



Bulgarian Academy of
Sciences and Arts

Българска Академия на
Науките и Изкуствата

Bulgarian *medicine*

ISSN 1314-3387

Българска медицина

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Contents

• Andrey Kotzev, Margarita Kamenova Etiology and pathogenesis of gastric cancer	4
• Jivka Ivanova, Mihail Boyanov, Andon Toshev Association of the FokI and BsmI Polymorphisms of the Vitamin D Receptor Gene with the Bone Mineral Density in Postmenopausal Bulgarian Women	12
• Nadejda Madjirova, Stoyanka Velkova Circadian rhythm of temperature and pulse of Moclobemid, Venlafaxine, Fluoxetine and Citalopram	22
• Todor Stoev Psychological Influences Under Stress – Strategy for Prevention and Control	34
• Valeria Tananska Medicine – basics and relevant issues	39
• Svetlin Tsonev, Temenuga Donova, Maria Milanova Women with Cardiac Syndrome X- Depressive Symptoms and a Possibility to be Assesed	44
• R. Dimova, B. Marinov, A. Nachev, A. Tomova, Ph. Kumanov Mayer-Rokitansky-Küster-Hauser syndrome. A case report with brief review of the literature	50
• Chronicle	56
• Author's guidelines	61

Съдържание

• Андрей Коцев, Маргарита Каменова Етиология и патогенеза на стомашния рак	4
• Живка Иванова, Михаил Боянов, Андон Тошев Връзка между полиморфизмите FokI и BsmI на витамин D рецепторния ген и костната минерална плътност на постменопаузални българки	12
• Надежда Маджирова, Стоянка Велкова Циркадиален ритъм на температурата и пулса при лечение с Моклобемид, Венафаксин, Флувоксамин и Циталопрам	22
• Тодор Стоев Психически въздействия при стрес – стратегия за превенция и управление	34
• Валерия Тананска Медицина: основа и съответни проблеми	39
• Светлин Цонев, Теменуга Донова, Мария Миланова Жени с Кардиологичен синдром X- депресивни симптоми и възможност за оценката им	44
• Р. Димова, Б. Маринов, А. Начев, А. Томова, Ф. Куманов Синдром на Mayer-Rokitansky-Küster-Hauser: описание на случай с кратък обзор на литературата	50
• Хроника	56
• Указания за авторите	61

Etiology and Pathogenesis of Gastric Cancer

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Етиология и патогенеза на стомашния рак

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Abstract

Gastric cancer is neoplasm with poor prognosis and high mortality rates. Gastric cancer is the third most prevalent cancer worldwide. There are a lot of studies, but the etiology and pathogenesis of gastric cancer are not yet fully understood. Diet, *H. pylori* infection, inflammation and genetic polymorphism play basic role in the gastric pathogenesis. Gastric carcinogenesis is a multistep and multifactorial process involving genetic end epigenetic disturbances, altered expression of numerous cytokines, proto-oncogenes, tumor-suppressor genes, cell-adhesion molecules and cell-cycle regulators. The lost balance between cell proliferation and cell apoptosis is a key event in initiating stages of gastric carcinogenesis. The influence of the external factors upon the different and complex molecular gastric cancer pathways must be investigated in order to reveal completely the biology of gastric cancer.

Резюме

Стомашният рак е неоплазия с неблагоприятна прогноза и висока смъртност. Стомашният рак е на трето място по честота в света сред малигнените заболявания. Етиологията и патогенезата на стомашния карцином са обект на много проучвания, но все още не са напълно изяснени. Хранителните фактори, инфекцията с *H. pylori*, възпалението и генетичния полиморфизъм играят основна роля в стомашната патогенеза. Патогенезата на стомашния рак при човека е многоетапен процес, който е свързан с генетични и епигенетични нарушения, с променена експресия на многобройни цитокини, прото-онкогени, тумор-супресорни гени, клетъчни адхезионни молекули и регулатори на клетъчния цикъл. Изгубеното равновесие между клетъчната пролиферация и апоптоза е ключов момент в иницирането на стомашната карциногенеза. За по-пълното разкриване на

Key words:

gastric cancer, carcinogenesis, *H. pylori*, inflammation, apoptosis, proliferation

Introduction

Gastric cancer is the third most common cancer worldwide and has very poor prognosis. Although the incidence of gastric cancer is declining, this aggressive disease is still the second cause of cancer mortality. The incidence of gastric cancer varies geographically, with highest rates in Japan, China, Eastern Asia, Eastern Europe, Central and South America, while North America, Western Europe, Australia and parts of Africa are considered as low-risk areas (29). Adenocarcinoma of the stomach comprises 90-95% of all stomach malignancies and histologically is classified as intestinal or diffuse subtype (33). Intestinal subtype is usually sporadic well differentiated cancer and occurs in endemic areas, whereas diffuse subtype is predominantly poorly differentiated or undifferentiated, and much rarer. In recent years, the incidence of the cardia and gastroesophageal junction cancers is steadily increasing (15). Atrophic gastritis, gastric adenomas and subtotal gastrectomy are pre-malignant conditions for developing of the gastric cancer. Patients with familial adenomatous polyposis (FAP), hereditary non-polyposis colon cancer (HNPCC), and Peutz-Jeghers syndrome are at elevated risk of gastric cancer.

Pathogenesis of gastric cancer

The pathogenesis of gastric cancer is a multifactorial process, which is still not fully elucidated. Environmental factors and genetic abnor-

биологията на стомашния рак е необходимо да се изследва влиянието на външните фактори върху различните и сложни молекулярни пътища на стомашната карциногенеза.

Ключови думи:

стомашен рак, карциногенеза, *H. pylori*, възпаление, апоптоза, пролиферация

malities promote the gastric carcinogenesis. The intestinal subtype of cancer is characterized with a multistep progression through chronic atrophic gastritis, intestinal metaplasia, dysplasia, and ultimately to cancer. In contrast, diffuse subtype of gastric cancer does not follow such a consecutive pattern and no known precursor lesion is found. The most important exogenous risk factors for gastric cancer are infection with *H. pylori* and diet.

H. pylori and gastric cancer

H. pylori infection is the primary cause for gastric carcinogenesis. *H. pylori* has been labeled as a group I carcinogen for the development of noncardia gastric cancer by the International Agency for Research on Cancer (IARC) in 1994 (2). Epidemiological data and animal studies demonstrate that *H. pylori* infection is strongly associated with gastric cancer. The incidence of gastric cancer is high in countries with high incidence of *H. pylori* infection, and this increases the risk for developing gastric cancer two- to eightfold (30). The development of atrophic gastritis is a key event in the initiation of the gastric cancer, and regression of gastric atrophy and intestinal metaplasia is reported as a result of eradication of *H. pylori* (12). In most infected patients, *H. pylori* causes a mild pangastritis, but some people develop predominantly antral or corpus gastritis. Host acid output is associated with the topographical distribution of *H. pylori* invasion. The individuals with low acid output are predisposed to develop multifocal atrophic

gastritis and have a higher risk for gastric cancer than people with high acid output in whom *H. pylori* colonization and inflammation are restricted to the antrum. The mechanisms of tissue damage elicited by *H. pylori* include release of cytotoxins, phospholipase, toxic ammonia, proinflammatory cytokines and reactive oxygen species (ROS) in the gastric epithelium (24). ROS could injure the genomic DNA and promote the cancer carcinogenesis. Consequences of *H. pylori* infection are also elevated gastrin levels, downregulated vitamin C levels and achlorhydria, which are additionally prerequisites for the development of gastric cancer (46). It seems that the inflammatory answer started by *H. pylori* is more harmful to the host than the bacteria.

H. pylori plays a dual role for sustenance of the equilibrium between apoptosis and proliferation in gastric mucosa. Initially, *H. pylori* infection increases apoptosis directly or indirectly by inducing the expression of Bak, Fas receptor (CD95), CD95L, INF- γ , IL-1 β , TNF- α , TNF- α receptors, cytochrome c and TGF- β , and induces atrophy. Nevertheless, this event is transitory and enhanced cell turnover occurs as a compensatory reaction, probably combined with lessened apoptosis. Induction of proinflammatory cytokine macrophage migration inhibitory factor (MIF) by *H. pylori* infection leads to increased Bcl-2 expression, and downregulation of p53 and apoptosis (6). A study found that *H. pylori* directly promotes gastric carcinogenesis by diminishing the expression of E-cadherin, but recent data challenge this finding (44, 5). Finally, if hyperproliferative state of the gastric cells remains permanent, malignant process could be triggered. Therefore, it could be presumed that *H. pylori* infection stimulates cell cycle progression and could lead to disturbed balance between apoptosis and proliferation in the pathogenesis of gastric cancer.

Virulence factors are very important for the oncogenic role of *H. pylori*. Cytotoxin-associated gene-A (CagA) is the main virulence factor of *H. pylori*. CagA is injected in the gastric epithelial cells by the type IV secretory system followed by tyrosine phosphorylation and activation of the cytoplasmic Src homology-2 domain containing protein tyrosine phosphatase-2 (SHP2). The complex CagA-SHP2 stimulates cell

turnover, migration and adhesion. CagA induces the expression of nuclear factor κ B (NF- κ B), which produces the proinflammatory cytokine IL-8 (23). NF- κ B serves as a key mediator in promoting and sustaining the unrestrained cell proliferation in the history of *H. pylori*-associated gastritis. Toll-like receptors (TLRs) identify pathogen-associated molecular patterns and play a crucial role in the initial innate immune response to *H. pylori* infection. TLRs are activated by multiple parts of the bacterium, including CagA, followed by induction of inflammatory signaling pathways and expression of NF- κ B, mitogen-activated protein kinase (MAPK), IL1, IL8, TNF- α , iNOS, COX-2, vascular endothelial growth factor (VEGF), chemokines, matrix metalloproteinases and adhesion molecules (48) (Fig. 1). CagA promote and cell motility by the activation of the HGF/scatter factor receptor c-Met (11). p53 tumor suppressor pathway is suppressed in patients with CagA-positive *H. pylori* strains and these patients have higher risk of gastric cancer compared with patient with CagA-negative strains (8). Vacuolating cytotoxin (VacA) is another virulence factor of *H. pylori*, which acts like an anion channel. The precise role of VacA in gastric carcinogenesis is obscure, but strains with sl/ml allele are connected with increased risk of gastric cancer (13).

