratins 1, 10 and involucrin in the spinous layer. Two distinct coactivators are involved in VDR transactivation during keratinocytes differentiation: in¬teracting proteins (DRIP/mediator) and the p160 steroid receptor coactivator family (SRC/p160). Interestingly, in prolifer¬ating keratinocytes, the VDR binds selectively to the DRIP/mediator complex of coactivators. During differentiation DRIP's expression is decreasing and VDR switches partners in favour of SRC/p160 **(Fig. 1)** [23].

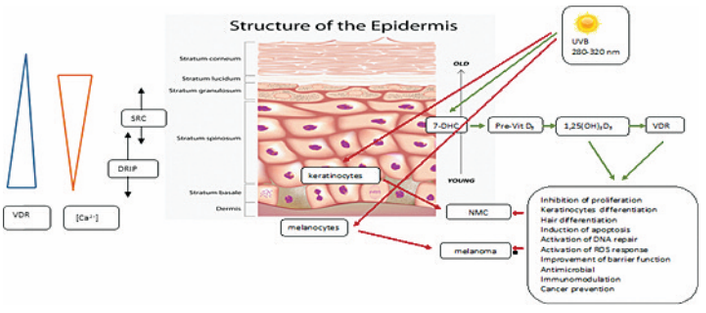

Figure 1: ***Regulation of keratinocytes functions, proliferation and differentiation by calcium and VDR with its co-activators. Vitamin D and its metabolites show an inhibitory effects on non-melanoma (NMSC) and melanoma skin cancer (modification of Piotrowska A et al., 2016 [23]).***

Genomic Actions of Vitamin D - regulation

All the genomic actions of 1α,25(OH)2D3 are mediated through its binding to VDR (1,25-dihydroxyvitamin D$_3$ receptors). Upon binding to a ligand such as 1,25(OH)$_2$D$_3$, VDR is stabilized as a result of phosphorylation at serine 51 and serine 208 [19]. Although nuclear receptors (VDR) can function as monomers, the active form are homodimers or heterodimers in complex with RXR, and recognizes specific DNA elements known as vitamin D response elements (VDREs) located in the promoter region of vitamin D responsive genes to exert its genomic actions [7]. The RXRα/VDR heterodimer was activated in a synergistic manner in the presence of both 9-cis-retinoic acid and 1α,25(OH)$_2$D$_3$ by a concerted interaction between both co-receptors and one molecule of SRC1. It is now well-established that VDR regulates at least 229 genes through binding to at least 2,776 genomic DNA binding sites. The genes include those involved in anti-proliferation, pro-differentiation, anti-inflammation, pro -apoptosis, immune regulation and many other functions in a tissue- and cell-specific manner [2,19].

Furthermore, the VDR-mediated gene expression is modulated by a multiple of coactivators and co-repressors. As 1α,25$(OH)_2D_3$ binds to VDR, phosphorylation occurs, carried out by protein kinase C (also Ser-119, and Ser-125) and casein kinase II over serine 51 located in the DNA-binding domain and serine 208 located in the hinge region, leading to subsequent conformational change of VDR, which, in turn, results in the release of co-repressors, including NCOR1 and NCoR2/SMRT and recruitment of co-activators, such as HAT and steroid receptor coactivator 1 (SRC1), to remodel chromatin and initiate gene transcription in a ligand-dependent manner [2]. Mutation of Ser-51 markedly inhibited transcriptional activation by the vitamin D hormone, suggesting that phosphorylation of Ser-51 by PKC (PKCβ in CV-1 monkey kidney cells) could play a significant role in vitamin D-dependent transcriptional activation [7].

Mutation of serine 51 to glycine (S51G) or to aspartic acid (SSlD), as well as altering the basic residues flanking serine 51 abolished the interaction of hVDR with the vitamin D-responsive element (VDRE) as monitored by gel mobility shift analysis. Thus, Hsieh JC et al. [8, 9] concluded that unmodified serine 51 and its surrounding basic residues are crucial not only for PKC-β substrate recognition but also for the optimal VDRE binding of native hVDR. The authors therefore had speculated that posttranslational modification of hVDR at serine 51 may constitute a negative regulatory loop which could be operative when target cells are subject to PKC activation events [8].

Mitogenic agents, such as basic fibroblast growth factor and phorbol esters, were found to cause significant decreases in VDR abundance, while substantially stimulating proliferation of NIH-3T3 mouse fibroblasts. Potent phorbol esters, such as phorbol myristate acetate (PMA) and phorbol-12,13-dibutyrate, whose biological actions have been shown to be mediated through the activation of protein kinase-C, down-regulated VDR in a time- and dose-dependent manner. Desensitization of protein kinase-C by prolonged exposure of cells to phorbol esters eliminated the PMA-mediated down-regulation of VDR. Staurosporine, an inhibitor of protein kinase-C, blocked the actions of PMA in keratinocytes inhibit PKCα and PKCη). Oleoyl acetyl glycerol, a synthetic diacyl glycerol, and A23187, a calcium ionophore, were both able to suppress VDR abundance alone and were additive in combination. The results suggest that activation of the protein kinase-C pathway and elevation of intracellular Ca^{2+} lead to significant down-regulation of VDR. The inhibitory effect of PMA appears to be exerted at the level of VDR mRNA expression [17]. The Ca^{2+}-dependent PKC izoforms in human species are classical PKCs, activated also by PMA and inhibite by Staurosporine, and these PKC izoforms could be responsible for VDR down-regulation (author's remark). The only cPKC expressed in human keratinocytes is PKC-α, therefore we have decided to search data concerning cross-talk between PKCα (and other PKCs) and VDR.

Protein kinase C α (PKCα) activators such as phorbol-12-myristate-13-acetate (PMA) were reported to up-regulate VDR expression in rat osteosarcoma cells. However, these results are in contrast to the earlier reports showing a decrease in the expression of VDR in rat osteosarcoma cells by PMA. The PKCα activator, phorbol-12-myristate-13-acetate (PMA) also induces the expression of VDR in the rat liver, and the induction of VDR by 1,25$(OH)_2D_3$ and CDCA (Chenodeoxycholic acid) was inhibited by the PKCα inhibitor, bisindolyl maleimide I [15]. The data suggests that VDR might be regulated at the level of mRNA and protein in rat, mouse and man by glucocorticoids, PTH (parathyroid hormone), through the activated protein kinase A (PKA) pathway in mouse osteoblast and osteosarcoma cell lines, PKCα and 1,25$(OH)_2D_3$ [15], 17β-estradiol (E2) in tissues such as the uterus, liver, and human breast cancer cells, through ERK1/2/ AP-1pathway in the osteoblastlike cell line ROS 17/2·8 and in rat colonocytes, duodenocytes [6], but the regulation seems to be species and tissue specific. The regulation of VDR expression is still incompletely understood [15].

Khan AA *et al.* studied the regulation of VDR mRNA expression by ligands for VDR, farnesoid X receptor (FXR), glucocorticoid receptor (GR) and protein kinase C α (PKCα) in rat and human ileum and liver using preci-

sioncut slices. 1,25(OH)2D3 induced VDR expression in rat ileum and liver, and human ileum but not in liver. Chenodeoxycholic acid (CDCA), but not lithocholic acid (LCA) and GW4064 induced VDR mRNA expression in rat ileum and liver. Their results show that the expression of VDR is likely to be regulated by PKC but not by FXR or VDR activation at least in the rat liver. Thus, CDCA might potentiate the toxicity of LCA by inhibiting its metabolism [15].

UV light

It is well established that sunlight (mainly ultraviolet type B (UVB)) is required for the efficient production of vitamin D. Paradoxically, the same solar radiation is considered as one of the most harmful factors for the skin. The UVB (280–320 nm) causes direct DNA and cell damage, thus contributes to the development of skin neoplasia. On the other hand, the UVA (320–400 nm) is mainly responsible for the skin aging. Thus, for the last decades physicians and scientists are warning against the potential danger concerning sunbathing. As a result, people all over the world avoid the sun, but meanwhile we are facing the global vitamin D deficiency, with noticeable outburst of rickets. Surprisingly, the number of new cases of melanoma and none melanoma skin cancer have constantly increased over the last years [23].

Recent studies also suggested that occupational exposure to the solar radiation is actually a protecting factor, but one also has to take under consideration, that the history of sunburns dramatically increases the probability of melanoma [23].

Interestingly, it is not possible to overdose vitamin D by sunbathing, because the excessive exposition to UV light leads to structural rearrangements of vitamin D and its subsequent photodegradation. The main products are 5,6-transvitamin D3, and suprasterols I and II. Moreover, irradiation of 7-DHC and its analogues, called 5,7-dienes, may result in formation of 5,7,9(11)-trienes, which were described as a photosensitizing agents, thought to be responsible for generation of reactive oxygen species. It is still unknown, whether vitamin D has any anti-oxidative or pro-oxidative properties, because several groups presented contradictory results and concepts [23].

For instance, recent studies showed protective effects of 1α,25$(OH)_2D_3$ and its analogues against UVB-induced DNA damage. The best studied effects of 1α,25$(OH)_2D_3$ require VDR activation and lead to alteration of gene expression, including several genes involved in reactive oxygen response and DNA repair. Vitamin D is also considered as a stimulator of melanogenesis, thus contributing to the skin protection against UV irradiation. Secondly, genomic and non-genomic effects has to be distinguished. Finally, the effect of vitamin D might be strongly dependent on cell type and modulated by internal and external factors, pathological conditions or genetic background [23].

The association between decreased sun exposure/vitamin D deficiency and the risk of chronic diseases, including many types of cancer, indicates that maintaining adequate vitamin D nutrition should be a paramount priority for men and women of all ages. Interestingly, it is not possible to overdose vitamin D by sunbathing, because the excessive exposition to UV light leads to structural rearrangements of vitamin D and its subsequent photodegradation [23].

VDR-Dependent Anti-cancer Actions

Several approaches have been used to demonstrate that VDR is required for the antiproliferative effect of 1α,25(OH)2D3 in cancer cells. Using JCA-1 prostate carcinoma cells stably transfected with cDNA that encodes VDR to increase its concentration, Miller and colleagues were able to demonstrate that the degrees of 1α,25$(OH)_2D_3$-induced antiproliferative action and CYP24A1 upregulation were proportional to VDR concentrations within the transfected cells. Conversely, stable transfection of antisense VDR cDNA to ALVA-31 prostate cancer cells to knockdown VDR attenuated the ability of 1α,25$(OH)_2D_3$ to inhibit cell growth and induce CYP24A1 expression. Using a different approach, Zinser *et al.* studied cancer cells derived from VDR knock-out animals and showed that the cells were completely resistant to 1α,25$(OH)_2D_3$-mediated growth arrest and apoptosis over the range of 0.01-100 nM

1α,25(OH)$_2$D$_3$. Overall, these studies demonstrate that the 1α,25(OH)2D3-dependent induction of cell cycle arrest, CYP24A1 up-regulation, and apoptosis in cancer cells are dependent on VDR [2].

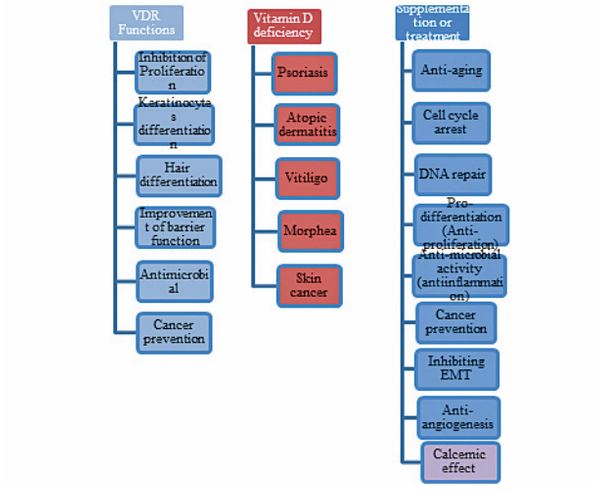

Figure 2: ***Effect of vitamin D deficiency on development of skin diseases and potential advantages of proper supplementation (modification of Piotrowska A et al, 2016 [23]).***

Cell Cycle Arrest

The cyclin-dependent kinase inhibitor p21waf1/cip1 was first suggested as a VDR target gene in the human promyelocytic HL-60 leukemia cells by Jiang *et al*. Later, multiple VDREs were identified within the p21$^{waf1/cip1}$ promoter at -770 in relation to the transcriptional start site in the human myelomonocytic cell line U937 and in MCF7 human breast cancer cells. Induction of p21waf1/cip1 mRNA occurred within 2 h of 1α,25(OH)$_2$D$_3$ addition and is a direct effect of liganded VDR. The expression of other CDK inhibitors, such as p27^{kip1} and the Ink4 family member p15, p16, and p18, were also found to be induced by the ligand. In pancreatic cancer cells, 1α,25(OH)$_2$D$_3$ inhibited their proliferation through cell cycle arrest at the G0/G1 phase, which in turn is mediated through the up-regulation of p21$^{waf1/cip1}$ and p27^{kip1}, followed by the down-regulation of cyclins, CDKs and CDKI. Interestingly, data from studies using LNCaP prostate cancer cells and HepG2 liver cancer cells indicate that the up-regulation of p27kip1 proteins induced by 1α,25(OH)$_2$D$_3$ or its analogs may not involve new p27^{kip1} mRNA synthesis [2,19].

Briefly, 1α,25(OH)$_2$D$_3$ has been shown to regulate the cell cycle in eukaryotic cells at 3 different check points: 1) inducing the expression of CDKIs, including p21, p27, p18 and p19, which in turn block CDK4 activation and arrest cells in G1 phase; (2) inhibiting CDK2 activity and leading to arrest in the S phase, and 3) down-regulating CDK1 leading to the arrest in the G2 phase [2].

DNA Repair and the Prevention of Tumor Initiation and Progression

Ting *et al.* utilized a well-established in vitro model of chemical carcinogenesis. In this model, they found that 1α,25(OH)$_2$D$_3$ promoted the expression of the DNA repair genes RAD50 and ataxia telangiectasia mutated (ATM), both of them are known to be critical for mediating the signaling responses to DNA damage. They also found that 1α,25(OH)$_2$D$_3$ protected cells from genotoxic stress and growth inhibition by promoting doublestrand break DNA repair. They noted that depletion of VDR reduced the observed genoprotective effects and caused malignant transformation that could not be prevented by 1α,25(OH)$_2$D$_3$ in a xenograft mouse model, indicating an essential role for VDR in mediating the anticancer effects of 1α,25(OH)$_2$D$_3$. Since genotoxic stress can activate ATM and VDR through phosphorylation of VDR, mutations in VDR at putative ATM phosphorylation sites impaired the ability of ATM to enhance VDR transactivation activity, leading to decreased induction of ATM and RAD50 expression by 1α,25(OH)$_2$D$_3$. Taken together, the authors propose a positive feedback signaling loop between ATM and VDR to enhance the up-regulation of DNA repair proteins and thereby prevent tumor initiation and progression resulting from DNA damages [2].

Apoptosis

1α,25(OH)$_2$D$_3$ induces apoptosis in a variety of cancer cells to exert anti-tumor effects by repressing the expression of the anti-apoptotic proteins Bcl-2 and Bcl-XL, or inducing the expression of pro-apoptotic proteins, such as BAX, BAK and BAD [2].

Zinser *et al.* showed that cancer cells derived from VDR knock-out animals were completely resistant to 1,25D-mediated

growth arrest and apoptosis. Danielsson *et al.* found that VDR ligands could induce apoptosis only in certain melanoma cell lines, suggesting that the effects of VDR ligands on the inhibition of the cell cycle and on the induction of apoptosis are mediated by different genes [19].

Anti-inflammation

Animal studies have linked the anti-cancer effects of 1α,25$(OH)_2D_3$ to its ability to regulate inflammation. In colon cancer cells, 1α,25$(OH)_2D_3$ can interrupt the Wnt-mediated crosstalk between tumor epithelial cells and macrophages in the tumor microenvironment by blocking the production of IL-1β, an inflammatory cytokine produced by tumor-associated macrophages. 1α,25$(OH)_2D_3$ up-regulated the expression of NAD+-dependent 15-hydroxy-prostaglandin dehydrogenase (15-PGDH) gene and down-regulated cyclooxygenase-2 (COX-2; PGHS-2 - Prostaglandin G/H Synthase) expression. Since prostaglandins are known to play a role in the development and progression of many cancers] the ability of 1α,25$(OH)_2D_3$ to decrease prostaglandin concentration strongly suggests that one mechanism of anti-cancer effect of vitamin D may be mediated through its anti-inflammatory action [2].

Moreover, VDR ligands inhibit expression of pro-inflammatory cytokines produced by T lymphocytes, such as IL-2, IFN-γ, IL-6 and IL-8, which are responsible for the exacerbation of the skin inflammation. Apart from that, 1α,25$(OH)_2D_3$ enhances expression of anti-inflammatory cytokine, IL- 10, within the psoriatic lesions, as well as the expression of its receptor in keratinocytes [23].

Transforming Growth Factor-beta (TGF-β) Pathway

Wu *et al.* reported two direct repeats of VDRE in the human TGF-β2 gene promoter, which can bind to VDR/RXR heterodimers. Furthermore, they demonstrated that 1α,25$(OH)_2D_3$ up-regulated the expression of TGF-β type II receptor mRNA and protein in human breast cancer MCF-7 cells. The later finding is in agreement with a previous report showing that 1α,25$(OH)_2D_3$ up-regulated the expression of TGF-β1 and its latent binding protein in cultured breast carcinoma BT-20 cells. TGF-β1-dependent pathway through up-regulation of Smad4 and phosphorylated-Smad3 levels was also shown to mediate the synergistic growth inhibition of MCF-7 breast cancer cells in the presence of melatonin and 1α,25$(OH)_2D_3$ and the up-regulation of VDR expression following butyrate treatment in CaCo-2 colon cancer cells. TGF-β is a potent growth inhibitor of many epithelial cell types in vitro [2].