Diet and gastric cancer

The role of diet in gastric cancer has been investigated in many epidemiological studies. Most of these studies reveal that high consumption of salt, red and processed meat, refined carbohydrates, salted food, pickled or smoked foods and saturated fat is connected with elevated risk of gastric cancer (1, 20). Contradictory results are available from the studies investigating the impact of the presumable protective diet on gastric cancer. Evidence for the protective role of fresh vegetables and fruits, as well as fiber rich cereals, whole grain, green tea, and micronutrients (vit. A, vit. B6, vit. C, vit. E, carotenoids, flavonoids, selenium, zinc) intake exists, but the outcome of some studies question these data (1, 25). The role of cigarette

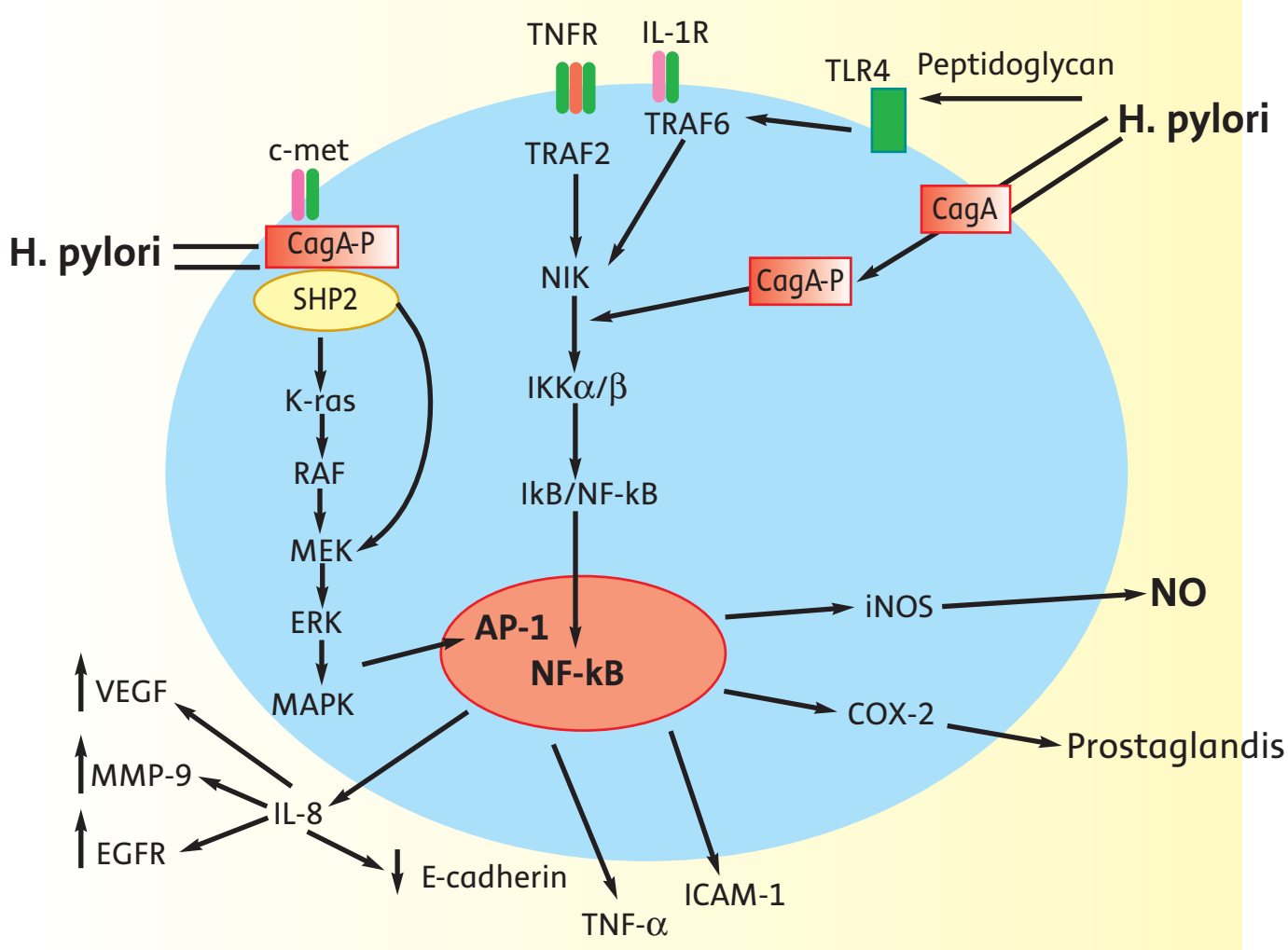


Figure 1. Mechanism of action of *H. pylori* in the gastric epithelium. ICAM= Inter-cellular adhesion molecule-1; MMP-9=Matrix metalloproteinase-9; iNOS=inducible nitric oxide synthase; SHP2= Src homology-2 domain containing protein tyrosine phosphatase-2. AP-1=Activating protein-1.

smoking and alcohol intake in pathogenesis of gastric cancer is disputable, but recent data demonstrated their significance as risk factors (36). Acetaldehyde exposure, associated with cigarette smoking and alcohol consumption, is accepted as a local carcinogen and risk factor of gastric cancer (41). Consumption of dietary nitrosamines also increases the risk of stomach cancer (32).

Host genetic polymorphism and gastric cancer

Genetic polymorphism of the host plays a significant role for the development of gastric cancer. Polymorphism of the genes that code

pro-inflammatory cytokines could influence the extent of inflammatory response to *H. pylori* infection. IL-1 β is a powerful pro-inflammatory cytokine and acid inhibitor. IL-1 β -511T and TNF- α -308A carriers are at increased risk of gastric cancer, although some ethnic differences exist. (18). Genetic polymorphism of metabolic enzymes defines the individual differences in ability to detoxify chemical carcinogens.

CYP2E1 is a cytochrome P450 isoenzyme which participates in metabolism of carcinogens and catalyzes the activation of different nitrosamines. CYP2E1 C2 Asian carriers are at elevated risk of developing gastric cancer (7). GSTM1 is a major part of the glutathione S-transferase (GST) superfamily and evidence exists that

GSTM1-null genotype is associated with elevated gastric cancer risk (49). Single nucleotide polymorphisms (SNPs) have been detected in the enzyme folate metabolism methylenetetrahydrofolate reductase (MTHFR). MTHFR 677C>T increases the gastric cancer risks in East Asians, but not Caucasians (51). It could be presumed that genetic susceptibility of the host interacts with the environmental risk factors, exert essential influence and modulate the gastric cancer risk.

Host immune response and gastric cancer

Host T cell immunity is crucial for the pathogenesis of *H. pylori* infection. The progression to gastric atrophy and cancer in patients with *H. pylori* infection is dependent on a Th1 (pro-inflammatory) immune response (3). Intracellular pathogens, including bacteria, induce Th1 responses, whereas extracellular pathogens like helminthic parasites provoke polarized Th2 responses. A recent study found that *S. japonicum* co-infection with *H. pylori* is associated with alterations in IgG responses to *H. pylori* and less gastric atrophy (17). Possible reason is that concurrent helminthic infection downregulates the acute proinflammatory Th1 immune response. Intestinal helminthiasis is widespread in people in developing countries, and therefore predominantly Th2 response to infection with *H. pylori* is observed, whereas people in developed countries mount predominantly Th1 response.

These data could explain and the so called „African enigma“, characterized with high rates of *H. pylori* infection in some parts of Africa, but with low incidence of gastric cancer.

Molecular biology of gastric cancer

The considerable advance in the knowledge of the molecular basis of human cancer improve the understanding of multiple molecular alterations in the gastric cancer. The pathogenesis of gastric cancer is connected with

genetic and epigenetic abnormalities. Disturbed balance between cell proliferation and apoptosis is a hallmark of stomach cancer, which is associated with overexpression of growth factors and protooncogenes, and enhanced suppression of proapoptotic pathways. Amplification of the EGF receptor type II (c-erbB-2/neu) is documented in intestinal-type gastric cancers, but is rarely seen in diffuse type (40). In contrast, K-sam oncogene, a member of the fibroblast growth factor receptor family, is expressed predominantly in diffuse type of gastric cancer, and K-sam positive patients have unfavorable prognosis (45). C-met oncogene which encodes a tyrosine kinase receptor for hepatocyte growth factor is also overexpressed mainly in diffuse gastric cancers and associated with poor survival (16). Overexpression of the C-myc oncogene has been reported in over 40% of gastric cancers and is found in early-onset gastric cancers (45). BMI1 which is a key protein in the Polycomb group (PcG) proteins, acts as an oncogene in gastric cancer and BMI1 overexpression is correlated with advanced clinical stage and lymph node metastasis (50). Signal transducers and activators of transcription (STAT) act as signal transducers in the cytoplasm and as transcription factors in the nucleus, and STAT3 expression in gastric cancer indicates poor prognosis (14). The expression of anti-apoptotic protein Bcl-2 is increased in gastric dysplasia as well as carcinoma (9). The expression of the proliferative marker Ki-67 is also elevated in gastric cancer, and is correlated with higher rate of metastatic lymph nodes and advanced disease (47). Telomerase reactivation is a common event in gastric adenocarcinoma, but it is not related to histopathological parameters (22). Expression of the cell surface receptor CD44v6 and matrix metalloproteinase-7 (MMP)-7, and VEGF-D in gastric cancer is correlated with distant metastatic disease (38, 42).

Inactivation of tumor suppressor genes and proteins is an inherent feature of the stomach carcinogenesis. p53 is the most frequently mutated tumor suppressor gene in gastric carcinogenesis. Overexpression of p53 protein is associated with poor survival, aggressive pathological features and could be used as a predictive

biomarker for developing and recurrence of gastric cancer (27). Aberrant methylation of the tumor suppressor and cell-cycle regulator p16 is observed frequently in lymphatic-invasive gastric carcinomas (21). The loss of expression of pRb is correlated with poor outcome, whereas p21 expression is associated with favorable prognosis in patients with gastric cancer (31). Reduced expression of cyclin-dependent inhibitor p27 is detected in nearly 50% of gastric cancers (39). Runt-related transcription factor 3 (RUNX3) acts like a tumor suppressor of gastric cancer and is implicated in gastric carcinogenesis. RUNX3 expression is reduced or lost in 60% of the primary gastric cancers and loss of the RUNX3 is detected in nearly 90% of advanced gastric cancers (34). The frequency of RUNX3 methylation is significantly increased in patients with advanced gastric lesions and could be used as an independent prognostic factor and a possible therapeutic target for gastric cancer (10). Suppression of proapoptotic Bax protein is early molecular event during gastric carcinogenesis (9). Tumor hypoxia is an indispensable attribute for the development of gastric cancer. Increased expression of hypoxia-inducible factor (HIF)-1 α is reported in carcinogenesis of stomach cancer, and HIF-1 α and p53 positive tumors are often presented with undifferentiated type and infiltrative growth pattern (43). Inactivation of E-cadherin by mutation and hypermethylation is found predominantly in cases with diffuse gastric cancer, whereas E-cadherin mutation is rarely found in intestinal type. Germ-line mutations in E-cadherin (CDH1) are associated with loss of E-cadherin function and the familial form of diffuse gastric cancer, hereditary diffuse gastric cancer (4). Patients with abnormal expression of E-cadherin and positive expression of MUC1 have unfavorable prognosis (37).

Chromosomal abnormalities and loss of heterozygosity (LOH) are considered nonspecific in the development of both subtypes of gastric adenocarcinoma. Gastric cancers with high-level microsatellite instability (MSI-H) are characterized by antral location, intestinal type of cancer and have better prognosis (19). In contrast, a recent study found that mitochondrial microsatellite instability (mtMSI) in gastric dysplasia is associated with poor prognosis (28).

The role of stem cells in oncology has been extensively investigated during the last years. A study found that stem cells of bone marrow origin migrated and proliferated in the mouse stomach epithelium after *Helicobacter* infection. After 20 weeks these marrow-derived cells differentiated in gastric epithelial cells, and finally by 52 weeks early gastric cancer occurred (26). These data support the putative role of the stem cells in the pathogenesis of gastric cancer, but more studies are needed this hypothesis to be validated.

Although the great progression in elucidation of the molecular biology of gastric cancer, the pathogenesis of gastric cancer still remains unknown. It is difficult to be determined with certainty which of the changed signaling pathways is characteristic only of gastric cancer. It is still impossible also to separate the causal molecular alterations from the incidental ones and from those which are simply result of the tumor development. Gene-environment interaction is of extreme importance in pathogenesis of gastric cancer. Hence, future studies must explore not only the genetic aberrations in this lethal disease, but also the influence of exogenous risk factors (*H. pylori*, diet, lifestyle) upon intimate molecular mechanism in carcinogenesis of gastric cancer.

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Association of the FokI and BsmI Polymorphisms of the Vitamin D Receptor Gene with the Bone Mineral Density in Postmenopausal Bulgarian Women

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Връзка между полиморфизмите FokI и BsmI на витамин D рецепторния ген и костната минерална плътност на постменопаузални българки

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Abstract

Numerous studies on vitamin D receptor (VDR) gene polymorphisms differ with conflicting data in various populations. The aim of this research project was to study the association of the FokI and BsmI polymorphisms of the vitamin D receptor (VDR) gene with the bone mineral density (BMD) in postmenopausal Bulgarian women.

Methods: 400 women referred for bone densitometry participated. BMD was measured by X-ray absorptiometry (on a DTX-100 device, Osteometer Meditech USA, and on a QDR 4500

Резюме

Различните проучвания върху полиморфизмите на витамин D рецепторния (VDR) ген са дали противоречиви резултати.

Цел на настоящето проучване бе да изследваме връзката между полиморфизмите FokI и BsmI и костната минерална плътност (КМП) на постменопаузални българки.

Методи: участваха 400 жени, насочени за остеогензиометрия. КМП беше измерена с рентгенова абсорбциометрия (апарати DTX-100 на фирма Osteometer

A device, Hologic Inc., Bedford, USA). The genetic analysis was performed on lymphocytes from whole blood.

Results: The calculated relative risk (RR) for low bone mineral density is higher in the presence of the FokI marker (3,67) compared to the BsmI marker (2,30). The association between the investigated polymorphisms and low BMD on a population level was expressed by the etiological factor: EF=0,55 for the FokI marker and EF=0,45 for the BsmI marker. We concluded that the FokI and BsmI polymorphisms were closely related to low BMD at the forearm, lumbar spine and femoral neck. Further studies of larger cohorts and in ethnically diverse subgroups are necessary to assess the role of both polymorphisms as genetic markers determining osteoporosis risk.

Key words:

osteoporosis; vitamin D receptor; bone mineral density; polymorphisms; FokI and BsmI

Mediatech, USA и QDR 4500 A на фирма Hologic Inc., Bedford, USA). Осъществи се генетичен анализ на съответните полиморфизми в лимфоцити от венозна кръв.

Резултати: Изчисленият относителен риск (RR) за ниска КМП е по-висок в присъствието на маркера FokI (3,67 пъти) отколкото в присъствието на BsmI (2,30 пъти). Връзката между изследваните полиморфизми и ниската КМП на популационно ниво, оценена посредством етиологичния фактор (EF), бе значима – EF=0,55 за маркера FokI и EF=0,45 за BsmI. В заключение, полиморфизмите FokI и BsmI на VDR гена са свързани с ниската КМП на лумбалните прешлени, бедрената шийка и предмишницата. Необходими са допълнителни проучвания в етнически различни и големи групи за цялостна оценка на генетичните полиморфизми като фактор за ранно откриване на повишен риск от остеопороза.

Ключови думи:

остеопороза, рецептор за витамин D, костна минерална плътност, полиморфизми FokI и BsmI

Introduction

Bone mineral density (BMD) is a major determinant of fracture risk and has an important genetic background. The first gene suspected to determine BMD was the vitamin D receptor (VDR) gene (15,17). It is localized on chromosome 12q13-14 in a region that contains other genes of interest to bone molecular biology - the collagen type2 α 1 (COL2A1) and the 1 α -hydroxylase genes (2,14). The VDR gene has at least 11 exons and spans 60 kb (10, 18). Several polymorphic variants described so far include a cluster of linked sites near or in exon IX (BsmI, ApaI, TaqI and Long/Short polyA track) and a FokI site in exon II, containing the initiation codon (10).

In 1992 Morrison et al. found an association between bone turnover and several polymorphisms at the 3'-end of the VDR gene

defined by the restriction enzymes BsmI, ApaI, and TaqI (16). Two years later they reported a close association between VDR-BsmI genotype BB and low BMD in a twin study (15).

In 1996 another locus of the VDR gene was associated with BMD. Gross et al. identified an initiation start codon polymorphism at the 5'-end of the VDR gene associated with an individual's risk for osteoporosis (8). These polymorphisms defined by the restriction enzyme FokI showed a translation product of VDR with a difference in length of three amino acids depending on the allelic variants (7).

Since the initial data for an association of these VDR polymorphisms with BMD, studies on different populations have showed controversial conclusions and some investigators have

found an association of these polymorphisms with BMD (7,8,12,13,15,16) while others have not (6).

The human *VDR* DNA presents two potential translation initiation (ATG) codons in exon II (8). A T/C polymorphism (ATG to ACG) has been shown at the first ATG which is referred as a start codon polymorphism (SCP). Initiation of translation from the second AUG shortens the *VDR* by three amino acids (1). Such a difference might contribute to altered receptor function in contrast to the silent polymorphism in intron VIII and exon IX. In the study of Aria et al. the shorter form of the *VDR* showed 1,7-fold greater transcriptional activation in HeLa cells than the longer form (1). The restriction endonuclease FokI can detect the SCP (7-9). *VDR* alleles with the first ATG are described by f (the longer form of 427aa, named M1) and *VDR* alleles without the first ATG are described by F (the shorter form of 424aa, named M4). The shorter form F or M4 is present in 65% of *VDR* alleles in humans.

The aim of this study was to examine the association of these *VDR* gene polymorphisms with BMD in a random population sample of Bulgarian menopausal women. This work is the final report of a study, whose preliminary results were published elsewhere (11).

Materials and methods

Subjects

400 unrelated menopausal Bulgarian women were recruited for the study. Diseases and medications known to affect bone metabolism were used as exclusion criteria. All subjects were grouped according to their BMD. 220 participants had low bone density (and were referred to as cases) and 180 had normal BMD (and were used as controls). The age of the participants ranged between 36-56 yrs among the cases and between 34-58 years in controls respectively. All participants gave their informed consent. This work has been approved by the responsible authorities at the Alexandrovska Hospital.

Bone densitometry

BMD was measured at the distal forearm by single-energy X-ray absorptiometry (SXA) on a DTX-100 Unit (Osteometer Meditech, USA) and at the lumbar spine (L1-L4) by dual-energy X-ray absorptiometry (DXA) on a Hologic QDR 4500 A device (Hologic Inc., Bedford, MA, USA).

On the DTX-100 the distal region of interest begins at the 8 mm separation point between radius and ulna and then continues proximally for a distance of 24 mm. The ultra-distal site extends from the radial endplate proximally to the 8 mm point. The manufacturer's Danish database was used (issued 1994).

BMD of the lumbar spine and femoral neck in the posterior-anterior (PA) projection was measured on a Hologic QDR 4500 A densitometer; with software version 8.26:3 (Hologic, Inc., Bedford, USA). The manufacturer's American reference database was used (issued 1991) (42).

Genotyping

DNA was isolated from whole blood.

Primers and PCR conditions for amplifying exon 2 of the *VDR* gene were designed accordingly to Gross et al. (18). The region of genomic DNA containing the BsmI polymorphic site in intron 8 was amplified as described by Ingles et al. (6).

dATP, dCTP, dTTP, dGTP – 1,25 mM each were used to amplify exon 2 and intron 8 with Taq DNA Polymerase. 100 ng of the DNA were used as template in the PCR reactions.

Primers (2a and 2b) flanking exon II were used to amplify a 265 bp PCR product that is then digested with FokI. Digestion of the PCR product with FokI generates two fragments of 196 bp and 69 bp. Individuals homozygous for the FF genotype have a single uncut 265 bp fragment, while homozygous for the ff genotype have two fragments of 196 bp and 69 bp. The heterozygotes Ff have all three bands.

The polymorphic region was located in intron VIII at 280 bp from the 5' start of the intron without amino acid change but disappearance of the restriction site for BsmI. BsmI

cuts the b allele of the *VDR* gene but not the B allele. The primers (U and L) were used to amplify 821 bp PCR product which was then digested with *BsmI*. Digestion of the PCR product with *BsmI* generates two fragments of 650 bp and 175 bp. Individuals homozygous for the BB genotype have a single uncut 821 bp fragment, while homozygous for the bb genotype have two fragments of 650 bp and 175 bp. The heterozygotes Bb have all three bands.

PCR products were digested with *FokI* and *BsmI* for 4h and electrophoresed through a 2% agarose gel. Individuals were scored as FF, Ff, ff and BB, Bb, bb according to the digestion pattern.

Statistical analysis

The relative risk (RR) was defined as:

$$RR = \frac{a \times d}{b \times c} \quad \text{where:}$$

a is the number of carriers among the cases

b is the number of not carriers among the cases

c is the number of carriers among the controls

d is the number of not carriers among the controls

The etiological factor (EF) was defined as:

$$EF = \frac{RR - 1}{RR} = \frac{a}{a + b}$$

The EF shows what part of the disease might be attributable to the studied polymorphisms on a population level.

Data were evaluated by χ^2 -test and presented as means \pm SD

Results

The *FokI* marker

The distribution of genotypes and alleles by *FokI* in the subgroups of cases and controls is

shown in Table 1. The genotype frequencies found in our preliminary report were 0,25 for FF, 0,43 for Ff, 0,32 for ff in cases and 0,52 for FF, 0,45 for Ff, 0,03 for ff in controls. The allelic frequencies were 0,47 (F), 0,53 (f) in cases and 0,75 (F), 0,25 (f) in controls (43). Allelic and genotype frequencies were calculated in cases (with low BMD and/or osteoporosis) and in controls (normal BMD), and in the homo- and heterozygotes (see Table 1). The results are statistically significant after χ^2 -test ($p < 0,05$).

When compared with genotype frequencies in cases with low BMD and in controls more common is ff in cases (28%) than in controls (6%). Less common in cases are found FF (25%) compared with controls (54%). The frequency of heterozygotes is higher in cases (48%) than in controls (40%). The statistical significance of these results was defined after a χ^2 -test (Table 1). P was $< 0,05$.

The correlation between the *FokI* genotype and BMD at the forearm site is shown in Fig. 1. Higher BMD was found in FF individuals and lower in ff individuals. The correlation remained unchanged when introducing BMD values (T-score) (see Fig. 1).

The relationship between the different genotypes *FokI* and BMD at the lumbar spine and femoral neck is shown in Fig. 2.

The *BsmI* marker

The distribution of genotypes and alleles by *BsmI* in the subgroups of cases and controls is shown in Table 2. The genotype frequencies found in our preliminary report were 0,33 for BB, 0,38 for Bb, 0,29 for bb in cases and 0,06 for BB, 0,44 for Bb, 0,50 for bb in controls. The allelic frequencies were 0,52 (B), 0,48 (b) in cases and 0,28 (B), 0,72 (b) in controls (43). Allelic and genotype frequencies by *BsmI* as defined in cases (with low BMD and/or osteoporosis) and in controls, and in the homo- and heterozygotes, are presented in Table 2. The results were statistically significant – the χ^2 -test ($p < 0,05$).

The BB genotype was more common in cases with low BMD and/or osteoporosis (35%) vs. 11% in controls with normal BMD and inversely the bb genotype was less common in cases (2%) than in controls (38%) (Table 2). The

Table 1. Distribution of genotypes and alleles by FokI

FokI	cases			controls		
	FF	Ff	ff	FF	Ff	ff
Genotype						
Number	54	105	61	98	72	10
Frequency	0,25	0,48	0,28	0,54	0,40	0,06
H0	0,50			0,38		
X ² / p	52,04 / p<0,05, df=2					
PIC	0,37					
Alleles	F	f		F	f	
Number	213	227		268	92	
Frequency	0,48	0,52		0,74	0,26	
X ² / p	55,98 / p<0,05, df=1					

heterozygotes Bb were more common in controls (51%) compared with cases (45%). Statistical significance of these results as defined from χ^2 -test was within the limits ($p < 0,05$).

The correlation between the genotype and BMD at the forearm is shown in Fig. 3. The relationship between these genotypes and the lumbar spine and femoral neck BMD is

shown in Fig. 4.

The published frequencies for both alleles under study are presented for visual comparison in Figs. 5 and 6.

The relative risk (RR) for low BMD and/or osteoporosis in the presence or absence of a given marker was as follows:

For the FokI marker - RR=3,67 and for the BsmI marker - RR=2,30

The EF showing what part of the illness is attributable to the associated factor on a population level was as follows:

For the FokI marker EF=0,55 and for the BsmI marker EF=0,45

The EF for both markers shows that a more substantial part of the illness is associated with the FokI (55%) than with the BsmI (45%). This suggests that the FokI marker might be more informative.