Anti-angiogenesis

Angiogenesis is generally applied to the formation of thin-walled endothelium-lined new blood microvessels from preexisting vessels, a process that is regulated by a range of endogeneous angiogenic factors and inhibitors. It plays an important role in reproduction, development and wound healing. It is usually focal and self-limited in time. On the contrary, pathological angiogenesis can last for years, and is necessary for tumors and their metastases to grow beyond a microscopic size. It can also cause bleeding, vascular leakage and tissue destruction. There are several hypotheses regarding the onset of tumor-induced angiogenesis. The most important one is hypoxia-induced angiogenesis. Inefficient vascular supply and the resultant reduction in tissue oxygen tension often lead to neovascularization in order to satisfy the needs of the tissue, which in turn stimulates the expression of hypoxia-inducible factors (HIFs). Tumor cells induce angiogenesis through a multistep process, called the „angiogenic switch", which ultimately tips the balance toward pro-angiogenic factors. HIFs can directly activate the expression of a number of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), VEGF receptors FLT-1 and FLK-1, plasminogen activator inhibitor-1 (PAI-1), angiopoietins, platelet-derived growth factor β, and matrix metalloproteinases MMP-2 and -9. There is considerable evidence that VEGF is a major tumor angiogenesis factor. Other than the hypoxia-induced pathological angiogenesis, it has been proposed that deficiency in MMP-19 may contribute to an earlier onset of tumoral angiogenesis, in contrast to most MMPs that promote tumor progression [2].

The evidence that vitamin D might exert

anti-angiogenic action was first provided by Merke *et al.,* who demonstrated VDR expression in venular and capillary endothelial cells of human skin biopsies. Subsequently, numerous studies showed 1α,25$(OH)_2D_3$ inhibited the proliferation of cultured endothelial cells and anti-angiogenesis in animal models. Furthermore, the expression of VEGF related protein has been shown to be down-regulated by EB1089, a less calcemic analog of 1α,25$(OH)_2D_3$, and 1α,25$(OH)_2D_3$ inhibited HIF-1 transcriptional activity as well as its target genes, including VEGF, ET-1, and Glut-1 in wild type human cancer cells, but failed to suppress VEGF expression in HIF-1α knockout human colon cancer cells. Taken together, the anti-angiogenesis effect of 1α,25$(OH)_2D_3$ in cancer cells is likely mediated by HIF-1/VEGF pathway. 1α,25$(OH)_2D_3$ can also up-regulate mRNA levels of the potent anti angiogenic factor thrombospondin 1 in human colon tumor cells [2].

Bernardi *et al.* have found calcitriol to inhibit the growth of tumor-derived endothelial cells (TDECs) in two tumor models at nanomolar concentrations. This may help to restore the homogeneousness of ECs in vessel wall thereby bringing the vessel architecture and function more towards normalcy and hence prevention of tumor angiogenesis and facilitation of chemotherapy- as well as hypoxia-induced tumor-cell apoptosis. They also found calcitriol to increase the number of VDRs and the level of apoptogenic protein p27 (Kip 1) in TDECs, thereby making them more vulnerable to its antiangiogenic and apoptotic action. The same authors have also investigated the action of calcitriol on VEGF-induced TDEC proliferation and found the vitamin to reduce phospho-ERK 1/ 2 and phospho-Akt levels in them. Such observation shows that calcitriol interferes with both the proliferation - transduction pathways induced by RTKs, thereby inhibiting angiogenesis [1].

Pro-differentiation role

It was shown that 1α,25$(OH)_2D_3$ was able to inhibit proliferation and increase the expression of a variety of differentiation markers, including involucrin, transglutaminase, loricrin and filaggrin, and enhance cornified envelope formation in cultured keratinocytes. By maintaining the ordered cellular proliferation and differentiated epithelium, 1α,25$(OH)_2D_3$ is able to contribute to skin cancer prevention [13].

1α,25$(OH)_2D_3$ and its analogs are potent mediators of keratinocyte differentiation in vitro. The precise mechanism of this action is still unknown. The nuclear transcription factor activator protein 1 (AP1) seems to play an important role in keratinocyte differentiation. In a time-course of a study of human keratinocytes incubated with 1α,25$(OH)_2D_3$ (10–7-10–11 M) a significant dose-dependent increase in activator protein 1 DNA binding activity as determined by electrophoretic mobility shift assay was seen after 36 h. This increase was followed by a significant dose-dependent decrease in activator protein 1 DNA binding activity after 72 h. The AP-1 binding sites of different promoters are not identical and may bind different AP-1 homo- and heterodimers, which in turn could account for their different regulation [5]. Johansen C. *et al.* results demonstrate that 1α,25$(OH)_2D_3$- and calcium-induced keratinocyte differentiation are accompanied by changes in activator protein 1 DNA binding activity. Protein kinase C activation appears to be essential for the calcium-dependent induction of keratinocyte differentiation, whereas a protein-kinase-C-independent activation of activator protein 1 DNA binding and keratinocyte differentiation is responsible for the 1α,25$(OH)_2D_3$-induced effects [13].

1,25D_3 potentiates differentiation through many of the same pathways as calcium, such as by increasing [Ca^{2+}]i (intracellular) levels and activating PKC and PLC signaling. Calcium and 1,25D3 synergistically activate transcription of the involucrin promoter that contains an AP-1 binding site necessary for calcium and 1,25D_3 induction and an adjacent vitamin D_3 receptor (VDR)-responsive element (VDRE). Conversely, dietary Ca^{2+} supplementation has been shown to up-regulate VDR expression in the epidermis of the vitamin D-deficient rat. VDR null and 25-hydroxyvitamin D_3 1-α-hydroxylase (CYP27B1)-deficient mice share similar epidermal phenotypes as EpidCaSR−/− mice: abnormal epidermal Ca^{2+} gradient, decreased LB (lamina basalis) numbers and secretion, delayed recovery of per-

meability barrier function, and defective differentiation, indicative of important roles for $1,25D_3$/VDR as well as calcium/CaSR in keratinocyte differentiation and permeability barrier homeostasis [28].

Typical of the type II nuclear receptors, $1,25(OH)_2D_3$-induced VDR activation inhibited proliferation and promoted differentiation of keratinocytes, though VDR/RXRα heterodimers can transactivate keratinocytic genes independent of $1,25(OH)_2D_3$ binding. Additionally, ΔNp63α, a critical regulator of epidermal biology, is known to directly control VDR expression in murine skin and mediate its function in a ligand-independent manner [10]. The loss of VDR observed upon the silencing of p63 led to the enhanced invasion of A431 cells (human squamous carcinoma), suggesting that the p63-mediated regulation of VDR has a role in inhibiting the migration and invasion of these cells [19].

HNSCCs typically express squamous epithelial marker cytokeratins 5/6 and p63, a basal cell/stem cell-like marker, is also often diffusely positive.The work-up of high-grade basaloid tumors includes cytokeratin positivity (negative in lymphomas and melanomas), p63 (positivity in SCC, negative in solid adenoid cystic carcinomas), and neuroendocrine markers (synaptophysin, chromogranin), which would be negative in SCC and positive in neuroendocrine carcinomas, small cell carcinomas, and merkel cell carcinomas [29]. According Ryvkin V *et al.* nuclear p63 protein was mainly restricted to basal layer KCs [25]. Moreover, positive and negative regulation of EGFR expression by p63 isoforms has been reported. Although further investigations are still needed, these studies suggest that isoform-specific regulation of EGFR expression by p63 is involved in the stem cell maintenance and tissue homeostasis in the epidermis [22].

The seminal discoveries in 1981 that $1,25(OH)_2D_3$ induced myeloid leukemia cell differentiation and inhibited melanoma cell proliferation prompted the interest in $1,25(OH)_2D_3$ as an anticancer agent. Subsequent observations have shown that $1,25(OH)_2D_3$ induces differentiation and apoptosis and inhibits proliferation, migration, invasion, and angiogenesis in cancer cells of different origin and in several animal models of cancer. However, the administration of $1,25(OH)_2D_3$ to cancer patients is restricted by its hypercalcemic effects at the therapeutic doses, enforcing the development of several analogs that maintain the antitumoral properties but have less calcemic actions. Currently, numerous clinical trials are ongoing using $1,25(OH)_2D_3$ or its analogs, alone or in combination with other anticancer agents, against several neoplasms (https://www.clinicaltrials.gov/) [18].

VDR and Epithelial to Mesenchymal Transition (EMT)

Typical EMT gene reprogramming is mainly orchestrated by key transcription factors including the zinc finger proteins SNAIL1 (Snail) and SNAIL2 (Slug), the double zinc finger and homeodomain factors ZEB1 and ZEB2, and the members of the basic-helix-loop-helix family TWIST1 and E47, all known as EMT transcription factors (EMT-TFs). They are activated by TGF-β, Wnt, Notch, NF-κB, ERK/MAPK pathways, and activation of PDGF, EGFR [31,20,21]. EMT-TFs are repressors of E-cadherin (encoded by CDH1 gene), that is the main component of adherens junctions and essential for th emaintenance of the epithelial state. Thus, E cadherin downregulation is considered a hallmark of EMT. In addition to the established EMT inducers, other transcription factors such as FOXC2, Goosecoid, KLF8, TCF4 (also known as E2-2), SIX1, HMGA2, Brachyury, and PRRX1 have been recently shown to induce or regulate EMT. Expression and/or activity of the transcription factors that drive EMT is induced and controlled by several signaling pathways that respond to extracellular cues, with a prominent role for transforming growth factor- β (TGF-β) signaling. The contribution of each transcription factor to the EMT depends on the cell or tissue type involved and the signaling pathway that initiates the EMT. Moreover, EMT-TFs often exhibit reciprocal control of their expressions and functional cooperation. Expression and/or activity of the transcription factors that drive EMT is induced and controlled by several signaling pathways that respond to extracellular cues, with a prominent role for transforming growth factor-β

(TGF-β) signaling. The contribution of each transcription factor to the EMT depends on the cell or tissue type involved and the signaling pathway that initiates the EMT. Moreover, EMT-TFs often exhibit reciprocal control of their expressions and functional cooperation [18].

In addition to colon cancer, E-cadherin is induced by $1,25(OH)_2D_3$ or analogs in normal mammary and bronchial epithelial cells and in tumor cell lines derived from breast, prostate, non-small cell lung, and squamous cell carcinomas, usually associated with an increase in epithelial differentiation, a reduction in cell migration and invasion, and the inhibition of Wnt/β-catenin signaling. The mechanism of E-cadherin induction by $1,25(OH)_2D_3$ in human colon cancer cells is transcriptional indirect and requires the transient activation of the RhoA-ROCK-p38MAPK-MSK1 signaling pathway [18].

$1,25(OH)_2D_3$ increases by a transcriptional indirec tmechanism the expression of Jumonji Domain Containing 3 (JMJD3), a histone H3 lysine 27 demethylase with putative tumor suppressor activity. JMJD3 mediates the induction of a highly adhesive epithelial phenotype, the antiproliferative effect, the gene regulatory action, and the antagonism of the Wnt/β-catenin pathway promoted by $1,25(OH)_2D_3$ in human colon cancer cells. Moreover, JMJD3 depletion upregulates SNAIL1, ZEB1, and ZEB2, increases the expression of the mesenchymal markers fibronectin and LEF1, and downregulates the epithelial proteins E-cadherin, claudin-1, and claudin-7. Accordingly, JMJD3 and SNAIL1 RNA expression correlate inversely in samples from human colon cancer patients. The induction of ZEB1 by JMJD3 depletion is associated with the downregulation of miR-200b and miR-200c, two microRNAs that target ZEB1 RNA and inhibit ZEB1 protein expression [18].

$1,25(OH)_2D_3$ directly induces the expression of cystatin D, an inhibitor of cysteine proteases of the cathepsin family encoded by CST5 gene. The authors found that cystatin D mediates the antiproliferative and prodifferentiation action of $1,25(OH)_2D_3$ in human colon cancer cells. In addition, ectopic cystatin D expression inhibits proliferation, migration, anchorage-independent growth, and theWnt/β-catenin pathway in cultured colon cancer cells and reduces tumor development in xenografted mice . Cystatin D represses SNAIL1, SNAIL2, ZEB1, and ZEB2, whereas it induces the expression of E-cadherin and other adhesion proteins such as occludin and p120-catenin. Accordingly, cystatin D and E-cadherin protein expression directly correlate in human colorectal cancer, and loss of cystatin D is associated with poor tumor differentiation [18]. Additionally, CST5 is a direct p53 target gene, upregulating CST5 on mRNA and protein levels. Treatment with calcitriol, the active vitamin D_3 metabolite, and simultaneous activation of p53 resulted in enhanced CST5 induction and increased repression of SNAIL, an epithelial-mesenchymal transition (EMT) inducing transcription factor. Furthermore, CST5 inactivation decreased p53-induced mesenchymal-epithelial transition (MET) as evidenced by decreased inhibition of SNAIL and of migration by p53. Furthermore, CST5 expression was directly repressed by SNAIL [9].

Recently, effects of $1,25(OH)_2D_3$ on the expression of several EMT-TFs have been described. $1,25(OH)_2D_3$ inhibits SNAIL1 and ZEB1 expression in non-small cell lung carcinoma cells, accompanied by an increase in E-cadherin expression, vimentin downregulation, maintenance of the epithelial morphology, and inhibition of cell migration. The low calcemic $1,25(OH)_2D_3$ analog MART-10 inhibits EMT and cell migration and invasion in breast and pancreatic cancer cells through the downregulation of SNAIL1 and SNAIL2 (Slug). In addition, MART-10 inhibits TWIST1 expression in breast cancer cells. Accordingly, Findlay *et al.* reported the inhibition of SNAIL1 and SNAIL2 by $1,25(OH)_2D_3$ in human colon cancer cells. Kaler et al. found that colon cancer cells stimulate tumor-associated macrophages to secrete interleukin- 1β (IL-1β), which in turn promotesWnt/β-catenin signaling, stabilizes SNAIL1 protein, and confers resistance to TRAIL-induced apoptosis in colon cancer cells. They also found that $1,25(OH)_2D_3$, by inhibiting the release of IL-1β by macrophages, downregulates SNAIL1 protein expression in colon cancer cells. Similarly, Zhang et al. showed that tumor-associated macrophages induce EMT in breast cancer

cells and that high VDR expression in cancer cells abrogates the macrophage-promoted E-cadherin loss, α-SMA upregulation, and increase in cell migration and invasion. 1,25$(OH)_2D_3$ attenuates the enhancing effect of TGF-β1 on cell motility and on SNAIL1, N-cadherin, and vimentin expression in human bronchial epithelial cells and inhibits theTGF-β1-stimulated EMT in rat lung epithelial cells [18].

Consistently with its inhibitory effect on EMT, 1,25$(OH)_2D_3$ downregulates the secretion of MMP2, MMP9, and MMP13 in prostate, breast, pancreatic, and squamous cell carcinoma cells and increases TIMP1 and TIMP2 activity in prostate and breast cancer cells. In addition, 1,25$(OH)_2D_3$ reduces the increase in MMP2 and MMP9 induced by TGF-β1 in human bronchial epithelial cells. Through these mechanisms, 1,25$(OH)_2D_3$ inhibits the capacity of cancer cells to degrade the extracellular matrix and invade the surrounding tissue and may thus reduce tumor cell metastatic potential. Remarkably, several studies from Pŕerez-Fernŕandez's group have demonstrated that 1,25$(OH)_2D_3$ represses the expression of the gene encoding the pituitary transcription factor 1 (PIT1) in breast cancer cells and that PIT1 silencing downregulates SNAIL1, MMP1, and MMP13 proteins. In agreement with this, high PIT1 protein expression correlates with elevated MMP1 and MMP13 levels, SNAIL1 protein expression, and presence of distant metastasis in invasive ductal breast carcinoma [18].

However, certain cancer cell lines do not express VDR and are unresponsive to 1,25$(OH)_2D_3$. Accordingly, VDR downregulation has been observed in a proportion of melanomas and colon, breast, lung, and ovarian tumors, which may jeopardize the response to therapy with vitaminD, 1,25$(OH)_2D_3$, or its analogs. These lines of evidence prompted the authors to study the mechanisms responsible for VDR downregulation in cancer. They found that SNAIL1 represses the expression of VDR by binding to three E-boxes in the human VDR gene promoter [18].

In addition to SNAIL1, it was reported by the authors that its family member SNAIL2 (Slug) represses VDR gene expression through the same E-boxes in the human VDR gene promoter and blocks the induction of an epithelial phenotype by 1,25$(OH)_2D_3$ in human colon cancer cells, they are also upregulated and downregulate VDR in in human osteosarcoma and breast cancer cells. Moreover, SNAIL1 and SNAIL2 show an additive repressive effect on VDR gene promoter. Remarkably, SNAIL1 and/or SNAIL2 RNA upregulation was detected in 76% of colon tumors and significantly correlated with diminished VDR RNA expression. Larriba MJ et al. also showed that SNAIL1 RNA overexpression in colon tumors diminishes VDR RNA expression in the histologically normal tissue adjacent to the tumor, suggesting that SNAIL1- expressing colon cancer cells secrete signals that modulate VDR expression in neighboring cells [18].