Figure 1.

BMD (in g/cm²) of the distal and ultradistal forearm for the different genotypes by FokI. Data are shown as means \pm 1SD.

$p=0,22$ for FF vs. Ff, $**p<0,01$ for FF vs. ff at the Distal forearm site, $*p=0,04$ for FF vs. Ff, $*p=0,05$ for FF vs. ff at the Ultradistal forearm site

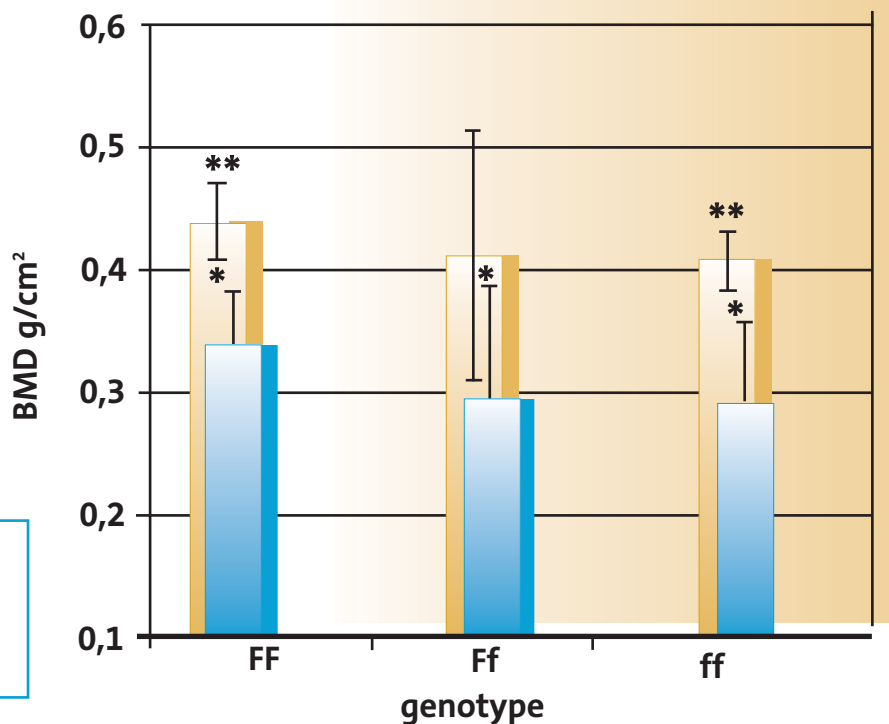
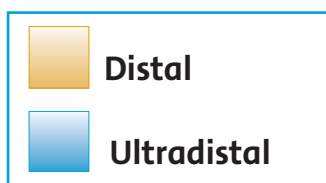


Table 2. Distribution of genotypes and alleles by BsmI

BsmI	cases			controls		
	BB	Bb	bb	BB	Bb	bb
Number	76	98	46	20	92	68
Frequency	0,35	0,45	0,21	0,11	0,51	0,38
H0	0,49			0,46		
χ^2 / p	33,44 / $p < 0,05$, $df=2$					
PIC	0,36					
Alleles	B	b	B	b		
Number	250	190	132	228		
Frequency	0,57	0,43	0,37	0,63		
χ^2 / p	32,23 / $p < 0,05$, $df=1$					

Discussion

The *VDR* gene is known as a candidate gene determining a part of the genetic basis of osteoporosis (3-5,19,20). While the other studied polymorphisms do not alter the sequence of the *VDR* protein, the FokI polymorphism encodes alternate proteins that differ in length by three amino acids (5,9). That probably influences

the function of *VDR* and contributes to the differences in BMD. The BsmI intronic polymorphism could be relevant for translational differences of the 5' *VDR* (a „functional polymorphism“ which is rather unlikely) or might act in strong linkage disequilibrium with a putative functional one in the *VDR* or a nearby gene.

The observed allelic frequencies in controls and cases were compared with published allelic frequencies in European populations (4,19) as follows: 60-69% for F allele and 31-40% for f allele, 37-45% for B allele and 52-67% for b allele; and with published allelic frequencies in Asian populations (32,34): 62% for F allele and 38% for f allele, and 5-12% for B allele and 88-95% for b allele respectively, normative for menopausal women (see Fig. 5 and Fig. 6).

Figure 2.

BMD (in g/cm^2) of the lumbar spine (L1-L4) and femoral neck for the different genotypes by FokI. Data are shown as means $\pm 1SD$. * $p=0,02$ for FF vs. Ff, * $p=0,03$ for FF vs. ff at the Lumbar spine $p>0,05$ for FF vs. Ff, $p>0,05$ for FF vs. ff at the Femoral neck

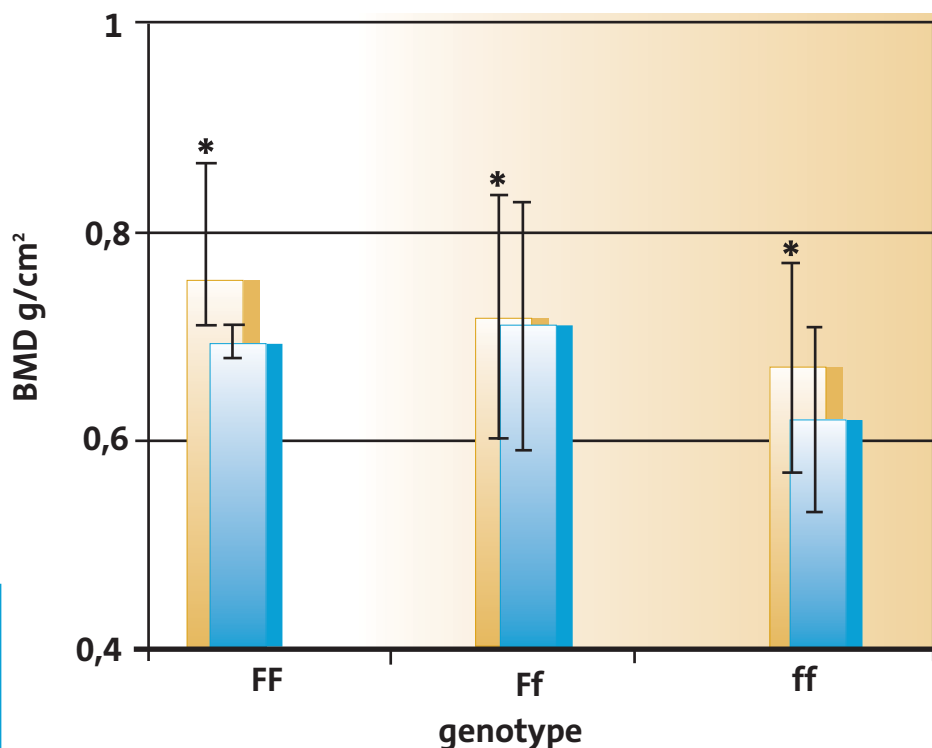


Figure 3.

BMD (in g/cm²) of the distal and ultradistal forearm for the different genotypes by BsmI. Data are shown as means ±1SD.

*p=0,03 for bb vs. Bb, **p=0,01 for bb vs. BB at the Distal forearm site, p>0,05 for bb vs. Bb, p>0,05 for bb vs. BB at the Ultradistal forearm site

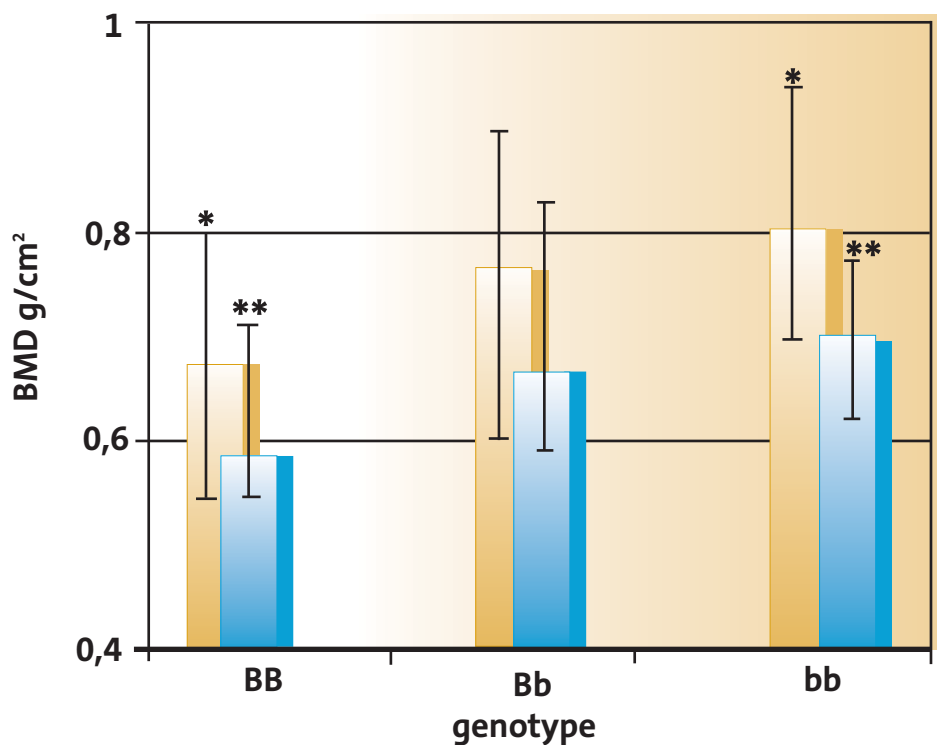
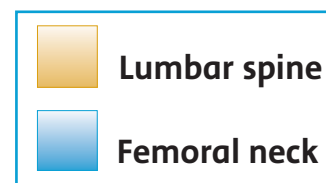
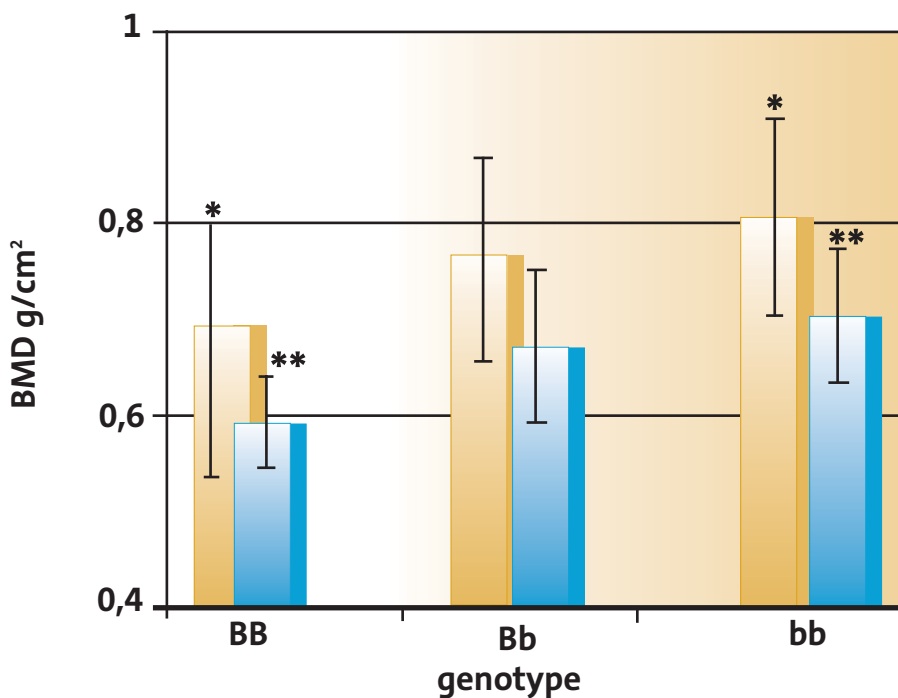


Figure 4.

BMD (in g/cm²) of the lumbar spine (L1-L4) and femoral neck for the different genotypes by BsmI. Data are shown as means ±1SD. p>0,05 for bb vs. Bb, *p=0,02 for bb vs. BB at the Lumbar spine p>0,05 for bb vs. Bb, **p=0,01 for bb vs. BB at the Femoral neck



The genotype frequencies of controls (N=180) from this study are 54% for FF, 40% for Ff and 6% for ff. In comparison they were 52% for FF, 45% for Ff and 3% for ff in the preliminary study in controls. The genotype frequencies of cases (N=220) are 25% for FF, 48% for Ff and 28% for ff (see Table 1). In comparison they were 25% for FF, 43% for Ff and 32% for ff in the preliminary study (43). The compared groups of

Bulgarian and European and Asian populations differ by distribution of allelic frequencies. We compared these frequencies with published genotype frequencies for European populations (4,19): 28-48% for FF, 41-58% for Ff and 6-16% for ff and with genotype frequencies for Asian populations (1): 36-37% for FF, 49-51% for Ff and 12-15% for ff (defined in menopausal women). Current allelic frequencies do not differ

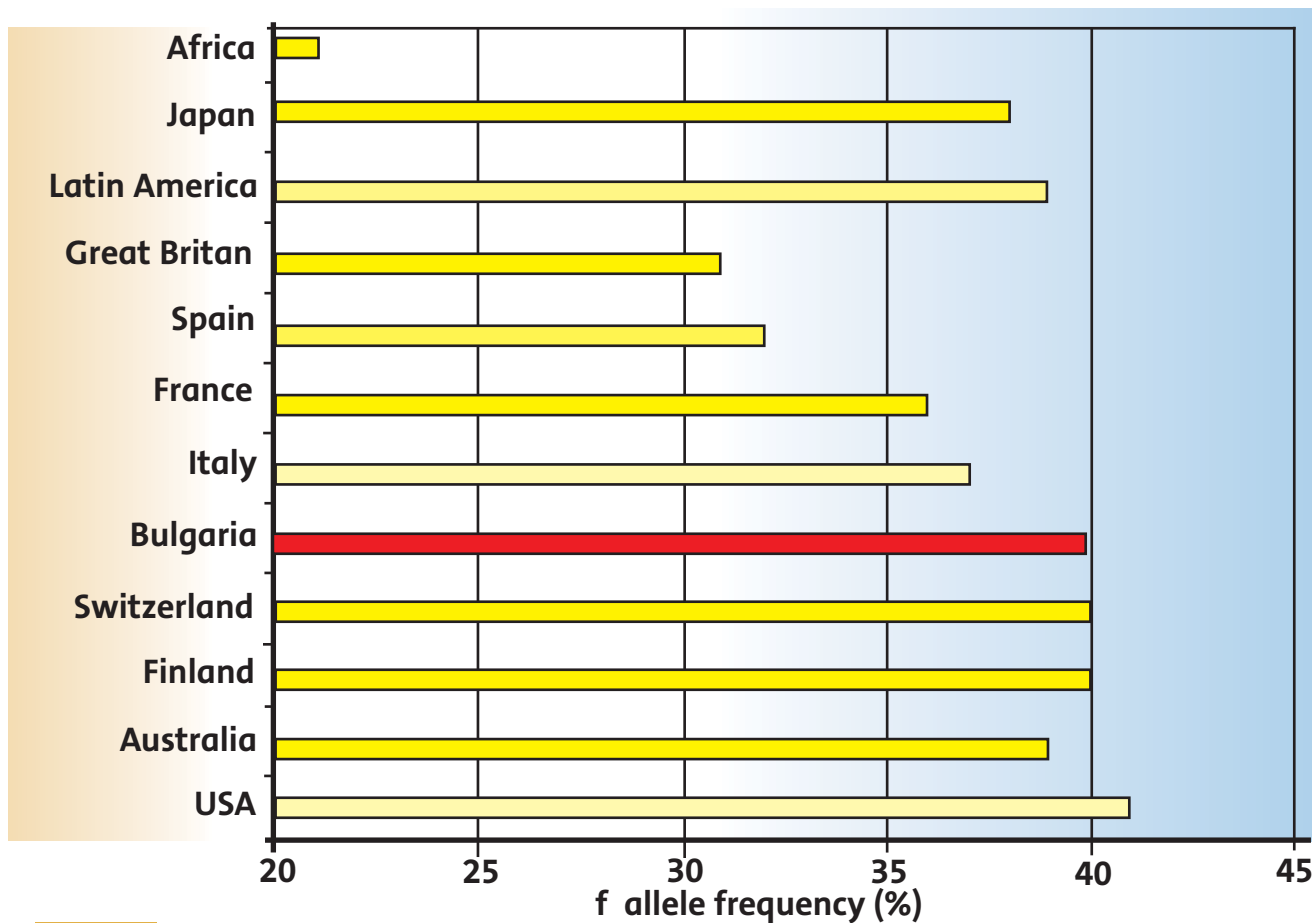


Figure 5. Genetic proximity between population groups from different races on the basis of determined frequencies of the f allele of the VDR gene.

from frequencies in our preliminary report (48 vs. 47% for F, 52 vs. 53% for f in cases and 74 vs. 75% for F, 26 vs. 25% for f in controls). Our data also do not significantly differ from data published by Gross et al. regarding the association of the genotype with low BMD (8). In their group (N=100) genotype frequencies differed from that of our group and were 37% for FF, 48% for Ff and 15% for ff and the differences are most significant for ff (8).

The genotype frequencies of controls (N=180) from this study are 11% for BB, 51% for Bb and 38% for bb. In comparison they were 6% for BB, 44% for Bb and 50% for bb in the preliminary study. The genotype frequencies of cases (N=220) are 35% for BB, 45% for Bb and 21% for bb (Table 2). In comparison they were 33% for BB, 38% for Bb and 29% for bb in the preliminary study. The observed allelic frequencies in our study population did not differ significantly from data in European populations (4,19) and data in our preliminary report (57 vs. 52% for B, 43 vs. 48% for b in cases and 37 vs. 28% for

B, 63 vs. 72% for b in controls). Comparing Bulgarian and Asiatic population the differences were significant (1). The frequencies were compared with published genotype frequencies for European populations (4,19): 12-25% for BB, 39-72% for Bb and 16-48% for bb and in Asian populations (1): 0-1% for BB, 10-22% for Bb and 77-90% for bb defined in groups of menopausal women). Our data did not differ from data published for European populations but they differed significantly from those published for Asian populations.

Our BMD data confirm the results from other population studies. In line with other investigators we observed higher BMD in FF carriers and lower BMD in ff carriers (4,5). The same trend was found with higher BMD in bb and lower values in BB carriers.

In our study group these with the ff genotype (6% of the controls and 28% of the cases) have 11,8 % lower BMD at the forearm than the subjects with FF (54% of the controls and 25% of the cases). The heterozygotes Ff (40% of the controls and 48% of the cases) have an inter-

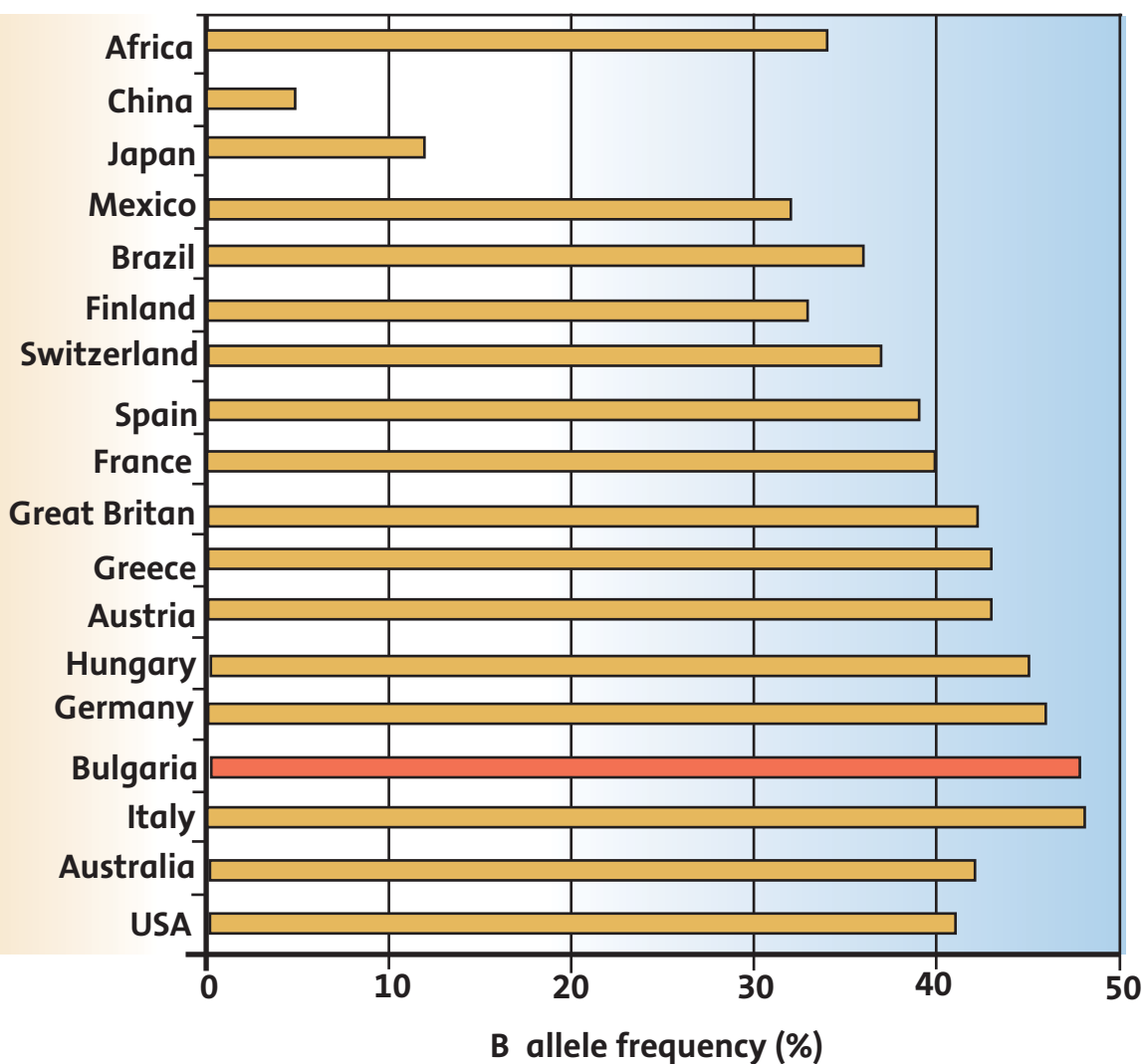


Figure 6.

Genetic proximity between population groups from different races on the basis of determined frequencies of the B allele of the VDR gene.

mediate BMD. The association between BMD and the genotype was also confirmed at the lumbar spine and femoral neck. Individuals with genotype ff have 11,7% lower BMD at lumbar spine and 7,7% lower BMD at femoral neck than FF. In our population the reduction in lumbar spine and femoral neck BMD was more substantial than that in forearm BMD (-36,5% and -33,3% vs. -27,4% at the forearm in ff and -24,8% and -25,6% vs. -15,6% at the forearm in FF).

Those participants carrying the BB genotype (11% of the controls and 35% of the cases) have 5,1% lower BMD at the forearm than subjects with bb (38% of the controls and 21% of the cases). The heterozygotes Bb (51% of the controls and 45% of the cases) have an intermediate BMD. The association of BMD with the genotype was confirmed also for the lumbar spine and femoral neck. Individuals with geno

type BB have 13,2% lower BMD at lumbar spine and 12% lower BMD at femoral neck than bb. In our population the reduction in lumbar spine and femoral neck BMD was more substantial than that in forearm BMD (-36,2% and -37% vs. -23,6% at the forearm in BB and -23% and -25% vs. -18,5% at the forearm in bb).