Studies in breast and colon cancer cells found that VDR, β-catenin, and Snail are interrelated. When VDR is activated, it will compete with β-catenin to combine with transcription factor 4, thus inhibiting the activity of β-catenin in colon cancer. VDR, which activates CDH1 expression upon ligand binding, is repressed by Snail but induced by ZEB1. As ligand-activated VDR induces epithelial differentiation and the expression of CDH1/E-cadherin and other intercellular adhesion genes, VDR repression by Snail1 and Snail2 (Slug) guarantees the induction of EMT, even in the presence of 1,25$(OH)_2D_3$. This effect seems to be specific to the Snail family of transcription factors, since other EMT inducers such as ZEB1, ZEB2, E47, and Twist1 do not inhibit the human VDR gene promoter [19].

Over-expression of FoxM1 caused the acquisition of the EMT phenotype via upregulation of mesenchymal cell markers, including ZEB1, ZEB2, Snail2 (Slug), and vimentin, in PDAC (pancreatic ductal adenocarcinoma) cells in a study. Meanwhile, KLF4 (Kruppel-like transcription factors 4) was studied as a negative regulatory factor of EMT, and increased expression of KLF4 led to the downregulation of Slug and Snail, while knockdown of KLF4 did the opposite. Of note, the increased KLF4 expression significantly upregulated VDR expression and sensitized the cells to the inhibitory effects of 1,25$(OH)_2D_3$ The mouse and human gastric tumors had

reduced expression of KLF4 and increased expression of FoxM1 compared with healthy gastric tissue, and expression of KLF4 suppressed the transcription of FoxM1 [19].

To support the link between EMT and CSCs (cancer stem cells) in PDAC, Shah et al. found that EMT-type cells have increased expression of the stem cell markers CD24, CD44, and epithelial specific antigen. Tsang and Lo revealed that PDAC cells with the EMT phenotype have increased sphere-forming capacity and high expression of CSC surface markers such as CD44 and epithelial cell adhesion molecule. Their studies also indicated that the overexpression of VDR by either gene transfection or lentiviral gene transfer caused the downregulation of stem cell markers, including c-Met and CD44, and suppressed the spheroid formation of PDAC cells [19].

Table 1*: List of 1,25(OH)2D3 -regulated proteins involved in EMT (from [25] (at the end of the article).*

Protein	$1,25(OH)_2D_3$ effect	Reference
Tight junction components		
Occludin	Upregulation	[26–28]
Claudin-1	Upregulation	[27, 29]
Claudin-2	Upregulation	[29, 30]
Claudin-7	Upregulation	[31]
Claudin-12	Upregulation	[30]
ZO-1	Upregulation	[26, 27, 29]
ZO-2	Upregulation	[26]
Adherens junction proteins		
E-cadherin	Upregulation	[26, 29, 31–46]
N-cadherin	Downregulation	[37, 41, 42, 47, 48]
P-cadherin	Downregulation	[37]
Vinculin	Upregulation	[26, 31]
Focal adhesion members		
Integrin α_3	Upregulation	[31]
Integrin α_V	Upregulation	[37]
Integrin β_5	Upregulation	[37]
Integrin α_6	Downregulation	[37]
Integrin β_4	Downregulation	[37]
Paxillin	Upregulation	[31, 37]
FAK	Upregulation	[37]
Cytoskeleton-related proteins		
Filamin A	Upregulation	[49]
Ezrin	Upregulation	[50]
α-SMA	Downregulation	[37, 43–46, 51–54]
Keratin-13	Upregulation	[49]
Vimentin	Downregulation	[40–42]
Plectin	Upregulation	[49]
Extracellular matrix proteins		
Fibronectin	Downregulation	[44, 45, 51, 54]
Collagen type I	Downregulation	[44, 45, 51, 53–57]
Collagen type II	Downregulation	[56]
Collagen type III	Downregulation	[44, 51, 54, 58]
MMPs and inhibitors		
MMP2	Downregulation	[39, 41, 42]
MMP9	Downregulation	[39, 41, 42, 59, 60]
MMP13	Downregulation	[48, 61]
TIMP1	Upregulation	[59, 60]
TIMP2	Upregulation	[59]
EMT-TFs		
SNAIL1	Downregulation	[40–42, 44, 61, 62]
SNAIL2	Downregulation	[42, 61, 62]
ZEB1	Downregulation	[40]
TWIST1	Downregulation	[61]
Wnt/β-catenin target genes		
MYC	Downregulation	[26, 32, 63, 64]
TCF1	Downregulation	[26, 32]
CD44	Downregulation	[26, 32]
Cyclin D1	Downregulation	[31, 33, 55, 64]
AXIN2	Downregulation	[65]
LEF1	Downregulation	[65]
Other EMT-related proteins		
JMJD3	Upregulation	[66]
Cystatin D	Upregulation	[67]
Cathepsin L	Downregulation	[68]
Sprouty-2	Downregulation	[69]
PIT1	Downregulation	[70]
IL-1β	Downregulation	[71]
TGF-β	Downregulation	[44, 54, 56, 58]
TGF-β receptor type I	Downregulation	[44]
Cadherin-17	Downregulation	[72]

The loss of VDR observed upon the silencing of p63 led to the enhanced invasion of A431 cells (human squamous carcinoma), suggesting that the p63-mediated regulation of VDR has a role in inhibiting the migration and invasion of A431 cells. Also, the upregulation of the zinc-finger transcription factor Snail has been shown to be linked to the acquisition of the migratory/invasive phenotype, which promotes invasion and metastasis by repressing multiple proteins, including E-cadherin and ΔNp63α. Interestingly, VDR is known to be repressed by Snail, and a negative correlation between Snail and VDR has been reported in a colon cancer cell line. It is thus likely that Snail represses the ΔNp63α - VDR-E-cadherin axis to promote the invasiveness of cancer cells. VDR has also been shown to inhibit the invasiveness of prostate cancer cells through binding to vitamin D_3. Tokar et al. found that vitamin D_3 exerted its anti-invasive effects by upregulating VDR and decreasing matrix metallopeptidase 9 (MMP9) and matrix metallopeptidase 2 (MMP2) activity [19], degrades type IV colagen in the Basal membranes [4,14,16] and major proteinases associated with increase invasive activity [26,32,27] (increased in both SCCs and BCCs [12]). It has been demonstrated that invasive melanoma cell lines show higher MMP-9 expression and higher activity when compared to non-invasive cell lines. In primary melanomas, MMP-9 is variably expressed in radial but not in the vertical growth phase and the de novo expression seems associated with early invasion. MMP-2 was evident by immunohistochemistry in malignant melanoma lesions, but not in benign and atypical nevi. Corte et al. found an association between MMP-13 expression with mitotic index ($p = 0.002$) in CMM [3].

p63-null mice show a complete lack of all stratified squamous epithelia and their derivatives, including epidermis, mammary glands, prostate, and other tissues. In human epidermis, DNp63a is specially expressed in the stem cell compartment. Importantly, p63 is essential for significant proliferative potential of the human keratinocyte stem cells [22].

In mature epithelium, expression of p63 (p53 family member) is highest in basal epithelial cells, where it functions as an inhibitor of NOTCH1 expression, and becomes downregulated during terminal differentiation coincident with NOTCH1 upregulation. Reactivation of p63 expression is observed in the suprabasal layers of dysplastic mucosa, and overexpression and/or genomic amplification of the TP63 locus is observed in the majority of invasive HNSCCs [24]. PKC-δ played as a protective role in SCC partly by down-regulating p63, leading to the suppression of SCC cell proliferation, attenuation of the activity and expression of CSCs (cancer stem cells) in SCC cells [33].

Notch signaling, which is also mutated in 75% of cutaneous SCCs [30], has been linked to multiple biological functions, including regulation of self-renewal capacity, cell cycle exit (in part through upregulation of p21/CDKN1A expression), and cell survival. In the stratified epithelium, Notch has a central role in promoting terminal differentiation, negatively regulated by EGFR, which is mediated through both direct effects (e.g., on activation of suprabasal keratins) and indirect effects on the Wnt, hedgehog, and interferon response pathways [24,11].

In this regard loss of VDR observed upon the silencing of p63 into A431 cells is a little bit contravertial.

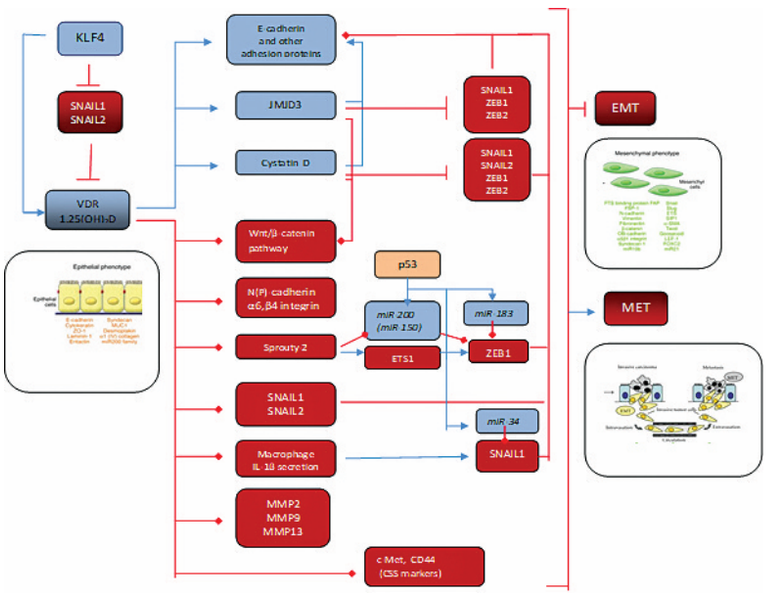

Figure 3: ***Scheme showing the mechanisms involved in the reciprocal regulation between 1,25(OH)2D3 and EMT in human colon cancer cells. Proteins and pathways displayed in blue are associatedwith an epithelial phenotype, while those shown in red are related with amesenchymal phenotype. Blue lines- induction and red lines - repression, (authors' modification from figure of Larriba MJ et al., 2016 [18]).***

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Нови стратегии за превенция на детското затлъстяване в България - Проект EPHE

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New Strategies to prevent childhood obesity in Bulgaria - EPHE project

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Bulgarian association for the study of obesity and related diseases (BASORD)

РЕЗЮМЕ:

Детското затлъстяване в България драстично се е увеличило през последните десетилетия. Според СЗО България е на пето място в детското затлъстяване сред 53-те страни-членки на Европа. Причините за тези поразителни числа са: обездвижване и небалансирано хранене. Целта на нашето изследване беше да се направи оценка на поведението на децата и учениците в училища в София.

Седем европейски държави са част от проекта EPHE (Белгия, България, Франция, Гърция, Португалия, Румъния и Холандия). В програмата взеха участие 205 деца от 6 до 9 години заедно със своите семейства и учители от три училища в София.

Децата от семейства с добри доходи имат значително по-добри данни за консумацията на плодове, плодови сокове и безалкохолни напитки в сравнение с децата от семейства с недостатъчни доходи (p

ABSTRACT

Childhood obesity in Bulgaria has dramatically increased during the last decades. According to WHO Bulgaria is on the fifth place of childhood obesity among 53 member countries in Europe. The reasons of these striking numbers are clear: a sedentary lifestyle and a poor nutrition. Thereafter, the aim of our study was to evaluate the nutritional and physical activity behaviour of school-aged children in Sofia.

Seven community-based programs are part of the EPHE project (Belgium, Bulgaria, France, Greece, Portugal, Romania and the Netherlands). A total of 205 children from 6 to 9 years together with their families and teachers from three schools in Sofia participated in the program.

Children from good income families had significantly higher fruit, fruit juices and soft drinks consumption compared to the children from not good income families (p< 0.001,

<0.001, p <0.05, p <0.05, съответно). Децата от семействата с недостатъчни доходи прекарват значително повече от общото си време пред екрана в сравнение с децата от семействата с високи доходи, съответно 25.7 часа срещу 18 часа, съответно, p <0.05.

Нашето проучване показа положителна тенденция към по-здравословно хранене, основано на висока консумация на плодове. Въпреки това, заседналият начин на живот е много популярен сред децата в училищна възраст в София, което може да увеличи риска от затлъстяване и метаболитните усложнения.

Ключови думи: детско затлъстяване, превенция, училище за здраве.

p<0.05, p<0.05, respectively). The children from not good income families spent significantly more total screen time compared to the children from high-income families, 25.7 hours vs. 18 hours, respectively, p<0.05.

Our study has demonstrated a positive trend towards a healthier nutrition based on the high fruit consumption. However, the sedentary lifestyle is very popular in school-aged children in Sofia which could increase the risk for obesity and its metabolic complications.

Key words: childhood obesity, prevention, school of health.

Introduction

WHO data form 2015 shows that Bulgaria is in the fifth place for childhood obesity in the European region (16,17). In this regard Bulgarian Association for the study of Obesity and Related Diseases (BASORD) participated in the three-year EPHE evaluation study (5,15). The aims of the project were to identify inequalities in the energy-balance related behaviours of children and to use the EPODE methodology to prevent childhood obesity. (3,4,22,23,24)

Materials and methods

Seven community-based programs are part of the EPHE project (Belgium, Bulgaria, France, Greece, Portugal, Romania and the Netherlands) (22,23,24). A total of 205 children from 6 to 9 years (46,8% - boys and 52,7% - girls, with a mean age of 7.97) together with their families and teachers from three schools in Sofia participated in the program. The EPHE parental questionnaire was distributed and filled in from each family. The main pillars of the questionnaire covered the dietary habits (fruit and vegetable consumption, soft drinks and fruit juices consumption, water intake), sedentary behaviour, and their family-environmental determinants, sleep habits of the child, and socio-demographic characteristics of the family. In addition on a national level BASORD organized three „Schools for health – for children, parents and teachers" from 2013 to 2017, in Albena on the Black Sea coast. This one-week initiative focused on lectures on a healthy and balanced nutrition and physical activities and games and covered more than 350 families (18).

Programme

In 2012 the Healthy Kids in Bulgaria program was developed, in order to combine the efforts of public, private and community partners to help prevent childhood obesity in the country.

The primary goal of the program was to make balanced nutrition and physical activities accessible and fun for Bulgarian families and primary school students by showing how fun a healthy lifestyle could be. Through the use of various interaction methods specifically created to match the characteristics and needs of each target group, the program aims to inspire and initiate healthier behaviour based on informed choices, balanced nutrition, daily physical activity and family & community initiatives. (1,2,10,11,12,19)

The coordination team developed a strategic plan for an educational program, supported by various in-school and after-school activities, in order to educate children between 7 and 13 how to make their own daily food

choices and to promote an active lifestyle among families. The aim was to raise awareness and engage school communities, professionals, celebrities, parents and teachers to be active participants in the discussion on the health risks of overweight and obesity among children. Healthy Kids in Bulgaria aimed to motivate people to consider and implement a change in their daily nutrition and physical movement choices. Interactive activities in each stage of the program were designed to change children's nutrition and physical habits and teach them to be proactive in their selection of healthy food and ways to exercise. (13,14,20,21).

SETTINGS

The Healthy Kids in Bulgaria activeties were tailored to the needs of each community. Outdoor settings included the schools' surroundings and playgrounds and public areas such as parks and city squares. Indoor activities were held in the schools' classrooms, sport halls, conference rooms and other public indoor areas. The combination of outdoor and indoor activities allowed a maximum utilisation of each venue and ensured a fun educational environment for the participants, suitable for each part of the program.

In order to assure the activities' success and influence, BASORD has conducted a two-day training of selected students, which were chosen to be Healthy Kids animators in Bulgaria. The program's animators held a number of fun and interactive lessons with games and competitions at school and outdoors. The use of diverse and interactive training and games engaged children and sparked their interest in a balanced diet and sports.

PARTICIPANTS:

After the pilot year of implementation, Healthy Kids in Bulgaria widened its range to include another region in the programme. By the end 2016, 20 schools took part in the activities and over 8500 families were directly involved in the interventions.

METHODOLOGY OF WORK (4,6,8,9)

Healthy Kids in Bulgaria was implemented in periods, in accordance with the schools' schedule. Each period was divided in stages, implemented once a year for the overall period of 5 consecutive years. The methodology was developed to ensure activity continuity and proper evaluation with result measurement. At the beginning and at the end of each period the results are measured and progress reports have been made.

- Introduction: The program is introduced to the school community, teachers and parents and their agreement for children to be involved is requested;
- Research and plan development: The children's BMI and nutritional knowledge are measured. A nutritional and educational plan is developed, in accordance with the government guidelines and the specific needs of the school;
- Communication and development: The program is communicated to the media and general public by means of public relations and social marketing activities;
- Distribution of basic information: basic nutritional information is distributed to the participants. The informational materials are developed according to each target group's specific characteristics and informational needs;
- Implementation of the program activities: Distribution of informational materials for children, professionals, teachers and parents; school branding and sports facilities renovation, organization of nutritional and cooking classes, organization of regular parents-teachers meetings and discussions, organization of physical activities opportunities and games for the children, open-air family days, etc.
- Results measurement: parents, teachers and children's knowledge and understanding of the information distributed, lessons learned, lifestyle change.

TARGET GROUPS

The predominant primary target group was primary school children of ages 7 to 13, along with their parents, and the teachers. Most of the targeted children and their parents come from middle class families with average income. They live in a respective area (region)

in capital that contains both low and high socio economic backgrounds. This target group focuses on children who study at public primary schools, including kids with disabilities in the same school classes. The parents come from various educational backgrounds and have high influence in the family decisions and lifestyle choices.

The project was also directed to people working in local authority offices, doctors and university students with the aim to attract their attention to the benefits of a healthy lifestyle and the need for action. Neighbours and friends of families and professionals involved in the program are also involved via Healthy Kids in Bulgaria. Media representatives play a significant role in the behavioural change and healthy lifestyle perception. Their attention and reflection on the problems of obesity are essential in the process of addressing the issue to help achieve recognition and further growth.