In conclusion, this is a final report from a study examining the prevalence of two VDR genotype polymorphisms and their association with the forearm, lumbar spine and femoral neck BMD. We were able to show that the prevalence of the polymorphisms under study was similar to that in the typical European population and different from that in an Asian population. The impact of the different genotypes on BMD was substantial both at the lumbar spine and femoral neck. Our data underscore the potential benefit of screening subjects at risk for osteoporosis for their genetic predisposition.

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Circadian Rhythm of Temperature and Pulse of Moclobemid, Venlafaxine, Fluvoxamine and Citalopram

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Циркадиален ритъм на температурата и пулса при лечение с Моклобемид, Венлафаксин, Флувоксамин и Циталопрам

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Категора по психиатрия и медицинска психология

Abstract

The aim of the present investigation is to study the circadian rhythm of temperature, pulse, mood and vigor in patients treated with antidepressants from the new generation and to make a comparison with the classical antidepressants. For this purpose were examined 44 patients (25 were on moclobemide, 7 on venlafaxine, 7 on fluvoxamine and 4 were on citalopram). Temperature, pulse, mood and vigor were registered each hour from 7 AM to 10 PM with 2 or 3 night measurements. Data were analyzed by Halberg's cosinor method. Chronobiological characteristic of Moclobemid is near to Imipramin and co-medication of imipramine and amitriptyline, and could disturb the pathological stable rhythm. The established synchronization between rhythms of temperature, pulse, mood and vigor is a predisposition for good

Резюме

Целта на настоящото изследване е да проучи циркадиалния ритъм на температурата, пулса, настроението и подтиците при пациенти, лекувани с антидепресанти от ново поколение в сравнение с оглед сравнение с класическите антидепресанти. За тази цел ние изследвахме 44 пациенти (25 на моклобемид, 7 на венлафаксин, 7 на флувоксамин и 4 на циталопрам). Температурата, пулсът, настроението и подтиците бяха регистрирани всеки час от 7 до 22 ч. с две до три нощни измервания. Данните бяха анализирани чрез косинор-метода на Халберг. Хронобиологичните характеристики на Моклобемид са близки до тези на имипрамин и комедикацияс имипрамин и амитриптилин и могат да променят патологично стабилния ритъм.

therapeutic effect in AD, panic disorders and depressive states. In Fluoxetine temperature, pulse, mood and vigor are relatively synchronized, because of the absence of peak-hours of these parameters. Temperature and pulse peaks in Fevarin are desynchronized and their mesors are not significantly higher in comparison with the healthy persons. Mood and vigor are desynchronized too (mood is with a peak at 21,01 h, whereas vigor is without daily rhythm). The good therapeutic effect of Citalopram could be explained with the fact that could normalize the values of mood and vigors and the mesors of temperature and pulse, even when these four parameters are desynchronized. These data show that patients must use the drugs for a long period of time after clinical improvement in order to recover their disturbed circadian rhythm. Maybe future studies in greater number of patients will deliver more information about their use in psychiatric patients.

Key words:

circadian rhythm, temperature, pulse, moclobemide, venlafaxine, fluvoxamine, citalopram

Introduction

During the last 5-6 decades chronotherapy takes a serious place in the treatment of some psychiatric disturbances. It was established that typical for depression is the disturbed circadian rhythm of some biological human functions. It has been emphasized in recent studies (Stoyanov, 2008, Stoyanov, Machamer, Schaffner, 2010) that chronobiological factors and arguments are of

Установената синхронизация между ритъма на пулса, температурата, настроението и подтиците е добра предпоставка за терапевтичен ефект при тревожни и депривни разстройства. При лечението с флуоксетин пулса, температурата, настроението и подтиците са относително синхронизирани поради липса на пикови часове на тези параметри. Пиковите на температурата и пулса при лечение с фебарин са десинхронизирани и техните мезори не са значимо по-високи от тези на здрави лица. Настроението и подтиците са десинхронизирани, като настроението е с пик в 21,01 ч., докато подтиците са без генонощен ритъм. Добрият терапевтичен ефект на циталопрам може да бъде обяснен с факта, че той може да нормализира стойностите на настроението и подтиците, както и мезорите на температурата и пулса, дори когато тези четири параметъра са десинхронизирани. Тези данни показват, че се налага пациентите да продължат да проемат медикаментите продължителен период след настъпване на клинична ремисия, за да могат да възстановят своя циркадианен ритъм. Вероятно бъдещи изследвания върху по-голям брой пациенти ще разкрият нова информация за приложение на психотропните препарати.

Ключови думи:

циркадианен ритъм, пулс, температура, моклобемид, венлафаксин, циталопрам, флувоксамин

critical importance in interpretation of correlations among neuroscience and psychiatry.

For the first time F. Halberg in 1968 stressed on the hypothesis about desynchronized rhythms in Affective disorders. Desynchronized process could be: phase advance, phase delay, phasen instabilitat (T. Wehr et al., 1980; B. Pflug & W. Martin, 1980; B. Pflug, 1987; T. Wehr & F. Goodwin, 1983; R. Wever, 1983; A. Lewy et al., 1984; 1985; Anna Wirz Justice, 2009;), shift

Seroquel XR – СТЬПКА КЪМ ХАРМОНИЯ

XR за X-перти

Шизофрения,
вкл. превенция на рецидив

БАР мания

БАР депресия

БАР превенция
на рецидив

Голям Депресивен
Епизод

Seroquel XR – СТЬПКА КЪМ ХАРМОНИЯ

КРАТКА ИНФОРМАЦИЯ ПО ПРЕДПИСВАНЕТО

Seroquel XR/ Сероквел XR 50 mg, 200 mg, 300 mg
таблетки с удължено освобождаване
quetiapine/кветиапин

Показания

- Лечение на шизофрения, включително:
 - предотвратяване на рецидив при стабилизирани пациенти с шизофрения, които получават поддържаща терапия със Seroquel XR.
- Лечение на биполарно афективно разстройство:
 - за лечение на умерени до тежки манийни епизоди при пациенти с биполарно афективно разстройство.
 - за лечение на големи депресивни епизоди при пациенти с биполарно афективно разстройство.
 - предотвратяване на рецидив при пациенти с биполарно разстройство, чиито манийни или депресивни епизоди са отговорили на лечение с кветиапин.
- Допълнение към лечението на големи депресивни епизоди, при пациенти с рекурентно депресивно разстройство (РДР), при които е постигнат субоптимален отговор на монотерапия с антидепресанти. Преди да започне лечение, клиницистът трябва да прецени профила на безопасност на Seroquel XR.

Дозировка и начин на приложение

Шизофрения и умерени до тежки манийни епизоди, асоциирани с биполарно разстройство:

I ден - 300 mg, II ден - 600 mg. Препоръчителната дневна доза е 600 mg. Тя трябва да се коригира в ефективния дозов диапазон от 400 mg до 800 mg дневно в зависимост от клиничния отговор и поносимостта на пациента.

Депресивни епизоди при биполарно афективно разстройство: Seroquel XR трябва да се прилага вечер преди лягане. Дневната доза за първите четири дни е 50 mg (Ден 1), 100 mg (Ден 2), 200 mg (Ден 3) и 300 mg (Ден 4). Препоръчителната дневна доза е 300 mg. В клиничните проучвания не е наблюдавана допълнителна полза в групата на лечение в доза 600 mg в сравнение с групата на лечение в доза 300 mg. При отделни пациенти може да има полза от лечение в доза 600 mg. В случай на проблеми с поносимостта, може да се обсъди понижаване на дозата до минималната доза от 200 mg.

Предотвратяване на рецидиви при биполарно разстройство: В зависимост от клиничния отговор и поносимост на всеки пациент - 300 - 800 mg на ден.

Допълнение към лечението на голям депресивен епизод при РДР Seroquel XR трябва да се приема преди лягане. Началната дозировка е 50 mg/дневно на първия и втория ден и 150 mg на третия и четвъртия ден. Антидепресивен ефект е наблюдаван в дози 150 и 300 mg дневно в краткосрочни клинични проучвания за допълнение към лечението (с амитриптилин, бупропион, циталопрам, дулоксетин, есциталопрам, флуоксетин, пароксетин, сертралин и венлафаксин) и в доза 50 mg/дневно в краткосрочни клинични проучвания на монотерапия. При по-високи дози съществува повишен риск от развитие на нежелани реакции. Поради това клиницистите трябва да са сигурни, че използват най-ниските ефикасни дози, като лечението се започва с 50 mg/дневно. Необходимостта от повишаване на дозировката от 150 на 300 mg/дневно трябва да се базира на оценката на всеки отделен пациент.

Пациенти в старческа възраст: Seroquel XR трябва да се прилага с повишено внимание, особено в началния период на дозиране. Честотата на титриране на дозата на Seroquel XR може да е необходимо да става по-бавно, а дневната терапевтична доза да е по-ниска, отколкото при по-млади пациенти. При пациенти в старческа възраст трябва да се започне с доза от 50 mg дневно.

Деца и юноши: Приложението на Seroquel XR при деца и юноши на възраст под 18 години не се препоръчва.

Чернодробно увреждане: Лечението трябва да започне с 50 mg дневно. Дозата трябва да се повишава с 50 mg дневно до достигане на ефективната доза.

Противопоказания: Съпътстващото приложение на инхибитори на цитохром P450 3A4, каквито са HIV протеазните инхибитори, азоловите антимикотици, еритромицин, кларитромицин и нефазодон е противопоказано.

Специални предупреждения и специални предпазни мерки при употреба:

Тъй като депресията се асимилира с повишен риск от суицидни събития, пациентите трябва да са под постоянно наблюдение до настъпване на подобрение. Трябва да се вземе под внимание потенциалния риск от суицидни събития след внезапно спиране на лечението с кветиапин.

Seroquel XR трябва да се прилага с повишено внимание при пациенти с известно сърдечно-съдово заболяване, мозъчно-съдова болест или други състояния, предразполагащи към хипотония.

Препоръчва се повишено внимание при лечение на пациенти с анамнеза за гърчове. При симптоми на късна дискинезия, трябва да се обмисли намаляване на дозата или преустановяване на Seroquel XR.

Невролептичният малигнен синдром се свързва с лечението с антипсихотични лекарства, включително quetiapine. Кветиапин трябва да се прилага с повишено внимание, когато е предписан заедно с лекарства, които удължават QTc-интервала, особено при пациенти в напреднала възраст, или такива със синдром на вродено удължаване на QT-интервала, застойна сърдечна недостатъчност, сърдечна хипертрофия, хипокалиемиа или хипомагниемиа.

Препоръчва се постепенно спиране на лечението с кветиапин, поради възможност за реакции на отнемане.

Seroquel XR не е одобрен за лечение на психоза, свързана с деменция.

Seroquel XR трябва да се използва с внимание при пациенти с рискови фактори за мозъчен инсулт.

Взаимодействия с други лекарствени средства и други форми на взаимодействие

Seroquel XR трябва да се прилага с внимание в комбинация с други лекарства с централно действие и с алкохол.

Не се препоръчва приема на quetiapine със сок от грейпфрут. Едновременното приложение на кветиапин с карбамазепин или тиоридазин или фенитоин значително е увеличило клирънса на кветиапин.

Бременност и кърмене

По време на бременност Seroquel XR трябва да се употребява само ако ползата оправдава потенциалния риск. Трябва да се избягва кърменето по време на приема на Seroquel XR.

Ефекти върху способността за шофиране и работа с машини

Пациентите трябва да се съветват да не шофират или да не работят с машини до изясняване на индивидуалната им чувствителност.

Нежелани лекарствени реакции

Най-честите нежелани реакции при quetiapine са сомноленост, световъртеж, сухота в устата, лека астенция, запек, тахикардия, ортостатична хипотония и диспепсия. Както при другите антипсихотични средства, лечението със Сероквел може да се съпровожда с увеличаване на телото, синкоп, невролептичен малигнен синдром, левкопения, неутропения и периферни отоци.