The efforts were concentrated in the city of Sofia to ensure project sustainability and to focus on achieving the objectives and desired results, Local Authorities and NGOs were actively involved in the activities plan development.

In 2012 Healthy Kids in Bulgaria was launched at 10 schools in Sofia. For less than a year the program attracted more than 3,500 families with children between the ages of 7 and 13 from two districts in Sofia – Triaditsa and Studentski. Events and activities targeted families, school stakeholders and the society through the involvement of private and public partners. Today, 8500 families participate in program and it is held in 20 schools in Sofia.

Social Marketing Activities (25,27,28,29)

Each year the central coordination team develops an integrated communication plan, which is implemented by each local coordinator, responsible for the schools in the respective region.

The central team conducts a kick off meeting with partners, local authorities, school directors and local coordinators, in order to present the school's strategy for the upcoming year.

Fig. 1 – ***Main data for EPHE program***

When all stakeholders are aware of the project plan, the local coordinators hold field meetings, develop the actions' schedules, and distribute the animators' roles and engagements. They are also responsible for the informational campaign in the schools, such as:

- Official Letters to school stakeholders and parents;
- Regular meetings with teachers and school directors;
- Personal contacts and program presentations to the parents held by famous Bulgarian sportsmen;
- Declarations for participation, which each of the parents' signs, in order to give permission for participation in the program.

It was followed by:

- In-class balanced nutrition lessons for all children, explaining the basics of balanced nutrition, the nutritional pyramid, fruits and vegetables consumption;
- In-class balanced nutrition games and activities for all children, giving practical knowledge and engaging children;
- Healthy Cooking Classes in school, teaching

all children how to prepare a balanced school sandwich;
- Open Sports lessons by celebrity sportsmen and sports tournaments at schools;
- Fast, Brave, Skilled and Healthy celebrations for all children in the school yard, which participate in cooking and sports batons;
- Picnics in the mountains for the schools stakeholders, etc.

The interactive balanced nutrition lessons and games were supplemented by healthy cooking classes where children prepared a balanced school menu. Celebrity chefs visited each of the schools and organized games and practical lessons to teach students how to prepare a balanced school lunch.

Besides the school-based activities, numerous public events for the whole family are organized, in order to reach the local communities. Public and private partners take part in the activities; and media are invited to visit some of the events. The celebrity endorsement is very valuable, as well. Children are influenced by famous role models who help them understand the importance of active lifestyle and balanced nutrition.

Interventions Undertaken

1. Preparation Step: from the End of 2012 to June 2013

Within the first year of the project the focus was on preparing the community for the project implementation and to further gain support from the local partners. A motivation and awareness campaign was developed, in order to motivate families to get involved and take part in implementing the first evaluation phase.

Official letters to Local Authorities (Ministry of Health, Ministry of Education and Science, Local Mayors) were sent and a meeting with the Ministry of Education, Youth and Science was organized. During the meeting, the EPHE project was presented and the Municipality was officially invited to support EPHE Actions. Having the support of the Ministry, an official Invitation letter from the Ministry was sent to all school directors so they felt very comfortable with EPHE implementation.

In order raise awareness on the project in Bulgaria, BASORD organized and press conference on the occasion of the World Obesity Day. The press conference were followed by free check-ups for weight, fat mass and cardiovascular risks from October 30 to November 3 at the NPC in Sofia and a scientific conference on the developments in the fight against obesity were held in the same day.

School for health – for children, parents and teachers (5,6,18)

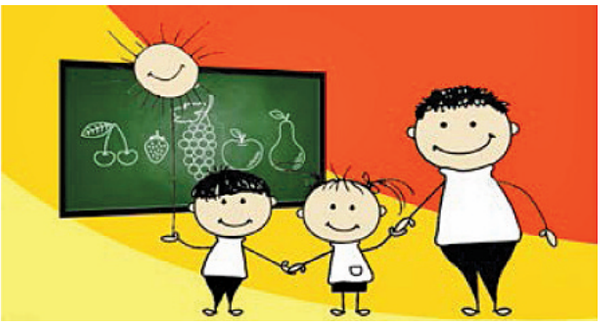

Fig. 2 – ***Logo of the School for health – for children, parents and teachers***

Once having the schools involved, the focus was on the families' motivation, in order to ensure a high participation rate and dedication on behalf of the parents and teachers. The main motivation for most of the families was the healthy camp „School for health – for children, parents and teachers", organized by BASORD in one of the most famous seaside resorts of Bulgaria, in 2013. A lottery amongst all families who participated in the project was drawn. 5 families and a teacher from each school were chosen to take part in the camp. They have spent a healthy week at the seaside with a professionally developed schedule, including balanced nutrition classes, entertaining sports activities and sports games for the whole family and personal nutritional consultations by. Besides the four main pillars of EPHE intervention, the nutritional approach was based on two more important dietary elements for the Bulgarian children: the consumption of milk and dairy products (which is the lowest in EU among children) (26), and the traditions in the healthy and balanced diet (the so-called Balkan diet). This approach led to 94% participation rate within the data collection of the baseline. This has lead to the empowerment of EPHE **.

2. The Intervention Step: September 2013 to June 2014

After examining the baseline results, a detailed action plan was developed for the second year. The results showed low fruits and vegetables consumption, high soft drinks consumption and long screen exposure amongst Bulgarian children. The actions were designed to address the price influence, the habitual intake and home availability of fruits and vegetables, as well as the parenting practices such as nagging behaviour.

Each school had a dedicated intervention plan and it was implemented within the second evaluation period. As the first „School for health" began in September 2013, this was the best occasion to start the intervention and to turn 130 parents, children and teachers into active lifestyle and balanced nutrition ambassadors. Within the camp frame, personal anthropometric measurement and professional consultations on the healthy lifestyle and the specific health problems were held. Each family had the opportunity to ask specific questions, based on the measurement results. Specific health concerns were discussed. The EPHE Families and teachers spent a week on the seaside with a balanced menu and a fixed daily program. Morning gymnastics, fixed sleep and rest time and balanced nutritional menu were also a part of the program. Besides the four main pillars of EPHE intervention, the nutritional approach was based on two more important dietary elements for the Bulgarian children: the consumption of milk and dairy products (which is the lowest in EU among children), and the traditions in the healthy and balanced diet (the so-called Balkan diet)(17,26).

While parents had lectures, children painted their favourite fruits and vegetables with a smiley face and a frowny face with foods that they don't like. The animators explained why all fruits and vegetables are good for health and how they can be prepared, in order to be tastier for the children. Family games and beach sports activities were organized every day.

At the end of camp there was an official closing ceremony. The Minister of Education and Science gave special certificates to all participants in the School for Health initiative. National and local media representatives were invited to cover the event and EPHE project received publicity.

Fig. 3 - ***Dances during „School for health – for children, parents and teachers"***

Fig. 4 - ***Sport activities during „School for health – for children, parents and teachers"***

Fig. 5 - ***Lectures on healthy nutrition, part of „School for health – for children, parents and teachers"***

„School for health – for children, parents and teachers" initiative was presented at different scientific congresses (Albena 2014; Bucharest 2014; Varna 2015, Prague 2015, Vancouver 2016, Marrakesh 2016, Bratislawa 2017, Rome 2017). The „School for health – for children, parents and teachers"

Fig 6 - *„School for health – for children, parents and teachers"*

gained much attention from other countries, e.g. Romanian school-aged children would like to take part of it. (16, 17)

Within the school year all children took part in in-class activities:

- Balanced nutrition classes and games, based on the fruit and beverage consumption and the nutritional pyramid;
- Healthy cooking activities and participation in the European day for healthy cooking with children;
- Some of the famous sportsmen in Bulgaria, Olympic gold medallists and world champions visited the schools and gave open lessons to the children;
- Each school had a sports celebration and organized kids athletics tournament at the school yard;
- Lessons on water consumption and sleep importance were held by the program animators and the children developed their own „Dreams Diaries", which were signed by celebrity sportsmen;
- Water day with painting exhibition and paintings on the ground were organized, in order to focus on the importance of water consumption. The parents received informational materials on water and the children won special certificates, given by their favourite animated hero.

Main Results

The results from the questionnaire showed a positive tendency of high consumption of fruits and vegetables from the school-aged children in Bulgaria. Unfortunately, Bulgarian children spend roughly 25 hours weekly screen time which is the highest within the European countries participated in the study. The "School for health" initiative demonstrated a change in the everyday nutrition and physical activity behaviour in the families. Children from good income families had significantly higher fruit, fruit juices and soft drinks consumption compared to the children from not good income families ($p< 0.001$, $p<0.05$, $p<0.05$, respectively). The children from not good income families spent significantly more total screen time compared to the children from high-income families, 25.7 hours vs. 18 hours, respectively, $p<0.05$.

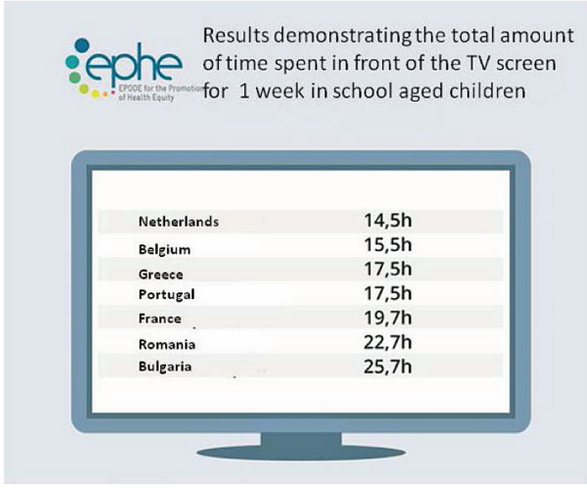

Fig 7

The data demonstrate that there is an evident improvement of the consumption of fruits and vegetables in the Bulgarian school-aged children **(Fig. 8),** but the lack of physical activity is one of the main causes of overweight and obesity **(Fig. 7)**

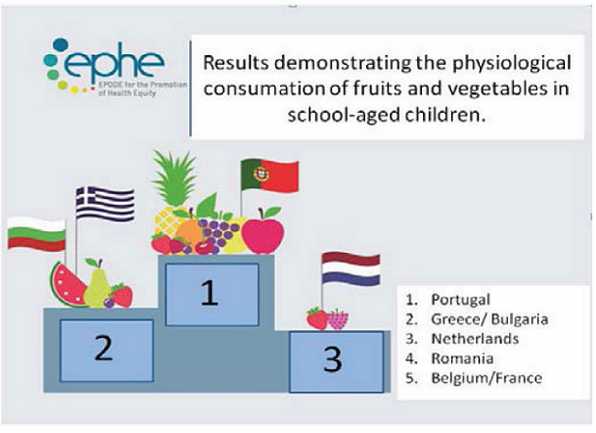

Fig 8

Conclusion

Many efforts on national and international levels, including research projects and different initiatives are needed to tackle the epidemic proportions of the childhood obesity not only in Bulgaria but in the European Union. Our study has demonstrated a positive trend towards a healthier nutrition based on a high fruit consumption. However, the sedentary lifestyle is very popular in school-aged children in Sofia which could increase the risk for obesity and its metabolic complications.

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Emotional and personal status in patients with bone tumor

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SUMMARY

The purpose of this study was to reveal personal characteristics and coping strategies and identify emotional status in patients with different forms of malignant bone tumor. Study participants (N=120) were aged 18 to 67 years old (average age at 41±3). General group was split into four groups depending on diagnosis: group of patients with osteosarcoma, patients with giant cell tumor, group of patients with chondrosarcoma and group of patients with bone metastases. Method «Dominant emotional condition» was used for diagnosis emotional status. Questionnaire «Big V» was used for identifying personal characteristics in patients with bone tumor. Coping behavior diagnosed using «Coping strategies». Questionnaire «Meaning of life orientations» was used for revealing value personality trend in patients with bone tumor. The results revealed in patients emotional status disposition to anxiety, reduced emotional stability, mood variability, irritability and prevalence of negative emotional tone. According the results of study personal characteristics patients with osteosarcoma can be described emotionally and personally as kind-hearted, benevolent and confiding persons. Patients with osteosarcoma have vast interests and luxuriant imagination. In concordance to data of coping strategies research, coping behavior in patients with osteosarcoma was characterized in terms of prevalence of problem escape strategies. Patients with osteosarcoma and bone metastases have disposition to bearings on transpersonal, philosophic comprehension of problem situation. Patients with bone metastases are more susceptible to search for social support, than patients with primary bone tumor. According the results of study value personality trend, patients with bone metastases are more inclined to make purposes for the future, than patients with giant cell tumor. Patients with osteosarcoma perceive their life as more interesting, emotionally intense and meaningful, than patients with giant cell tumor. The research is supported by RFFI grant № 17-36-00011 «a1».

Key words: bone tumor, emotional condition, personal characteristics, coping behavior, value personality trend.

Introduction

In present-day science biopsychosocial model replaces biological model of disease. Biopsychosocial model views somatic pathology not only from the perspective of pathobiological process, but it appreciates psychological and social factors of disease (Wasserman L.I., 2011).

Oncological diagnosis is a serious stressful factor for a patient (Sidorov P.I. 2006; Smulevich et al. 2007; B.J. Sadock et al. 2009). A number of authors note not mere significant influence of oncological pathology on patient mental state, but focuses on the part of psychological factors in genesis, course and outcome of disease (Lillberg K. et al. 2003; Palesh O. et al. 2007; Porcelli P., Sonino N. 2007; Steel J.L. et al. 2007; Chida Y. et al. 2008; Satin J.R., Linden W., Phillips M.J. 2009; Ross K. et al. 2009).

But not only diagnosis is traumatic. The oncological treatment includes surgical treat-

ment and chemotherapy. As noted by L.P. Urvantsev (1998), surgical treatment inflicts physical and psychological trauma. Besides painful senses waiting for surgery, doubts about its successful outcome, helplessness during the postsurgical period and other psychological factors influence on patient mind.

Among psychological problems which oncological patients have P. Porcelli, N. Sonino (2007) note certainty lost, emotional instability (fear, anxiety, sadness), prospects for the future change and possible death threat. A lot of scientists reveal prevalence depression, distress and anxiety among oncological patients (Breslau E.S. et al., 2001; Sadock B. J. et al., 2009).

T. Yonemoto et al. (2009) studied psychological characteristics in patients with bone sarcoma. They revealed that posttraumatic growth was specific for patients with osteosarcoma in remission. In the research the link older age of patients in the beginning of illness and amputation with posttraumatic growth was found.

Van Riel et al. (2004) report, that self-image of adolescents with sarcoma didn't differ from self-image of their healthy peers. R.S. Robert (2010) revealed, that assessment of body image was significantly worse in patients underwent amputation after unsuccessful limb-salvage surgery compared other patients.

Nevertheless, the number of studies reporting on emotional and personal status in patients with malignant bone tumors is limited. Most of existing works studied quality of life in children and adolescents with bone sarcoma. Major part of researches also aims to quality of life comparative analysis in patients with bone tumor after limb-salvage surgery and amputation. There are no quality of life researches in adults with bone tumor which pay attention to psychological factors of quality of life in contemporary literature. It determines need of complex research aimed at studying different aspects of emotional status, person and behavior in patients with bone tumor.

The purpose of this study was to reveal personal characteristics and coping strategies and identify emotional status in patients with different forms of malignant bone tumor.

The goals of this study were: 1) identifying characteristics of emotional status in patients with bone tumor; 2) revealing structural (typological) person characteristics in patients with bone tumor; 3) determining coping strategies; 4) revealing value personality trend in patients with bone tumor.

Experimental group characteristic and methods

Study participants (N=120) were inpatients of N.N. Blokhin Russian Cancer Research Center, Surgery Department of General Oncology. The patients were aged 18 to 67 years old (average age at 41±3). There were 76 (63%) males and 44 (39%) females in general group. According the research goals general group was split into four groups depending on diagnosis. The first one (A) included 41 patients diagnosed with osteosarcoma, stage IIB, average age at 27 ± 2. There were 25 (61%) males and 16 (31%) females in this group. The second group (B) included 31 patients with giant cell tumor average age at 36 ± 3. There were 13 (42%) males and 18 (58%) females. The third group (C) included 30 patients diagnosed with chondrosarcoma G1, G2 average age at 45±3. Males prevailed in this group; they were 24 (80%) patients. Females were 6 (20%) patients. The fourth group (D) included 18 patients with bone metastases (primary diagnosis is clear-cell carcinoma). There were 14 (78%) males and 4 (22%) females. The average age is 55±2.

Most patients with osteosarcoma, chondrosarcoma and bone metastases were males, females prevailed in the group of patients with giant cell tumor. The groups of patients vary by age. Patients with osteosarcoma are younger than other patients. Most patients with osteosarcoma (51,2%) and bone metastases (66,7%) don't work. Most patients with giant cell tumor (51,6%) and chondrosarcoma (60%) refer to middle age. They work. The duration of the disease in patients with bone tumor is given in **Table 1.**

Table 1 - *The duration of the disease in patients with bone tumor*

Duration of the disease	Osteosarcoma n=41 A	Giant cell tumor n=31 B	Chondrosarcoma n= 30 C	Metastases n =18 D
	M ± m	M ± m	M ± m	M ± m
Since diagnosed (months)	16,68 ±3,73	8,06± 2,1	18,03± 6,27	25,44± 6,7
Since symptoms appeared (months)	20,54±3,58	12,66±2,64	31,93±8,94	25,78±6,66

The results presented in table 1 show, that the longest period between symptoms appearance and diagnosis is in the group of patients with chondrosarcoma (13,9 months). The treatment in patients with giant cell tumor lasts the shortest time. The average treatment lasting since diagnosis is 8,06 months. Patients with osteosarcoma and chondrosarcoma have treated about half a year at research moment. The average treatment lasting in patients with bone metastases was 25,44 months at research moment. The course of the disease is given in **Table 2.**

The results presented in table 2 show, that most patients with primary bone tumor were referred for planned hospitalization. And most patients with bone metastases were admitted to oncological hospital with unfavorable course.

Most patients with osteosarcoma (70,7%), patients with giant cell tumor (51,6%), bone metastases (77,8%) and only 40% patients with chondrosarcoma undergo surgical procedure – resection with endoprosthesis. A half of patients with shondrosarcoma undergo limb-salvage surgery with alloplasty. 4,9% patients with osteosarcoma and 10% patients with chondrosarcoma undergo amputation. There were no amputations in the group of patients with giant cell tumor and bone metastases.

The assessment of general health status in patients with bone tumor on Karnovsky scale and ECOG is given in **Table 3.** The variables were extracted from medical records and show patients objective physical status.

Table 2 - *Course of the disease in patients with bone tumor*

Course of the disease	Osteosarcoma n=41 A	Giant cell tumor n=31 B	Chondrosarcoma n= 30 C	Metastases n =18 D
Planned hospitalization	35 per. (85,4%)	31 per. (100%)	28 per. (93,3%)	5 per. (27,8%)
Improvement	5 per. (12,2%)	0	2 per. (6,7%)	0
Unfavorable course	1 per. (2,4%)	0	0	13 per. (72,2%)

Table 3 - ***General health status in patients with bone tumor (objective physical status)***

Karnovsky index/ ECOG scale	Osteosarcoma n=41 A	Giant cell tumor n=31 B	Chondrosarcoma n= 30 C	Metastases n =18 D
	M ± m	M ± m	M ± m	M ± m
Karnovsky index	80 ± 1,79	78,89 ± 2,2	80,5± 2,15	71,18±2,63
ECOG scale	1,23 ± 0,1	1,41 ± 0,1	1,4 ± 0,12	2,12±0,12

The results presented in table 3 show, that objective physical status in patients with primary bone tumor significantly better than objective physical status in patients with bone metastases. And objective physical status in patients with different forms of primary bone tumor is the same.

Statistical analysis of study results

Statistical significance of distinctions between groups was calculated with t-criterion on Student test. MICROSOFT EXCEL 2007 was used for processing the results.

Methods description

The methods were:

1) «Dominant emotional condition» (Kulikov L.V., 2003);

2) Personal questionnaire «Big V» (Yanichev D.P., 2006);

3) «Coping strategies» (Wasserman L.I. et al., 2010);

4) «Meaning of life orientations» (Leontyev D.A., 2000).

Method «Dominant emotional condition» aims to determine mood characteristics and some other characteristics of mental state personal level using patient subjective assessments. The main purpose of the questionnaire is diagnosis relatively persistent (dominant) conditions. Widely using method «Big V» aims at revealing distinguished five global personal factors bound up with the levels of extraversion, self-consciousness, cooperation, emotional stability and personal resources. Method «Coping strategies» is an adapted edition of questionnaire «The Ways of Coping Questionnaire» – WOSQ by R. Lazarus and S. Folkman with obtaining normative data on native sample. The questionnaire based on cognitive theory of stress and coping by R. Lazarus and S. Folkman (Lazarus R. S., Folkman S., 1984). Method aims at revealing coping strategies in stressful and difficult situations person uses. The method «Meaning of life orientations» is an adapted edition of «Purpose-in-Life Test – PIL» by D. Krambo and L. Maholik. Purpose in life the method diagnosis is determined by the authors as person experience of ontological life significance (Leontyev D.A., 2000).

Results and discussion

The results of study emotional status in patients with bone tumor using the method «Dominant emotional condition» is presented in **table 4.**

Mathematics statistical analysis revealed there were high significant differences on all scales of «Dominant emotional condition» in each patients group with the standard (50-T points). The exceptions were scales «Active-passive relation to life» and «Life satisfaction-dissatisfaction».

Patients with bone tumor have close to standard points on the scale «Active-passive relation to life». And patients with osteosarcoma are susceptible to more active and positive relation to life, ready to overcome obstacles, than patients with giant cell tumor and chondrosarcoma. Patients with osteosarcoma are younger than patients of other group, that can influences on severity of intention to overcome obstacles and feel strengths for it in patients with osteosarcoma.

Patients with bone tumor have low points on the scale «Low-high tone» compared with the standard. Consequently patients with

Table 4 – *Emotional status in patients with bone tumor*

Scales of method «Dominant condition»	Osteosarcoma n=41 A	Giant cell tumor n=31 B	Chondrosarcoma n= 30 C	Metastases n =18 D	Reliable Differences
	M ± m	M ± m	M ± m	M ± m	
Active-passive relation to life	47,66 ± 2,09	43,5 ± 1,92	42,3 ± 1,81	42,77±3,1	AB* AC**
Low-high tone	28,85 ± 2,24	29,7 ± 1,84	28,55 ± 2,75	32±4,6	
Tranquility — anxiety	24,37 ± 1,86	28,6 ± 2,09	25,24 ± 2,67	24,11±3,5	AB*
Emotional tone stability-instability	30,24 ± 2,1	28,2 ± 1,96	29,52 ± 2,7	27,89±4,5	
Life satisfaction-dissatisfaction	42,1 ± 2,68	45,57 ± 2,14	38,28 ± 2,58	35,67±4,65	BC** BD*
Positive-negative self-image	30,44 ± 1,79	27,6 ± 1,35	24,76 ± 1,68	25,11±3,17	AC**

Note. ***In this table and subsequent ones in the column „Reliable Differences" sign * corresponds to the level of statistical significance 0,05<p<0,1; ** – p<0,05; *** –p<0,01.***

bone tumor have fatigue, inertia and low efficiency. The opportunity to be active is reduced in patients with bone tumor. Besides tendency to show asthenic reactions in response difficulties can be noted in these patients.

According the results patients with bone tumor also have low points on the scale «Tranquility—anxiety» compared with the standard. So the patients are susceptible to be concerned in a wide range of life situations, to see the threat to the well-being without precise evaluation of reasons, to anticipate future threat without clear awareness of its sources. At the same time patients with giant cell tumor are more confident in their abilities, than patients with osteosarcoma.

Low points compared with the standard are appropriate to patients with bone tumor on the scale «Emotional tone stability-instability». It evidences that the patients have reduced emotional stability, mood variability, increased irritable and negative emotional tone.

Patients with bone tumor have close to standard points on the scale «Life satisfaction-dissatisfaction». Generally patients with bone tumor are satisfied with their life. They are ready to take responsibility for happenings in their life. There were statistically significant differences between patients with giant cell tumor and the group of patients with chondrosarcoma and bone metastases on this scale. It means that patients with chondrosarcoma and bone metastases are more susceptible than patients with giant cell tumor to assess low their personal advancement, they have partial self-disclosure, lack of inner support sense and leaving in doubt.

Low compared with the standard data assessments in patients with bone tumor on the scale «Positive-negative self-image» tells about high criticalness in self-assessment and intention to be sincere. Besides the data tell about patients negative attitude to themselves, which is more pronounced in patients with chondrosarcoma than in patients with osteosarcoma.

Table 5 - ***Personal characteristics in patients with bone tumor***

«Big V» scales	Osteosarcoma n=41 A	Giant cell tumor n=29 B	Chondrosarcoma n= 28 C	Metastases n =17 D	Reliable Differences
	M ± m	M ± m	M ± m	M ± m	
Extraversion	25,44±0,83	25,83±0,8	25,43 ± 0,86	27,52±1,09	
Self-consciousness	30,46±0,85	31,14± 0,61	30,29 ± 0,92	30,88±1,48	
Cooperation	34,46±0,69	32,86±0,66	32,25 ± 0,97	32,18±1,32	AB* AC* AD*
Emotional stability	24,07±1,01	25,1±1,22	25,43 ± 1,21	25,06±2,02	
Personal resources	28,76±0,87	25,52±0,83	24,64 ± 1,17	26,47±1,62	AB*** AC***

Note: ***Standard data of method «Big V» obtaining on native normative sample were got by D.P. Yanichev (2006).***

Personal characteristics in patients with bone tumor were studied using personal questionnaire «BIG V». The results are given in **Table 5.**

Patients with osteosarcoma have higher values on the scale «Cooperation», than other patients. This describes patients with osteosarcoma as kind-hearted, benevolent and confiding persons.

Besides patients with osteosarcoma have higher values on the scale «Personal resources», than patients with giant cell tumor and chondrosarcoma. Patients with osteosarcoma have vast interests and luxuriant imagination. They can be described as creative persons.

The results of studying coping strategies in patients with bone tumor are presented in **Table 6.**

The results presented in table 6 show, that patients with bone tumor reasonable use coping strategies without specific strategy prevalence. Though there are differences between patients groups.

Patients with bone metastases are more susceptible to search for social support, than patients with primary bone tumor. Patients with bone metastases are inclined to looking for opportunity to use external resources for solving problem situation. For them focus on interaction with other people and expectation for support are specific. The drawback of search for social support is a chance of depending attitude formation, over expectations towards other people. But as patients with bone tumor have sufficiently high values on the scale «Acceptance of responsibility», so strategy of search for social support is adaptive in this group of patients. Patients with bone tumor actively engage external resources for solving problem situation and don't go into depend position. Besides, the age of these patients must be taken into account. Most patients with bone metastases are older 50 years and they don't work, that clarifies intention of the patients on using help of other people.

Patients with giant cell tumor are more inclined to accept their part in problem origin and their responsibility for solving it. The data are consistent with the results of questionnaire «Dominant emotional condition». Patients with giant cell tumor have higher values than patients with chondrosarcoma on the scale «Life satisfaction-dissatisfaction». This means that patients with giant cell tumor are more susceptible to accept responsibility for their life.

Strategy «Escape» is more prevalent among patients with osteosarcoma than in the group of patients with giant cell tumor and chondrosarcoma. Patients with osteosarcoma are inclined to try to overcome negative experience seeing the disease at the expense of avoidance.

Table 6 - *Coping strategies in patients with bone tumor*

«Coping strategies» scales	Osteosarcoma n=41 A	Giant cell tumor n=31 B	Chondrosarcoma n= 26 C	Metastases n =17 D	Reliable Differences
	M ± m	M ± m	M ± m	M ± m	
Confrontation	49,05± 1,57	47,42±1,6	47 ± 2,43	47,41± 2,6	
Distancing	49,68± 1,54	49,39 ±2,07	48,12± 2,78	50,94± 2,52	
Self-control	48,73± 1,63	47,84 ±2,08	45 ± 2,86	48,05± 3,2	
Search for social support	48,45± 2,03	49 ± 1,82	46,5 ± 2,07	55,12± 2,4	AD** BD** CD***
Acceptance of responsibility	45,1± 1,85	47,81± 1,56	44,12± 1,97	48,12± 2,45	BC*
Escape	52,48±1,14	48,19± 1,84	47 ± 2,55	49,82± 2,58	AB** AC**
Plan to solve the problem	48,8± 1,81	47,13± 1,89	47,58± 2,39	52± 2,92	
Positive reconsideration	51,45±1,87	47,81± 1,92	47,38± 2,19	54,59 ± 2,42	AB* AC* BD** CD**

Besides, there are differences between the groups of patients on the scale «Positive reconsideration».

Patients with osteosarcoma and bone metastases are more susceptible to bearings on transpersonal, philosophic comprehension of problem situation, inclusion it in broader context of person working at self-development, than patients with giant cell tumor and chondrosarcoma. Prevalence of this strategy gives patients with osteosarcoma and bone metastases an opportunity of personal growth in the process of coping. But there is also a chance of underestimation of realistic possibilities to solve the problem.

The results of study value and motivational orientation of person in patients with bone tumor are presented in **Table 7.**

Values on all scales of «Meaning of life orientations» do not significantly differ from standard data in patients with bone tumor. The exception is the scale «The common value of life meaningfulness». Patients with bone tumor have higher values on this scale compared with the standard data. Besides, there were differences between the groups of patients.

The results presented in table 7 show, that patients with bone metastases are more inclined to make purposes for the future, than patients with giant cell tumor. Present of purposes for the future in patients with bone metastases life makes their life to be meaningful, gives them the intension and future perspective.

Patients with osteosarcoma perceive their life as more interesting, emotionally intense and meaningful, than patients with giant cell tumor.

Patients with giant cell tumor and chondrosarcoma are surer in person opportunities to control the life, make own decisions and bring them to life, than patients with osteosarcoma.

Table 7 - *Value and motivational orientation of person in patients with bone tumor*

«Meaning of life orientations» scales	Osteosarcoma n=40 A	Giant cell tumor n=30 B	Chondrosarcoma n= 27 C	Metastases n =17 D	Reliable Differences
	M ± m	M ± m	M ± m	M ± m	
Purposes in life	27,93 ± 0,87	27,1 ± 1,11	28,15 ± 1,28	30,35±1,6	BD*
Life process	30,6 ± 0,97	28,37 ± 1,21	30,85 ± 1,25	30,71±1,71	AB*
Life potency	26,73 ± 0,83	24,73 ± 1,17	26,89 ± 1,14	27,65±1,55	
Control locus - Ego	21,7 ± 0,6	20,53 ± 0,92	21,04 ± 0,85	21,94±1,13	
Control locus - Life	28,45 ± 1,14	31,13 ± 1,3	31,22 ± 1,16	30,24±1,73	AB* AC*
The common value of life meaningfulness	135,4 ± 3,75	131,87 ± 4,91	138,15 ± 4,93	140,88±7,09	AE** BE* CE** DE**

Conclusion

Thus, patients with bone tumor have variety of emotional problems. Besides there is prevalence of maladaptive coping strategies in patients with bone tumor. So the patients should take part in psychological programs taking into consideration the psychological features in these patients.

1. Emotional status in patients with bone tumor of all studied nosological forms was studied. Disposition to be concerned in a wide range of life situations, reduced emotional stability, mood variability, irritability and prevalence of negative emotional tone were revealed.
2. Patients with osteosarcoma can be described emotionally and personally as kind-hearted, benevolent and confiding persons. Patients with osteosarcoma have vast interests and luxuriant imagination.
3. of escape strategy was revealed in patients with osteosarcoma coping behavior. Patients with osteosarcoma and bone metastases have disposition to bearings on transpersonal, philosophic comprehension of problem situation. Patients with bone metastases are more susceptible to search for social support, than patients with primary bone tumor.
4. Patients with bone metastases are more inclined to make purposes for the future, than patients with giant cell tumor. Patients with osteosarcoma perceive their life as more interesting, emotionally intense and meaningful, than patients with giant cell tumor.

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Тенденции в разпространението на заболяванията и небойните наранявания сред личния състав на българските въоръжени сили в периода 2008-2013

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Trends in the incidence of disease and non battle injuries amongst the bulgarian armed forces personnel, 2008-2013

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РЕЗЮМЕ:

Целта на изследването е да се анализира разпространението и тенденциите в заболеваемостта и небойните наранявания сред личния състав на българските въоръжени сили (БВС) за периода 2008-2013 г. Разпространението на заболяванията и небойните наранявания (ЗНбН) беше анализирано чрез определяне на показатели за честотата на разпространение и разпределение. Определянето на тенденцията на честотата на разпространение на ЗНбН бе извършено посредством регресионен анализ. Нашите резултати показват тенденция за значително намаляване като цяло на ЗНбН сред личния състав на БВС

ABSTRACT

The aim of the study is to analyze distribution and trends in the incidence of disease and non battle injuries (DNBI) amongst Bulgarian armed forces (BAF) personnel, during the period 2008-2013. The spread of DNBI was analyzed by determining indicators for incidence rate (per 1,000 combat-years) and distribution. Regression analysis was carried out for determining the trend of the DNBI incidence rate. A significant downward trend were established for of the overall DNBI incidence in BAF (y = -38.13x + 1049; R^2=0.629), diseases of respiratory system (y= -26.22x+377.8; R2=0.814), diseases of digestive system (y= -18.16x+168.8;

(y = -38.13x + 1049; R² = 0.629), както и на заболяванията на дихателната система (y = -26.22x + 377.8, R2 = 0.814), заболяванията на храносмилателната система (y = -18.16x + 168.8, R2 = 0.828) и небойните наранявания (y = -2.23x + 94.41, R2 = 0.429), докато заболяванията на кръвоносната система показват сигнификантна тенденция на увеличена честота +57.84; R2 = 0.684). Наблюдава се тенденция на повишена честота и на ендокринни и метаболитни заболявания (y = 3.320x + 5.771, R2 = 0.884), заболявания на мускулно-скелетната система (y = 2.892x + 47.47, R2 = 0.41) = 1.160x + 23.82; R2 = 0.262) и заболявания на пикочно-половата система (y = 0.954x + 43.84; R2 = 0.177). Въпреки намаляването на общата честота на ЗНбН сред личния състав на БВС бяха установени неблагоприятни тенденции в степента на разпространение на някои групи болести, а именно: заболявания на кръвоносната система, ендокринни и метаболитни заболявания, заболявания на окото, заболявания на пикочо-половата система, заболявания на опорно-двигателния апарат.