Опаковка: табл. 50 mg, 200 mg, 300 mg x 60;

Дата на последна актуализация на КХП: 2011-02-08

Моля, преди да изпишете Seroquel XR се запознайте с пълната информация по предписването.

Продуктът се отпуска по лекарско предписание.

Пълната информация по предписването можете да получите от:

АстраЗенека България, София 1057, бул. Драган Цанков 36,

тел.: (02) 971 25 33, 971 38 03,

факс: (02) 971 11 24

A 81/ 12.04.2011

Seroquel XR, Кратка характеристика на продукта, 08. 02.2011

BUL-FEB11-SER-3-ADV

AstraZeneca

Once Daily
Seroquel XR
quetiapine 50 mg, 200 mg, 300 mg

from circadian to ultradian rhythm (A. Bicakova-Rocher et al., 1989; 1996).

W. Duncan & T. Wehr (1988) supported the idea, that the „circadian pacemaker“ in endogenous depression is destroyed, and the use of some antidepressants could improve the disturbed circadian rhythm. In clinical practice sleep disturbances and depressive symptoms in depressive patients could be improved with effective antidepressant medication. Some of them could delay, others could advance the phase. So the most important idea of therapeutics is to synchronize the disturbed circadian rhythm in patients with Affective disorders/AD/, Schizophrenia. For this purpose are used specific chronobiological methods as sleep deprivation, bright therapy in patients with depression and bulimia nervosa, dark therapy for patients with acute mania (20), electric-acupuncture for depressive patients with pathologically stable rhythm (N. Madjirova et al. 1997).

The chronobiological characteristics of some antidepressants are investigated on animals. They put some theoretical aspects of their mechanisms, but the questions connected with practice are quite different and still undecided. The data about the new generation of antidepressants are not well studied. We had examined the circadian rhythm of temperature and pulse and the stability of rhythm of mood and vigor in various psychotropic drugs (antidepressants, neuropeptic and tranquilizers). Most of the publications of the new generation of antidepressants (citalopram, fluvoxamine) are connected with their clinical characteristic, but almost nothing is written about their chronobiological features.

The aim: in this study we would like to pay a special attention to antidepressants from the new generation as Moclobemid (Aurorix), Efexor (Effectin = Fluoxetine), Citalopram (Citalopram = Cipralex). Our aim is to establish the circadian rhythm of temperature and pulse and to some of them the stability of rhythm of mood and vigor in depressive patients and to make a comparison with the classical antidepressants such as Imipramine, Amitriptyline, Clomipramine and Maprotiline.

Material and method

In the present investigation were included 44 patients (27 were with AD and 15 with ND (anxiety and panic disorder). The patients were on moclobemid (26 patients, followed in 109 days, 11 were with AD, 15 were with ND), venlafaxine – 7 patients studied 63 days, fevarin – 7 patients studied 45 days and on citalopram – 4 patients followed in 38 days. The control group consists of 65 healthy individuals.

- Examination of the circadian rhythm of temperature, pulse, mood and vigor: temperature and pulse were measured each hour from 7 AM to 10 PM with 2 or 3 night measurements with mercury thermometer and the pulse rate was registered for 1 min.

Data were analyzed by the method of Halberg's Cosinor Analysis.

Hamilton's depressive scale was used to determinate the mental state of the patients.

We made a comparison with a control group of healthy persons and patients with AD and ND before and after medication with some of the classical antidepressants, from our previous examinations.

- Study the stability of the rhythm of mood and vigor in patients on Moclobemid, Venlafaxine and Citalopram.

The stability was measured by our own scale that was standardized on 481 psychiatric patients and 141 healthy persons. The examined persons registered the rhythm of mood and vigor every day for a period longer than a month (N. Madjirova, 1992; 1995; 2006). For part of the patients we have their rhythm of temperature and pulse. They were registered each hour from 7AM to 10 PM with 2-3 night measurements at least for a week.

- One-day examination of the circadian rhythm could not have measured the full cycle of the circadian rhythm predominating in the individual patients.

- When the follow-up was at least for two or three weeks, it was possible to identify the predominating rhythm (arrhythmic, morning, evening, fluctuating) in the individual patients and to characterize its stability. We studied each patient for stability of diurnal fluctuations of

emotions and vigor, and for some of them of temperature and pulse. Data were analyzed by Cosinor Method of Halberg.

- During the long-term investigation 3 types of stability of the circadian rhythm emerged (see graphic 1):

- Stable type rhythm – the substitution of one type of circadian rhythm for another over greater intervals of time, for 1 or 2 weeks (type I-a), and no change of the rhythm was observed during the study (type II).

Unstable rhythm – when changes occurred almost daily (type I-b), or periods of a single type rhythm alternated with frequent changes of the rhythm – type III (N. Madjirova, 1992; 1995; 2006).

Results and discussion

Data of temperature and pulse mesors and their peak-hours of the various drugs are given at table 1.

Moclobemid (Aurorix) – is a new generation (RIMA - Reversible Inhibitors of MAO-A). It is

with a very good effect for old patients, for anxiety disorders (R.G.Priest,1992), social phobia (M. Versiani u E. Nardi,1994) and it is with a good effect on the REM-phase of the sleep. Moclobemide was used in 26 patients (11 with AD – depressive episode and 15 with ND, social phobia with panic attacks). The daily dose was between 150 and 300 mg. in co-medication with carbamazepine (CBZ) about 200-600 mg. daily.

The circadian rhythm of temperature and pulse was followed in between 2 and 5 days. On table 1 is given its chronobiological characteristic: for the whole group temperature and pulse mesors and their peak-hours are not different from the group of healthy persons. In our previous study (N. Madjirova, 1997) was established that chronograms of the antidepressants are different when they are used by patients with AD and ND. So we make a comparison of the chronograms of moclobemid in patients with AD and ND.

We established that temperature and pulse mesors are higher, but not significantly, in the group of ND. The peak-hours in AD delay in

The types of stability of the rhythm

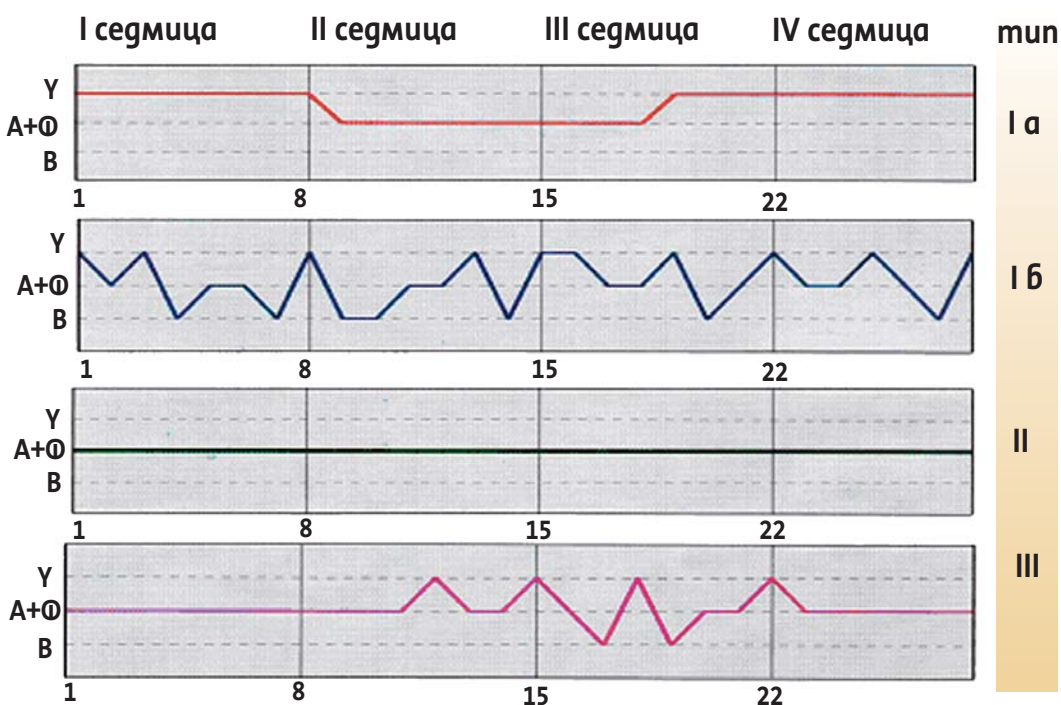


Figure 1.

Graphic view of the types of stability of the rhythm of mood and vigor: stable (I-a and II), unstable (I-b and type III).

A – ритмичен
B – вечерен
O – флукутиращ
Y – утринен

comparison with ND with 2,36 h for temperature and 2.09 for pulse. Typical for patients (AD and ND) on moclobemid is the synchronization between temperature and pulse chronograms (see table 1). The good improvement of ND with social phobia and panic attacks could be explained with synchronization between their peaks that are not different from the healthy persons (16,02 h; 16,18h for temperature and 14,58 h; 14,15 for pulse). This synchronization could also explain the good effect in old patients, who are characterized with phase delay in the old persons. Such kind of synchronization could not be found in patients who were treated with other drugs, with the exception of the mood stabilizer normothymine and co-medication of three-cycle antidepressants and carbamazepine. We have studied 1 patient, followed in for 1 week on normothymine and the peaks of temperature (17,23) and pulse

(18.21) were synchronized. The good therapeutic effect of depressive patients on normothymine is connected with its ability to normalize their phase.

I. Some chronobiological features of the circadian rhythm of Moclobemid:

1. Moclobemid (total in AD and ND):

- Temperature and pulse mesor are synchronized;
- The peak-hours of temperature and mesors are not different by the values of the healthy persons.

2. Chronobiological features of Moclobemid in AD:

1. Rhythm of mood and vigor are desynchronized – the peak-hour of mood is at 19,09, whereas the vigor is without daily rhythm.
2. Temperature and pulse peak-hours are

Table 1. Temperature and pulse mesors and their peak-hours of moclobemid, efexcor, fevarin and citalopram determined by F. Halberg cosinor analyzes.

Dg	Drug	n of patients	n of days	Temperature mesor	Peak Hour	Significance	N of days	Pulse mesor	Peak hour	significans	Synchronization
AD	Moclobemid	11	27	36,339	18,37	Yes	15	69,09	18,08	Yes	Yes
	Efexor	7	63	36,423	16,14	No	59	77,71	13,52	No	Yes
	Fevarin	7	45	36,447	19,1	Yes	40	79,06	3,03	No	No
	Citalopram	4	38	36,148	15,41	Yes	38	71,04	11,31	No	No
	AD- before therapy	100	100	36,416	16,01	Yes	100	82,91	11,44	No	No
ND	Moclobemid	15	82	36,592	16,02	Yes	58	83,30	14,58	Yes	Yes
	ND – before therapy	81	81	36,48	16,19	Yes	46	80,47	14,17	Yes	Yes
Healthy persons		65	65	36,387	16,18	Yes	55	74,83	14,15	Yes	Yes
Total	Moclobemid	26	109	36,47	17,17	Yes	73	76,19	16,35	Yes	Yes

* AD – affective disorders, ND – neurotic disorders

synchronized.

3. Individual chronograms of mood and vigor show that only 48,15% are synchronized, 22,22% are desynchronized and 29,62% show no daily rhythm. Temperature, pulse, mood and vigor are desynchronized in 84,62% of the group.

3. Some chronobiological features of Moclobemid in ND during therapy

- Mood and vigor exhibit no daily rhythm. They are relatively synchronized, because of the absence of daily fluctuation.

- Temperature and pulse peak-hours are synchronized and the values of their mesors and peaks are similar with the healthy persons.

- Temperature and pulse peak-hour are desynchronized with mood and vigor peak-hours.

- Individual chronograms show, that the 4-th parameters are desynchronized in 92,31%. Only 22,22% of pulse and temperature are synchronized, whereas mood and vigor are synchronized in 64,29%. Daily rhythm of temperature and pulse disappeared in 44,44%. Temperature and pulse are synchronized in 66,67% of the cases.