Ключови думи: честота, заболявания и небойни наранявания, въоръжени сили

R2=0.828) and non-battle injuries (y= -2.23x+94.41; R2=0.429), while the diseases of the circulatory system showed significant trend of increased incidence (y= 6.62x+57.84; R2=0.684). A trend of increased incidence was established also for endocrine and metabolic diseases (y= 3.320x+5.771; R2=0.884), diseases of the musculoskeletal system (y= 2.892x+47.47; R2=0.41), diseases of the eye (y= 1.160x+23.82; R2=0.262) and diseases of the genitourinary system (y= 0.954x+43.84; R2=0.177). Despite the reduction of the overall DNBI incidence amongst BAF personnel, unfavorable trends in the incidence rate of certain groups of diseases were established, namely: diseases of the circulatory system, endocrine and metabolic diseases, diseases of the eye, diseases of the genitourinary system and diseases of the musculoskeletal system.

Key words: incidence, disease and non battle injuries, military personnel

Introduction

Disease and Non Battle Injuries (DNBI) have always been and will continue to pose a risk to military personnel and thus to affect the success of military campaigns and operations [1,10, 8]. They also represent a permanent risk to the health of military personnel in peacetime as might adversely affect training process and combat readiness of the troops [6,7]. In this regard, preventive medicine and easy access to health care are fundamental aspects in maintaining health and combat readiness of military staff [4]. The study of trends in the incidence of DNBI is useful for planning preventative measures and maintaining combat readiness of the troops.

The aim of the study was to examine distribution and trends of DNBI incidence rates among Bulgarian armed forces (BAF) personnel during the period 2008-2013.

Materials and Methods

This study was based on data from the annual reports for health condition of BAF personnel. The spread of DNBI amongst military personnel was analyzed by determining indicators for incidence rate (per 1,000 servicemen-years) and distribution by diagnosis. Regression analysis was carried out for determining the trend of DNBI incidence rates. The least squares method was used for estimation of regression dependences and coefficient of determination (R2) – for statistical significance of dependence. The ICD-10 was used to classify DNBI.

Results

A total of 173,657 cases of DNBI among BAF personnel (military and civilian employees) during the period of January 2008 – December 2013 were registered. No deaths from DNBI were reported (fatality rate 0 %).

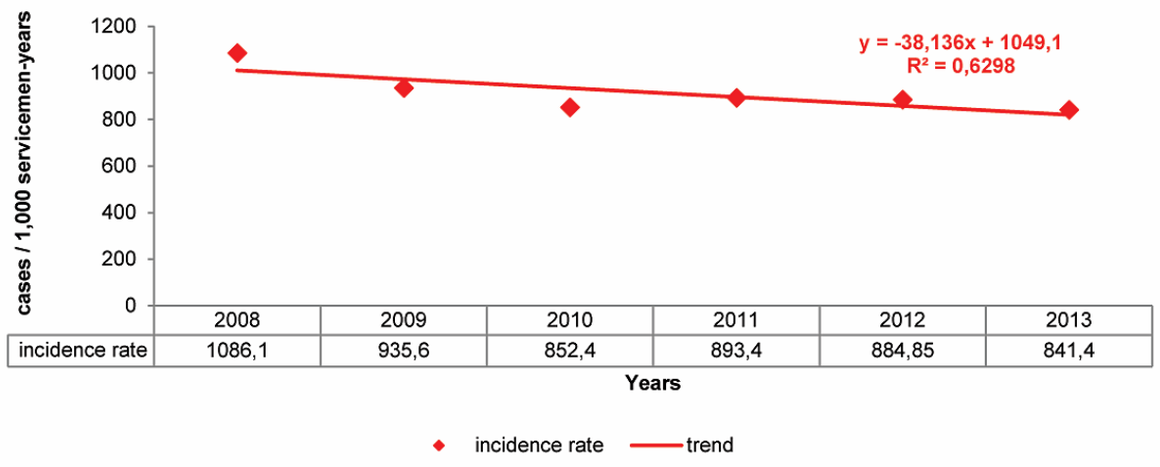

Figure 1 ***Incidence rates and trends of DNBI among BAF personnel, 2008-2013.***

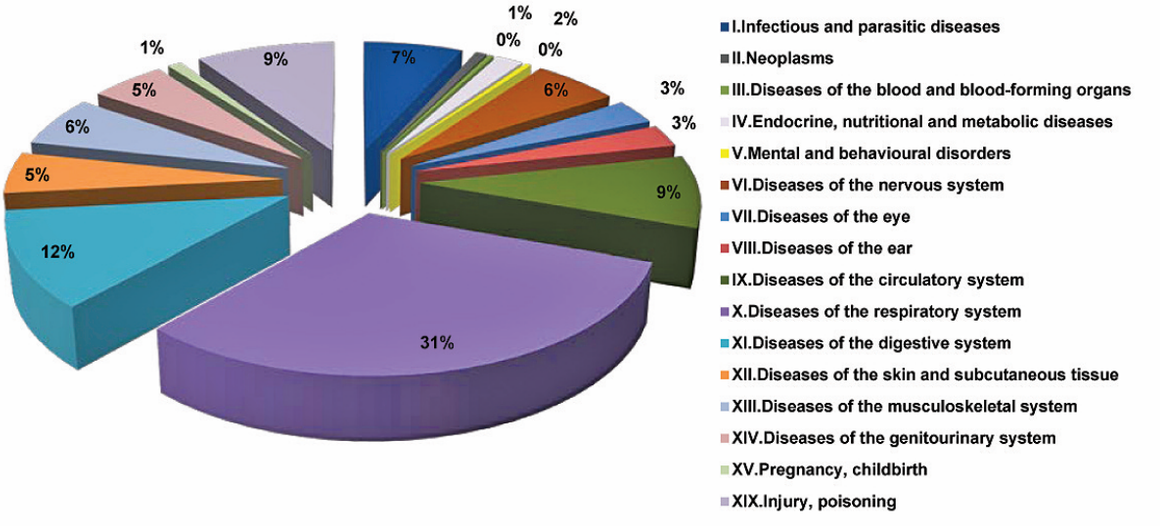

Figure 2 ***Distribution of DNBI (according to ICD-10) among BAF personnel, 2008-2013.***

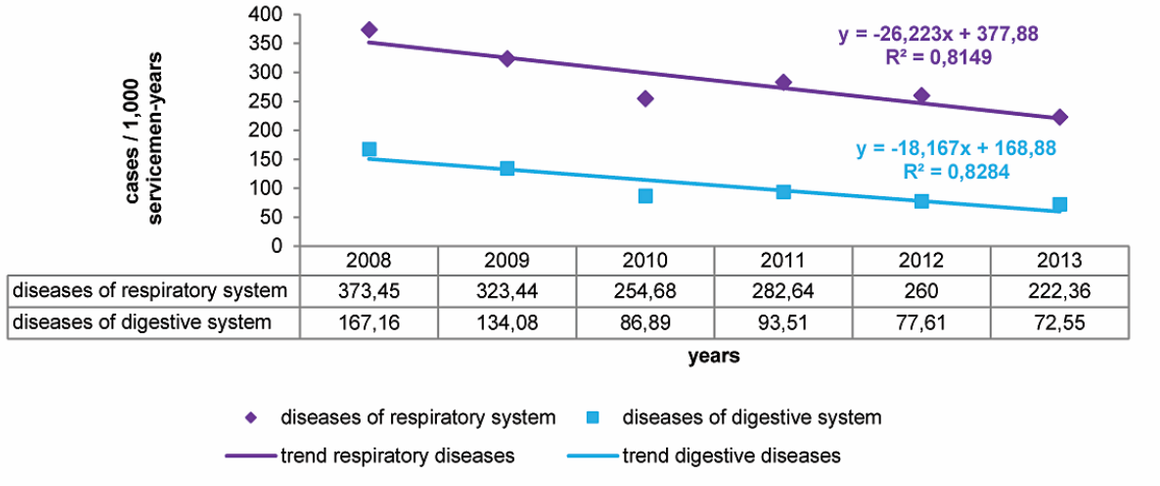

Figure 3 ***Incidence rates and trends of the diseases of respiratory system and the diseases of digestive system among BAF personnel, 2008-2013.***

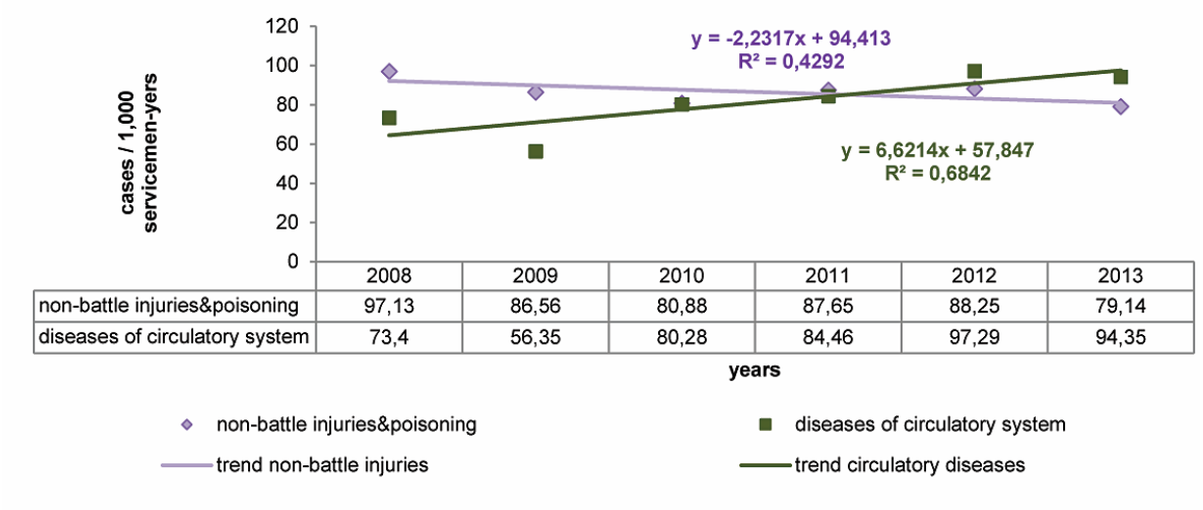

Figure 4 ***Incidence rates and trends of non battle injuries and diseases of the circulatory system among BAF personnel, 2008-2013.***

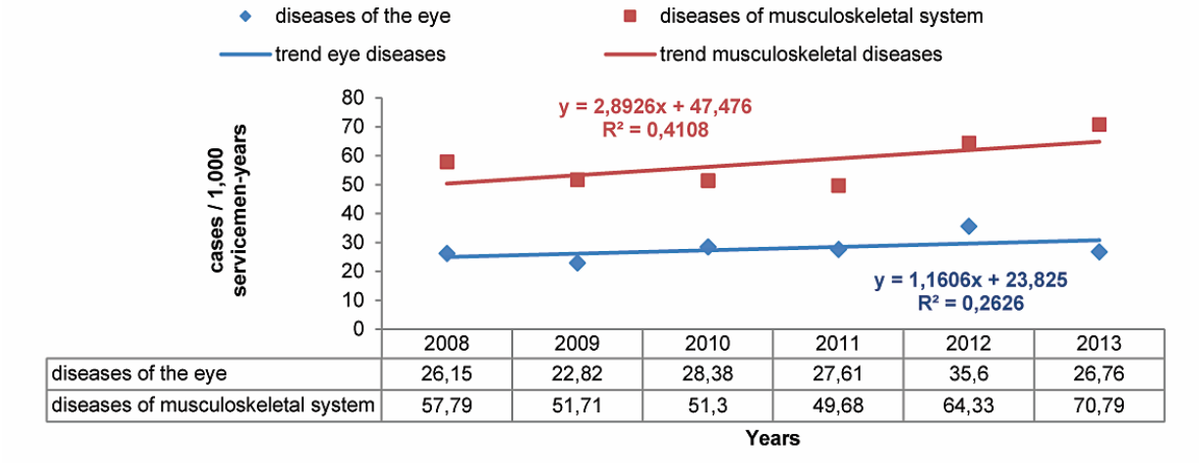

Figure 5 ***Incidence rates and trends of diseases of the musculoskeletal system and diseases of the eye among BAF personnel, 2008-2013.***

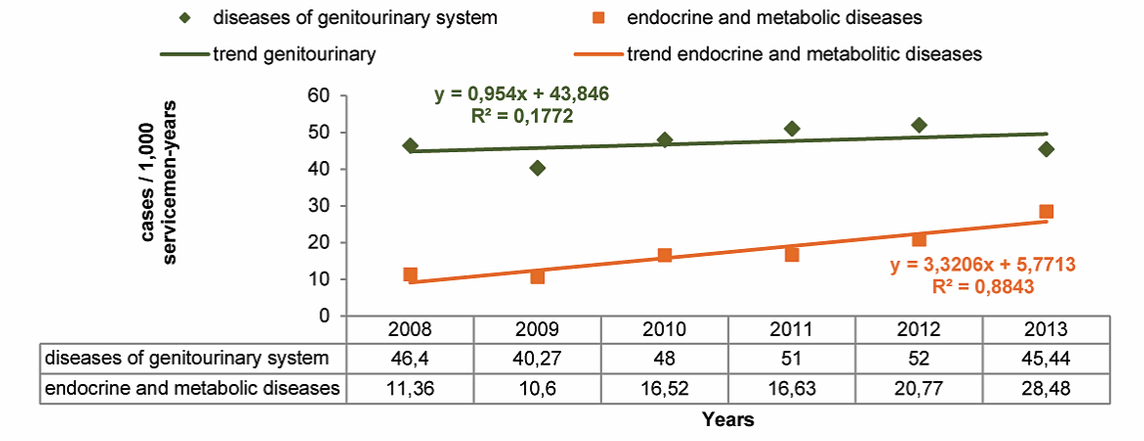

Figure 6 ***Incidence rates and trends of endocrine and metabolic diseases and diseases of the genitourinary system among BAF personnel, 2008-2013.***

The yearly average number of cases was 28,942.83. The most number of cases 33,213 was reported in 2008 г. and the least number of cases 19,147 in 2013.

The overall DNBI incidence rate among BAF personnel was between 1086.1/1,000 servicemen-years (2008) и 841.4/1,000 servicemen-years (2013), yearly average – 915.6/ per 1,000 personnel per year. A significant downward trend (y = -38.13x + 1049; R^2=0.629) of the overall DNBI incidence in BAF was established during the analyzed period **(Figure 1).**

The diseases of respiratory system dominate the structure of DNBI with 31.37%, followed by the diseases of digestive system with 11.57%, non battle injuries - 9.44%, diseases of the circulatory system - 8.71%, certain infectious and parasitic diseases - 6.75%, diseases of the musculoskeletal system - 6.15%, diseases of the nervous system - 5.82%, diseases of the genitourinary system - 5.14%, diseases of the skin and subcutaneous tissue - 5.02% etc. **(Figure 2).**

The diseases of the respiratory system were the most commonly reported (n=54,478) among BAF personnel. The acute upper respiratory infections dominated in the group (almost 40% of the cases). A significant trend (y= -26.22x+377.8; R2=0.814) for reduction of incidence of diseases of the respiratory system was established for the analyzed period.

Diseases of the digestive system were the second most common (n=20,086). Dental diseases were leading in the group with 51.66% rate (n=10,376). A statistically significant trend (y= -18.16x+168.8; R2=0.828) of decreasing the incidence of diseases of the digestive system was ascertained **(Figure 3).**

It should be noted that the most of the recorded cases from these two groups were acute diseases. They results usually with definitive recovery of patient's condition of health and activity.

Non battle injuries and poisonings (n=16,391) ranks third in incidence. A trend with moderate statistical significance (y= -2.23x+94.41; R2=0.429) for reduction of incidence rates was examined. Injuries of the extremities (more than 95% of cases) were leading among the traumatic injuries. The most injuries resulted from incidents during combat training or sport activities. Military personnel were affected by non battle injuries significantly more frequently than civilian employees.

The diseases of the circulatory system were the next most common (n=15,134). In contrast to previous groups of diseases, a statistically significant trend of increased incidence (y= 6.62x+57.84; R2=0.684) was found for these diseases **(Figure 4).** Mainly cases of arterial hypertension (nearly ¾ of numbers) were registered in the group. Other difference is that cardiovascular diseases are predominantly chronic diseases. It is important to note that the diseases of the circulatory system were with higher levels of incidence in the group of civilian employees when compared to military personnel. This in our opinion is due to the higher average age of the civilian employees in comparison to servicemen.

A trend of increased incidence was established also for diseases of the eye (y= 1.160x+23.82; R2=0.262), diseases of the musculoskeletal system (y= 2.892x+47.47; R2=0.41) **(Figure 5),** endocrine and metabolic diseases (y= 3.320x+5.771; R2=0.884) and diseases of the genitourinary system (y= 0.954x+43.84; R2=0.177) **(Figure 6)**.

The incidence for the rest groups of diseases (Infectious and parasitic diseases, diseases of the nervous system, diseases of the skin, diseases of the ear etc.) decreased for the analyzed period.

Discussion

Military conscription along with barrack accommodation of the personnel was abolished in Bulgaria on Dec 1, 2007. The hygiene condition in military units was improved. Catering services was introduced for a large part of the military staff. Professional soldiers' recruitment process was improved. All this in our opinion contributed to the reduction of DNBI incidence and was favorable for the health of BAF personnel. The reduction is mainly due to decrease in the incidence of the leading three groups of diseases: diseases of respiratory system, diseases of digestive system and non battle injuries and poisonings.

Significant reduction in the incidence was observed for the diseases of the respiratory system by 42.03%. The incidence rate decreased from 529/1,000 servicemen-years in 2006 to 222.36/1,000 servicemen-years in 2013. Despite the established trend of reducing incidence, the diseases of the respiratory system continued to dominate the DNBI among BAF personnel. This is particularly true for the military personnel deployed abroad during the period, where the registered incidence rates were higher compared to that among the personnel in the country. Acute upper respiratory infections were the most commonly registered in the group. Basically, these are contagious diseases with airborne mode of transmission. Their prevalence was mainly due to a number of conditions, favoring the airborne transmission of pathogens:

- More intense (daily) contact with the civilian population after the end of conscription;
- Activities under adverse climatic and microclimatic conditions affecting the natural body resistance;
- For the military personnel, deployed abroad:

o permanent battle stress, causing decline of immunological resistance;
o crowded military bases and premises;
o accommodation in dormitories (e.g. K-Spans);
o contact with individuals coming from different geographic regions and different microbial flora.

Data on the prevalence of the diseases of the respiratory system coincided with data of other researchers, which also established the leading role of respiratory diseases in peacetime DNBI rates. According to them, the incidence in different countries and different years varied between 70-80 to 200 or more/1000 personnel [2, 3].

More significant reduction in the incidence compared to the time of conscription service was found in the diseases of the digestive system. For instance, if in 2006 was registered incidence within 298/1,000 servicemen-years, in 2013 it was already 72.55 /1,000 servicemen-years, i.e. almost fourfold reduction in the rates. The reduction covered the whole range of diseases of the digestive system including dental cases. However, the incidence rates for the personnel deployed abroad were higher in comparison to that among the personnel in the country and this was mainly due to dental pathology (52.8% of the diseases of the digestive system and 9.25% of all DNBI). Similar data for the higher incidence of dental cases among deployed troops in Afghanistan and Iraq was established by US researchers [9,11] and by French researchers for troops deployed in Mali [5]. Although BAF personnel underwent pre-deployment dental examination and assessment of their dental fitness, the number of servicemen who had sought for and received dental care during missions abroad was still high. Because the deployed abroad Bulgarian Roles 1 were without dental capabilities, all dental cases were treated outside the national contingents. Considering that it is a costly external service which burdens the allocated national funding for the operation, it is necessary to carry out comprehensive dental treatment before deployment.

The incidence of non-battle injuries and poisonings among BAF personnel during the analyzed period was also with downward trend. This most likely was due to the improved organization of accident prevention as well as the better training and experience gained by professional soldiers, compared with military conscripts. The decreased number of field exercises compared to the past could also have an impact on the reduction of the incidence.

The incidence of the diseases of the circulatory system increased from 71/1,000 servicemen-years in 2006 to 94.35/1,000 servicemen-years in 2013. The increased incidence rate and proportion of this group of diseases was determined mainly by demographic processes (increased average age of personnel). The common modifiable risk factors for chronic diseases like unhealthy diet, physical inactivity and tobacco use were also of importance. The data for the incidence and causality were identical to those for the civilian population of the country. As an integral part of the population, both military personnel and civilian employees were also adversely affected by these risk factors. Such causality could be established also for the other groups of diseases with growing incidence: endocrine and

metabolic diseases, diseases of the musculoskeletal system, diseases of the eye and diseases of the genitourinary system.

The chronic course of the most of diseases from these groups could results in the development of complications, short term or permanent disability and consequently loss of the serviceman or civilian employee for the institution. Increase in the average age is usually associated with increased risk of co-morbidity with the occurrence of frequent episodes of short term or prolonged disabilities. All this could contribute to higher costs of medical care and overloading the military health system. The risk of sudden death is high of the diseases of the circulatory system.

Downward trend of incidence (y= -1.618x+67.41; R2=0.15) of the infectious diseases was established during the analyzed period. The reduction can be explained by advances in preventive medicine and improved surveillance and control of communicable diseases both in the country and BAF. The end of military conscription and barrack accommodation reflected favorably on the hygienic condition of the military environment by reducing the possibility of hygiene failures that could have adverse effects on the health of personnel. Changes in the BAF catering service and the reduced number of canteen users led to the reduction of possibilities for transmission of pathogens via contaminated food. Pre-exposure prophylaxis against hepatitis A and B for personnel deployed abroad significantly reduced the amount of susceptible hosts to these common diseases. Nevertheless infectious diseases are and will continue to be of importance for BAF. Certain specific conditions of military service will continue to contribute to the spreading of communicable diseases:

- military field activities – risk for vector-borne diseases;
- catering service in field operations – increased risk of foodborne illness due to unsafe food and water supplies and poor food safety management practices;
- increased mobility of the modern servicemen – risk of airborne transmitted infections;
- deployment in different geographic regions – risk for vector-borne diseases or imported infections.

Conclusions

The transition from conscript military service to professional army at the end of 2007 had a positive impact on DNBI incidence among the BAF personnel through its reduction. Despite the reduction of the overall DNBI incidence amongst BAF personnel, unfavorable trends in the incidence rate of certain groups of diseases were established, namely: diseases of the circulatory system, endocrine and metabolic diseases, diseases of the eye, diseases of the genitourinary system and diseases of the musculoskeletal system. The chronic course of the most of diseases from these groups is likely to lead to short or long term disability of the patients, their temporary or permanent loss for the military institution and could contribute to higher costs of medical care and overloading the military health system.

The authors declare that they have no conflict of interest.

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Ефекти на рисперидон и пробиотик в експериментален модел на аутизма: метаболитни въпроси

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Effects of risperidone and probiotic in experimental model of autism: metabolic issues

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РЕЗЮМЕ:

Диагностицирането на аутизъм се е увеличило съществено през последното десетилетие, а разработването на ефективна терапевтична програма все още е предизвикателство. Рисперидон е използван успешно за намаляване на сериозни поведенчески проблеми при деца със заболявания от аутистичния спектър, но данните показват че това лекарство може да предизвика метаболитни отклонения, които да увеличат риска от развитие на диабет, сърдечно-съдови заболявания и др. В същото време, множество експериментални и клинични изследвания проучват ролята на микробиотата в свързани с аутизъм поведенчески симптоми и метаболитни отклонения. Научните данни показват, че пробиотиците могат да имат благоприятни ефекти върху неврологични, имунологични и метаболитни аномилии при аутизъм.

Целта на настоящето проучване е да изследва ефектите на рисперидон и пробиотик върху наддаването на тегло и нивата на глюкоза на гладно при плъхове с експериментален модел аутизъм.

Ключови думи: аутизъм, пробиотик, рисперидон, наддаване на тегло, глюкоза на гладно.

ABSTRACT

Autism diagnoses have increased rapidly over the last decade and an effective treatment program is still a challenge. Risperidone has been successful in reducing serious behavioral problems in children with autism spectrum disorder (ASD), but data have shown that it can contribute to metabolic alterations that may predispose patients to diabetes, cardiovascular disease, etc. In the same time lots of experimental and clinical studies have been investigating the role of microbiome in autism-related behavioral symptoms and metabolic issues. Research data have shown that probiotics can have beneficial effects on neurological, immunological and metabolic anomalies in ASD.

The aim of the present study was to investigate the effects of risperidone and probiotic on weight gain and glucose levels of rats in experimental model of autistic disorder.

Key words: autism, probiotic, risperidone, weight gain, fasting glucose, rat

Introduction

Autism spectrum disorders (ASD) are neurodevelopmental disorders affecting about 1 % of the population (or 1 of 68 children) with predominant male prevalence at a boys:girls ratio of 4:1. Furthermore, the epidemiologic data shows rise in ASD frequency. The main features associated with autism are communication disorders, inability to establish verbal contact and stereotypical, repetitive actions and permanent disabilities in social skills. Also, most children with ASD have attention deficit, intellectual disabilities and cognitive challenges. The symptoms of autism are usually noticed between 18 months and 3 years of age and are often accompanied by other diseases such as hyperactivity and attention deficit, epilepsy, psychosis, sleep disturbances, gastrointestinal problems and others. [1] The animal model of autism with the use of valproic acid is one of the most common in the experimental medicine. [1] This model demonstrates many of the structural and behavioral characteristics seen in patients with autism. Valproic acid is an anticonvulsant and antimanic drug. Although the valproic acid is well tolerated and safe in adults, there are many evidences which prove teratogenic effects of this drug. In human and animal trials with valproate the pre-natal exposure to this substance might induce anomalies similar to the ASD. [2]

Children with autism are often affected by gastrointestinal problems such as abdominal pain, constipation and diarrhea. [3] This is why in the recent years there is a great interest in the use of probiotics in these patients. Risperidone has demonstrated efficacy for acute (8 week) and intermediate length (6 month) management of severe irritability and aggression in children and adolescents with autism. [4] In the same time it might induce metabolic changes with increased weight gain and disturbances in the glucose metabolism. [5] In this regard, we investigated the effects of risperidone and a probiotic on the fasting glucose levels and weight gain in rats with experimental model of autism.

Materials and methods

Pregnant Wistar rats were maintained under standard laboratory conditions (12 h light-dark cycle, temperature 20±0,5°C, humidity 65±1%). They were treated with physiological solution (i.p.), or valproic acid (600 mg/kg, i.p.) on the 12,5 day of pregnancy in order to develop the experimental model. The male offspring rats were separated from the mothers on the 23rd postnatal day and divided into 6 groups: 1. Control „c" (physiological solution, p.o.); 2. Control+probiotic **„c+prob"** (probiotic - 1%, 1ml/100 g, p.o.); 3. Control+risperidone **„c+risp"** (1 mg/kg, p.o.); 4. Autistic **„a"** (physiological solution, p.o.); 5. Autistic+probiotic **„a+prob"** (1%, 1ml/100 g, p.o.); 6. Autistic+risperidone **„a+risp"** (1 mg/kg, p.o.). The valproic acid and risperidone were of finest grade and were ordered from Sigma® company. The probiotic is a special strain of Lactobacillus Bulgaricus – „I. Bogdanov patent strain tumoronecroticance B-51" – ATCC 21815, LB51® in short and was ordered from Deodan® company. The substances were administered to the animals for 21 days and weight gain and fasting glucose levels were evaluated at the end of the period. ANOVA test was used for statistical evaluation of relative changes. $P<0,05$ was considered statistically significant.

The experiments were carried out in accordance with the Bulgarian regulations on animal welfare and in conformance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). BAFS license №154/11.11.2016

Results

The results showed considerable differences in the weight gain of the experimental groups. Autistic rats gained significantly less weight compared to the control animals. Treatment with risperidone and probiotic resulted in increased weight in the experimental rats. However, glucose levels were normal and comparable between the groups.

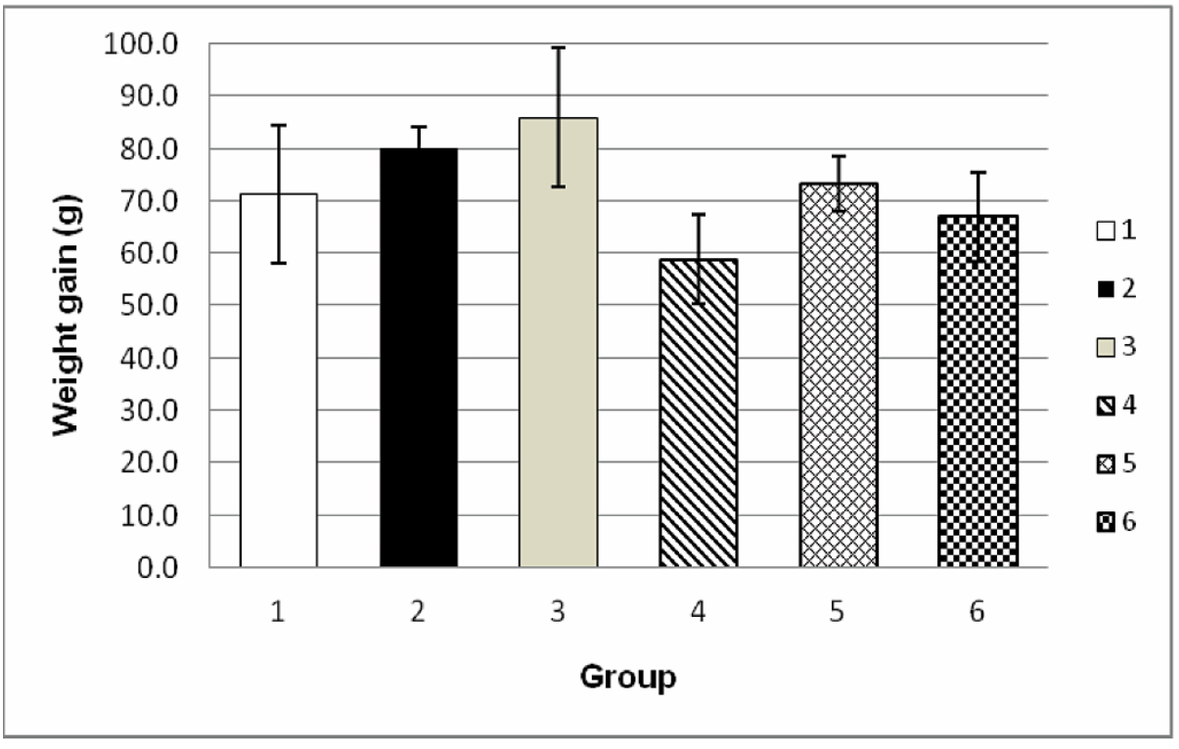

Fig. 1. ***Weight gain at the end of the experiment.***

1. Control **„c"**; 2. Control+probiotic **„c+prob"**; 3. Control+risperidone **„c+risp"**; 4. Autistic **„a"**; 5. Autistic+probiotic **„a+prob"**; 6. Autistic+risperidone **„a+risp"**

Figure 1 shows bigger weight gain of the control animals compared to the autistic groups. Although there is no statistical significance in the difference, there is a well seen tendency of the control groups (including the Probiotic and risperidone groups) to grow more than the autistic groups.

Discussion

The conventional antipsychotics are often used in patients with ASD, but they might induce severe adverse reactions. [6] Atypical antipsychotics are preferred because they combine efficacy in ameliorating some of the symptoms of ASD with a lower risk of adverse reactions. Risperidone in particular might be effective in reducing hyperactivity, aggressive behavior and repetitive movements without inducing severe side effects. [6] In the same time risperidone and some other second generation antipsychotics are known to worsen specific metabolic characteristics – they might increase the fasting glucose levels and the body weight. [7] Some of these modulations might be also due to epigenetic changes. [8]

Children with autism are often affected by gastrointestinal problems such as abdominal pain, constipation and diarrhea. [9] There is increasing evidence that host-microbe interactions play a key role in maintaining homeostasis. [10] Dysfunction of the microbiome-brain-gut axis has been revealed in neurodevelopmental disorders such as autism. [11] The concept of a microbiome-brain-gut axis is emerging, suggesting microbiota-modulating strategies may be a tractable therapeutic approach for developing novel treatments for CNS disorders. [11] There are some advances in modulating brain development and behavior with the help of probiotics, prebiotics, and diet through the gut microbiota-brain axis. A variety of mechanisms including immune, neural, and metabolic pathways may be involved in change of the gutmicrobiota-brain axis. [12] Probiotic supplementation in children with ASD is a relatively new strategy to modify the gut-brain axis. [12] Furthermore, it is an alternative therapeutic strategy with

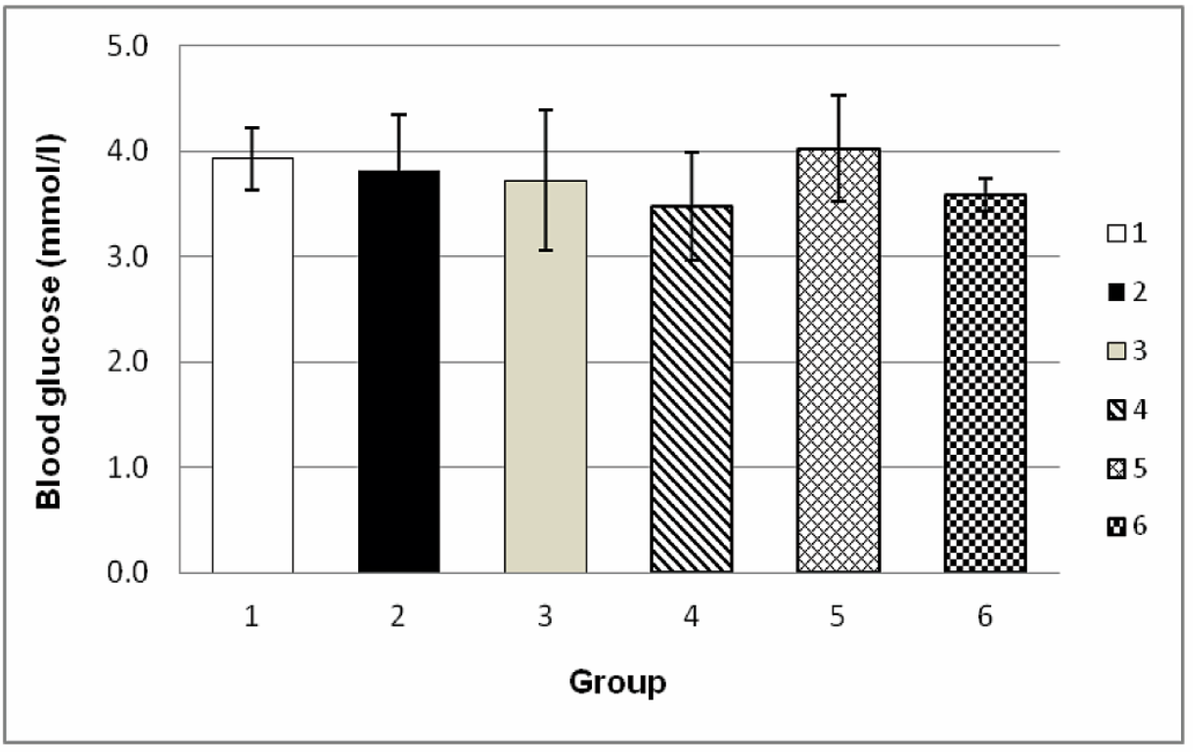

Fig. 2. ***Fasting blood glucose levels at the end of the experiment.***

1. Control **„c"**; 2. Control+probiotic **„c+prob"**; 3. Control+risperidone **„c+risp"**; 4. Autistic **„a"**; 5. Autistic+probiotic **„a+prob"**; 6. Autistic+risperidone **„a+risp"**

Figure 2 demonstrates the fasting plasma glucose levels in the different groups of rats at the end of the experiment. There is no statistically significant difference in the values of the plasma glucose levels between the different groups of rats.

very few or no side effects.

Children with autism spectrum disorder often have gastrointestinal problems resulting in changes in their body weight. [13] Overweight and obesity are more common than underweight in children with ASD. [14] Risperidone increases the risk for increased body weight and obesity. [4] Furthermore, it leads to significant increases in glucose ($p = .02$), hemoglobin A1c ($p = .01$), insulin ($p < .0001$), homeostatic model assessment-insulin resistance (HOMA-IR; $p < .001$), alanine aminotransferase ($p = .01$), and leptin ($p < .0001$). [15] As a conclusion, this second generation antipsychotic might induce the development of insulin resistance and metabolic syndrome but in the same time it decreases severe irritability and aggression in children and adolescents with autism. Probiotics ameliorate the gastrointestinal symptoms in children with ASD and in some research papers they demonstrate weight-lowering effect. [16] In general, however, the effects of probiotics in children with ASD on the body weight and fasting glucose levels are not clear and might be bidirectional.

Conclusions

These findings suggest the importance of increasing the expertise of professionals who work with patients with ASD regarding weight and metabolic issues to improve the quality of life of these individuals and work with them in maintaining healthy condition.

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Ранни промени в сърдечната функция при пациенти с чернодробна цироза и нивата на NT-PROBNP

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Early changes in cardiac function in patients with liver cirrhosis and NT-PROBNP LEVELS

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РЕЗЮМЕ:

Основните клинични признаци на циротична кардиомиопатия включват атенюирана систолна контрактилност в отговор на физиологични или фармакологични стимули, диастолна дисфункция, аномалии в проводимоста и хронотропна некомпетентност [1,7,8,14]. Целта на този дисертационен труд е да установи асоциацията между ранните промени в сърдечната функция при пациенти с чернодробна цироза и нивата на NT proBNP. 62-ма пациенти бяха включени в проучването и всички преминаха - абдоминална ехография, доплерово изследване на v. portae, гастроскопия, ехокардиография, плазмен анализ на NT-proBNP. SPSS версия 16 за Windows беще използван за обработка на данните. Наблюдавахме по-високи серумни нива на NT-proBNP при цироза в сравнение с контролите [5,6]. Също така открихме, че предсърдните обеми, фракцията на изтласкване и масата на лявата камера и ПАПс (систолното пулмонарно артериално налягане) са значително променени в

ABSTRACT

The main clinical features of cirrhotic cardiomyopathy include attenuated systolic contractility in response to physiologic or pharmacologic strain, diastolic dysfunction, electrical conductance abnormalities and chronotropic incompetence. [1,7,8,14]. The aim of this study is to establish the association between early changes in cardiac function in patients with liver cirrhosis and NT- proBNP levels. 62 cirrhotic patients were enrolled. All underwent abdominal ultrasound, upper GI endoscopy, ECG, echocardiography, plasma levels of NT-proBNP were determined. SPSS for Windows, version 16, was used for data analysis. We observed higher NT-proBNP serum levels in cirrhotic compared to controls [5,6]. We also found that atrial volumes, ejection fraction and partially left ventricular mass and PAPs (systolic pulmonary arterial pressure) are significantly altered, as compared to the hypertensive controls. Supporting previous studies [2,4,12], we also found that the mean QTc interval was prolonged in 65% of women and 96% of men. In conclusion, the

сравнение с контролите . Поддържайки предишни проучвания [2,4,12], установихме също, че средният QTc интервал е удължен при 65% от жените и 96% от мъжете. В заключение, настоящото изследване показва, че плазмените нива на NT-proBNP, LAD, E/A съотношението, EDT и E /E 'могат да бъдат надеждни показатели за степента на сърдечните аномалии при пациенти с цироза.

Ключови думи: чернодробна цироза, портална хипертония, циротична кардиомиопатия, диастолна дисфункция.

present study shows that plasma NT-proBNP levels, LAD , E/A ratio, EDT and E/E' may be reliable indicators of the extent of cardiac abnormalities in cirrhotic patients.

Key words: liver cirrhosis, portal hypertension, cirrhotic cardiomyopathy, diastolic dysfunction, prolonged QTc.

INTRODUCTION

At the 2005 World Congress of Gastroenterology at Montreal, a group of experts proposed diagnostic and supportive criteria for cirrhotic cardiomyopathy as follows: (1) systolic dysfunction: blunted increase in cardiac output on exercise, volume challenge or pharmacological stimuli or resting ejection fraction <55 %, (2) diastolic dysfunction: the ratio of early to late (atrial) phases of ventricular filling or E/A ratio <1.0 (age-corrected), prolonged deceleration time (>200 ms), or prolonged isovolumetric relaxation time (>80 ms), (3) supportive criteria: electrophysiological abnormalities, abnormal chronotropic response, electromechanical uncoupling/dyssynchrony, prolonged QTc interval, enlarged left atrium, increased myocardial mass, increased brain natriuretic peptide (BNP) and NT- proBNP, or increased troponin I [14]. Over the past few years, considerable attention has been paid to the role of heart dysfunction markers, BNP and its prohormone - NT pro BNP, which are released from the heart ventricles in response to muscle cell stretching [3,5,16,17]. Given the limited number of studies we were interested in examining the association of the severity of diastolic dysfunction, cirrhosis and NT proBNP levels in patients who were admitted to hospital at St. George Hospital in Plovdiv.

AIM

The aim of this study is to establish the association between early changes in cardiac function in patients with hepatic cirrhosis and NT proBNP levels.

MATERIAL AND METHODS

Fourty two consecutive hospitalized patients with viral -related cirrhosis were studied. All underwent abdominal ultrasound and upper GI endoscopy. We also evaluated a control group of 20 patients with arterial hypertension matched for age and sex. Table 1 shows demographic characteristics of patients included in our study.

Electrocardiography

A 12-lead surface EGG was obtained from all subjects in the supine position immediately before echocardiography by using machine. The ECG was recorded at a paper speed of 25 mm/s.

Echocardiographic assessment

All cirrhotic and control patients underwent M-mode, 2-dimensional, and Doppler echocardiography via trans-thoracic approach to study diastolic and systolic cardiac function.

Plasma NT-proBNP analysis

Blood was drawn from a forearm vein after at least 10 minutes of resting supine, collected in standard sampling tubes for NT-proBNP analysis, and in appropriate tubes for other laboratory determinations.

Statistical Analysis

SPSS for Windows, version 16, was used for data analysis. The qualitative data were analyzed by chi-square and Fisher's exact test. Continuous variables are presented as mean ± standard deviation (SD); categorical

Tabl. 1 ***Demographic characteristics of patients with LC and controls***

	Conrols	Child A	Child B	Child C
Total	20	6	19	17
Man	13	4	13	16
Woman	7	2	6	1
Age	52 ±10	56 ±8	54 ±9	57 ±9

variables are presented as percentages. P value <0.05 was considered significant.Continuous variables were summarized as mean ± SD; categorical variables as frequency and percentage. Univariate linear regression analysis was used to study relationships between NT-proBNP and clinical, biochemical and echocardiographic variables of cirrhotic patients.

Results

Cirrhotic patients 78,6% men had a median age of 62 years, 21,4 % woman had a median age of 60years. The control subjects (mean age 60,6 ± 8,4 years, 12 men и 8 women) were comparable for arterial hypertension prevalence to the cirrhotic population.

Cirrhotic patients had significantly higher NT pro-BNP plasma levels compared to controls. Similarly, left atrial volume (LAV) and left ventricular ejection fraction were significantly altered in cirrhotic patients as compared to controls, and a trend was also observed for left ventricular mass and Systolic Pulmonary Arterial Pressure (PAPs) [tabl.2].

By linear regression analysis, in cirrhotic patients, at univariate analysis, NT-pro-BNP levels were directly related to liver dysfunction and to renal impairment .The observed changes in the E/A ratio, DT, mean pulmonary pressure, LVEDV, interventricular wall thickness, posterior wall thickness (PWT), LV volume and EF are consistent with the progression of the liver disease.

Table 4 shows factors associated with presence of ascites. As expected cirrhotic patients with ascites had a higher impairment of both liver and kidney function compared to their counterpart without ascites. They also had higher NT pro-BNP and CK-MB plasma levels and a trend to larger atrial and to a higher E/A ratio.

The values of all QT interval-related parameters were higher (P < 0.001) in patients with cirrhosis than those in controls. QTc interval was prolonged in 65% of females and 96% of males, supporting the previous studies (31).

DISCUSSION

In our study we observed higher NT-pro-BNP serum levels in cirrhotic compared to controls We also observed that LVEDV is increased proportionally to the severity of the hepatic cirrhosis. We found that hypertrophy of LV, LV and LA dilatation, diastolic and systolic dysfunction of the LV are correlated to the severity of the hepatic cirrhosis. The emer-

Tabl. 2 ***NT pro BNP serum levels and echocardiographic features of 42 cirrhotic patients and 20 matched controls***

	NT pro BNP pg/ml	*CK-MB*	*Mean e' sept / e' lat*	*E Ve m/sl*	*E/e'*	*LA обем (мл)*	*E/A*	*LEVDV ml*	*EF%*	*PAPs mmHg*	*LV mass g/m2*
Patient with cirrhosis	420.2±103.2	43.8±25.6	0.10±0.03	0.77±0.24	8.2±3.1	60.8±27.3	1.07±0.40	92.3±32.3	61.7±6.7	31.4±4.8	83.3±25.0
Controls	68.8±76.6	13.8±9.6	0.11±0.07	0.69±0.18	7.2±2.5	42.5±13.1	1.04±.044	77.9±25.7	66.5±4.01	27.1±1.7	72.9±17.3
P	<0.001	<0.001	0.22	0.14	0.20	0.001	0.70	0.16	0.05	0.08	0.08

Tabl. 3 ***Relationship between NT-pro-BNP levels and clinical and echocardiographic data on linear regression analysis in cirrhotic patients***

	Age	*Sex*	*MELD*	*Child Pugh*	*Albumine*	*Bilirubine*	*PLT*	*INR*	*Creatinine*	*Ascites*	*Mean e' sept/ e' lat*	*E Vel*	*E/e'*	*LA volume(ml)*	*E/A*	*LEVDV*	*EF %*	*PAPs*	*LV mass*
B	0.201	−0.138	0.457	0.407	−0.332	0.191	−0.194	0.420	0.339	0.485	0.059	0.009	0.100	0.316	0.171	−0.008	0.123	0.185	0.210
S.E	0.15	111.38	9.425	22.443	87.110	17.913	0.783	150.649	180.265	88.936	1623.886	224.385	0.001	2.385	123.013	1.628	7.250	12.129	2.640
P	4.566	0.32	0.001	0.002	0.01	0.18	0.17	0.002	0.01	<0.001	0.70	0.94	0.43	0.03	0.24	0.95	0.38	0.28	0.89

Tabl. 4 ***Demographic, clinical, laboratory and echocardiographic features of cirrhotic patients according to presence or absence of ascites.***

	MELD	*Albumine*	*Bilirubine*	*PLT*	*INR*	*CK*	*CK – MB*	*Creatinine* mg/dl	*NT pro BNP*	*Mean e' sept / e' lat*	*E Vel*	*E/e'*	*LA volume (ml)*	*E/A*	*LEVDV*	*EF%*	*PAPs*	*LV mass*
Patients without ascites	5.7±2.9	3.6±0.5	1.0±0.5	98.2±66.6	1.2±0.1	78.9±25.9	15±5.9	0.6±0.1	181.9±155.9	0.09±0.03	0.72±0.20	8.7±3.9	54.7±21.2	0.93±0.41	82.7±38.9	61.6±9.0	29.2±4.5	87.5±24.2
Patients with ascites	12.6±3.9	2.8±0.4	3.0±3.2	85.1±63.3	1.3±0.3	163.2±89.5	35.8.±29.5	1.0±0.2	535.2±408.1	0.10±0.02	0.81±0.26	7.8±2.2	67.2±28.8	1.13±0.45	96.3±27.3	63.6±4.3	31.5±5.1	81.2±23.8
P	<0.001	<0.001	0.005	0.46	0.002	<0.001	<0.001	0.007	<0.001	0.19	0.19	0.32	0.08	0.09	0.13	0.29	0.16	0.38

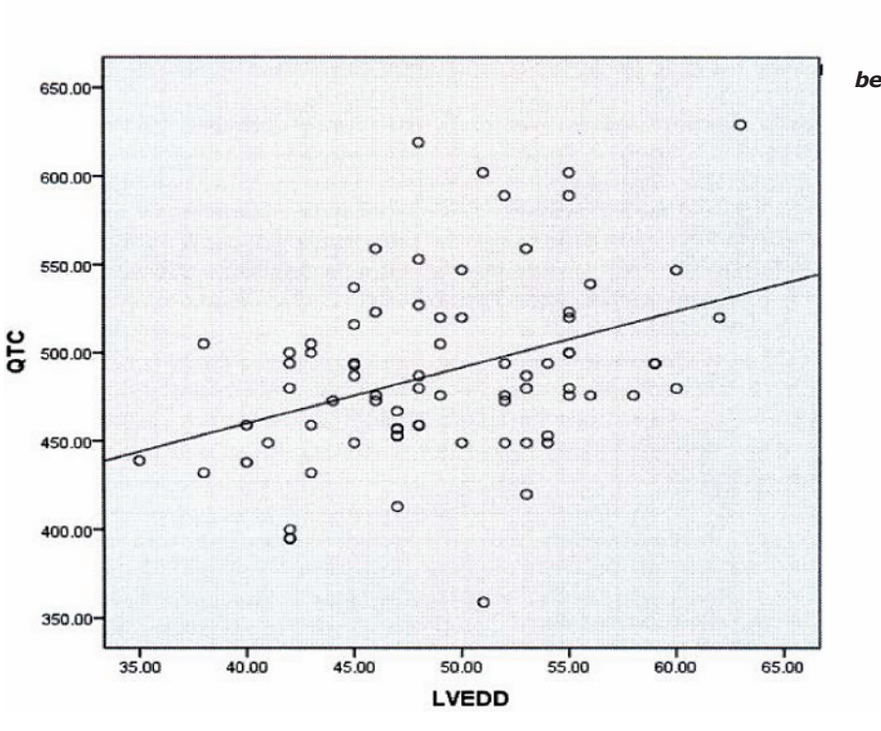

Fig. 12 ***Correlation between LVEDD и QTc***

gence of ascites is a very important moment. It's progression correlates significantly with the dilatation of the left chamber, the degree of diastolic and systolic dysfunction. In this study, we also observed that patients with ascites, compared to those without ascites, have higher serum levels of NT-Pro-BNP and respectively larger atrial volumes. According on published data and our own results, we can conclude that LV hypertrophy, LA and LV dilation as well as Doppler data showing impaired relaxation are early predictive factors for the development of CCM. Concerning the investigated biomarker NT proBNP, we found that its levels correlated with the stage of liver disease. Along this line we also observed higher NT-pro-BNP serum levels in cirrhotic compared to controls. Importantly, a significant correlation was observed between NT-proBNP and Child class, suggesting that plasma NT-proBNP levels are likely to be related to the severity of cirrhosis. Accordingly, our data confirm the hypothesis already reported by Henriksen et al [6], that NT pro-BNP levels could be a marker of cardiovascular diastolic dysfunction in patients with end stage liver disease, acting as mediator of splanchnic vasodilatation in liver cirrhosis [10,13]. In fact, in our cirrhotic patients NT pro-BNP levels were related not only to large atrial volumes, but also to indexes of liver and kidney function [9,11,15]. Overall these data suggest that NT pro-BNP is a marker of cardiac subclinical dysfunction participating to liver decompensation.

CONCLUSIONS

In conclusion, our study shows that cirrhotic patients have larger atrial volumes and biochemical changes (NT pro BNP) showing cardiac dysfunction realated to hepatic decompensation and ascites. It is clinically relevant that NT-proBNP plasma levels are increased proportionally to the severity of cirrhosis. NT-proBNP can be used for an early diagnosis of changes in cardiac structure and function that have already begun in patients with LC.

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Abbreviations

- IVSd - interventricular septum thickness in diastole
- LVPWd - left ventricular posterior wall thickness in diastole
- LVEDD - left ventricular end-diastolic diameter
- LVESD - left ventricular end-systolic diameter
- LVMI - left ventricular mass index
- LAD - left atrial diameter
- LAV - left atrial volume
- PAPs - Systolic Pulmonary Arterial Pressure
- MELD – model of end stage liver disease
- RAAS - renin-angiotensin-aldosterone system
- CCM – cirrhotic cardiomyopathy
- LC – liver cirrhosis
- A - peak late diastolic velocity of mitral inflow;
- E - peak early diastolic velocity of mitral inflow;
- E/A ratio - ratio of peak early diastolic velocity to late diastolic velocity of mitral inflow;
- E/e ratio - ratio of peak early diastolic velocity to early diastolic velocity at LV basal lateral segment
- DT – deceleration time of E wave mitral inflow
- EF - ejection fraction
- BNP - brain natriuretic peptide
- proBNP- pro-brain natriuretic peptide

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