As we see the individual chronobiological characteristic of patients on moclobemid are not so perfect in comparison with the chronobiological characteristic of the total group. But we must not neglect the fact that moclobemid is one of the antidepressants with very good chronobiological data of temperature and pulse, that are perfectly synchronized and the values of their peak-hours and mesors are the same as of the control group, especially for patients with ND. All this facts explain the good improvement of patients with ND (panic disorders).

Stability of rhythm of moclobemid:

Stability of the circadian rhythm was followed in 19 of the patients (8 were with AD, 11 with ND, from which 9 were with panic disorders). To all of them was followed in the rhythm of mood, vigor, temperature and for the ND and pulse. For 6 of the patients with AD we have their circadian rhythm before medication, and for 3 of them we have an impression for their stability. Data of the parameters are given at table 2.

Stability of the rhythm in patients with AD

- Before medication there is registered no daily rhythm of mood, vigor, temperature and pulse.

- After medication – in this group were included 8 patients with AD, who were on Moclobemid and CBZ, and were followed in at least 3 weeks. The values of mood and vigor are increased significantly and correlate with their clinical improvement. Their peak-hours are at 21,54 and 21,36 and are synchronized. This data support the idea of the old psychiatrists and our previous results that evening type rhythm dominates in depressions. Temperature mesor decreased after their improvement and the peak-hour is not different from the peak of healthy persons. For the rhythm of mood and vigor dominate unstable type rhythm (85,71%), whereas for temperature is 60,00%. This type of rhythm stability is near to the stability of Imipramine.

Stability of rhythm in patients with ND – the results in the group with panic disorders are very good – values of mood and vigor speak for a very good clinical improvement. The most important is that the 4 parameters (mood, vigor, temperature and pulse) are synchronized and their peak-hours are in the afternoon and not different from the peaks of the healthy group. This data indicates that co-medication of moclobemid and CBZ is very good for patients with panic disorders. The other 2 neurotic patients with depressive syndrome and vegetative symptoms show no daily rhythm of vigor, temperature and pulse. Even the examination is only on 2 cases, their values are not so perfect, as the results of the patients with panic disorders. The daily rhythm of temperature and pulse disappears, but mood and vigor are synchronized in co-medication of Moclobemid, CBZ and Antepsin in patients with AD.

Common for the group of Moclobemid is:

1. Mood and vigor, and temperature and pulse are well synchronized, that could explain the clinical improvement of the patients.

2. The stability of the rhythm: dominates

Table 2. Some chronobiological features of patients treated with Moclobemid.

Data parameters		AD-recurrent depression Before therapy	AD-recurrent depr. Co-medication with CBZ	ND-panic disorder Co-medication with CBZ	ND-depr. S yndrome and cephalgia	Co-medication with CDZ & Antelepsin
M O O D	n-patients	6	8	9	2	2
	n-day	17	175	71	13	23
	Mesor	140,97+/-120.	200,89+/-54	303,93+/-33	332,8+/-57,9	378.14+/-36.
	Peak-hour	18,07	21,54(17,29-23,21)	15,59(14.18-18.49)	15,59(14,18-18,49)	19,56(18.48-20.59)
	Significance	NO	YES	Yes	Yes	Yes
	n of R/Arhyth.	4/1	4/3	48/23	11/2	20/3
	<u>Stable rhythm</u>	2	<u>1 (14,29%)</u>	<u>2 (40,0%)</u>	<u>0</u>	<u>1(50,%)</u>
	Unstable	1	7 (85,29%)	5 (60,0%)	2 (100%)	1(50,0%)
V I G O R	n-patients	6	8	9	2	2
	n-day	17	175	71	13	23
	Mesor	124,32+/-111	192,92+/-54	290,20+/-34	246,09+/-65	376,52+/-38
	Peak-hour	15,20	21,36 (17,29-23,21)	15,39 (13,51-17,58)	12,04	19,51 (18,48-20,59)
	Significance	NO	YES	Yes	No	Yes
	n of R/Arhyth.	3/2	4/3	35/36	7/5	21/2
	<u>Stable rhythm</u>	<u>2</u>	<u>1 (14,29%)</u>	<u>2 (40,0%)</u>	<u>0</u>	<u>1 (50,0%)</u>
	Unstable	1	7 (85,29%)	5 (60,0%)	2 (100%)	11 (50,0%)
T e m p e r a t u r e	n-patients	6	8	9	2	2
	n-day	19	150	68	9	23
	Mesor	36.72+/-0.19	36,512+/-0.1	36,58+/-0,12	36,619+/-0,1	36,519+/-0,2
	Peak-hour	17.12	16,00 (14,20-18,13)	16,16 (15.12-18.08)	13,31	15,28
	Significance	NO	YES	Yes	No	No
	n of R/Arhyth.	5/1	4/3	42/26	8/1	18/5
	<u>Stable rhythm</u>	<u>2</u>	<u>3 (40,0%)</u>	<u>3 (50,0%)</u>	<u>0</u>	<u>1 (50,%)</u>
	Unstable	1	5 (60,0%)	3 (50,0%)	2 (100%)	1 (50,%)
P U L S E	n-patients			9	2	1
	n-day			46	9	6
	Mesor			84,42+/-1,4	70,72+/-19,2	93,21+/-19,3
	Peak-hour			15,47 (14,18-17,16)	12,40	23,46
	Significance			Yes	No	No
	n of R/Arhyth.			29/17	4/5	2/4
	<u>Stable rhythm</u>			<u>1 (33,33%)</u>	<u>0</u>	<u>1 (50,%)</u>
	Unstable			3 (66,67%)	2 (100%)	1 (50,0%)

Temper. - temperature

R/Arhyth. - relations between patients with rhythmic values of the parameters/arrhythmic values.

the unstable rhythm and this data are near to mipramin, co-medication of imipramine and amitryptiline stability and this effect could destroyed the pathologically stable rhythm, that

Idominates in depressive patients with AD and some ND.

Conclusion:

Chronobiological characteristic of Moclobemid is close to Imipramine and co-medication of imipramine and amitriptyline, and may disturb the pathologically stable rhythm. The established synchronization between rhythms of temperature, pulse, mood and vigor is a predisposition for good therapeutic effect in AD, panic disorders and other depressive states.

Fluoxetine

(Efexor = Effectin = Fluoxetine): this antidepressant is from the new generations of SSSR. In this group are included 7 patients with AD, aged between 25 and 54 years (middle age 41.6 years). 5 of them are with recurrent depression and 2 are with bipolar disorders (the present episode is depressive). 2 of the patients were followed for 2 days, 2 – for 4 days, 1 – 9 days, 1 – 17 and 1 – 25 days. So for 3 of them we have an impression for their stability.

Chronobiological characteristic:

1. Individual chronograms:

2. In 3 of the patients the daily rhythm of the four parameters (temperature, pulse, mood and vigor) disappeared and so they are relatively synchronized because of the absence of daily rhythm. The other 2 patients are desynchronized – there is no daily rhythm of mood and vigor, one patient is with peak-hour of temperature, and 1 is with a peak-hour of the pulse. So the 4 parameters in the 4 patients are desynchronized.

3. *Group characteristics:*

The mesors of the four parameters are almost the same as the mesors of the control group of the healthy persons. Temperature, pulse, mood and vigor are relatively synchronized, because of the absence of peak-hours of these parameters. If we make a comparison with the classical antidepressants we established that his chronobiological characteristic is almost the same as of imipramine, trimipramine, nomiphen-sine, hydiphen, co-medication of imipramine and amitriptyline. Their parameters are relatively

synchronized. Their pulse rate is not quickened and the values are the same as of healthy persons and patients on hydiphen. (N. Madjirova, 1995; 1997; 2006).

Conclusion:

There should be stressed the fact, that temperature, pulse, mood and vigor in are synchronized, because of the absence of daily rhythm of these parameters. So they are relatively synchronized.

Fluvoxamine = Fluvoxamine

Fevarin is an inhibitor of serotonin re-uptake. It is with a very good effect in obsessive-compulsive disorders. We studied 7 patients (5 with AD, recurrent depression, 2 with ND, panic disorders), aged between 29 and 49 years (the middle age is 39,4 years. Their dose was between 75 and 150 mg. daily.

L. Demisch et al. (1996) studied the chronobiological effect of fluvoxamine on rat pineal serotonin and melatonin metabolism. They established that its chronobiological effect is the most strong in rats when it is given at 4,00 h PM. This effect could be explained with the higher sensitivity of 5-HT in the CNS. They are connected with the phase changes of 5-HT, that appeared 7 hours after the disappearance of light.

W. Duncan, K. Johnson u T. Wehr (1995) established that antidepressants could delay the circadian rhythm of body temperature, without changing its amplitude and mesor in experimental animals (rats). But there were no data about these changes when the drugs are given for a longer period of time. They have established that hypothalamic temperature delayed the phase of temperature in rats treated with some antidepressants (Clorgiline, Fluoxetine, Lithium carbonate). These results correlate with our data – temperature peak-hour (17,10) 1,52 hours) is later 2,52 h in comparison with the healthy persons (see table 1).

Chronobiological characteristic:

- Individual chronograms: rhythms of mood and vigor in 6 of the patients, with the exception of 1 with panic disorder, are synchronized, whereas the rhythm of temperature and pulse are desynchronized. The relation between rhythmic and arrhythmic cases for mood and vigor are 71,43%/28,57%, whereas for temperature and pulse are the opposite (28,57%/71,43%).

- **Group characteristics:**

1. Temperature and pulse mesors are higher, but not significantly higher in comparison with the healthy persons. Pulse rate shows no daily rhythm, and is desynchronized with temperature rhythm (see table 1). Mood and vigour are desynchronized too (mood is with a peak-hour at 21,01 h, whereas vigour is without daily rhythm).

2. Stability of rhythm: stability has been registered in 4 of the patients.

Conclusion: the group chronobiological characteristic of Fevarin shows that the 4 parameters (temperature, pulse, mood and vigor) a desynchronized. Citalopram = Citalopram = Cipram

There were included in this group 4 patients (4 were with AD, recurrent depression, 1 of them was with psychiatric symptoms), aged between 49 and 59 years, the middle age is 54.5 years. 2 of them are followed in 1 week, 1 – 11 and 1 – 2 weeks. Most of the publications are on their clinical improvement and regulation of sleep.

Chronobiological characteristic:

- **Individual characteristics:**

Individual parameters show that ? of the days (78,95%) are desynchronized, and the other 21,05% are relatively synchronized, because of the absence of daily rhythm. About 50% of temperature, mood and vigor are rhythmic and the others – arrhythmic. For pulse only 18,42% are rhythmic and 81,58% are arrhythmic.

- **Group characteristics:**

Temperature peak is at 15,41 h and is not different from the control group (16,18 h), but it

is desynchronized with the other 3 parameters (pulse, mood and vigor), that show no daily rhythm. May be the good effect could be explained with the fact that pulse mesor and temperature peak-hour and mesor are not different from the healthy persons from one side and the relative synchronization between pulse, mood and vigor, because of the absence of daily rhythm from the other side.

Conclusion:

The good therapeutic effect of Citalopram could be explained with the fact that it normalizes the values of mood and vigors and the mesors of temperature and pulse, even these four parameters are desynchronized.

All these results lead us to the idea, that even clinical improvement rhythm processes are not well stabilized. So we must have in mind the experimental results on rat of M. Poppei et al. (1982) and R. Rose et al. (1982), that the treatment of animals with experimental neuroses for 1 month is not sufficient, as their desynchronization continues even their clinical improvement. According to them chronic desynchronization of the biological rhythm are one of the reasons for ND.

Some of the established chronobiological data put some important questions about the treatment of the psychiatric patients.

- How many days/months the antidepressant therapy must continue?
- Is not possible co-medication to course desynchronization?
- What is the effect of the various antidepressants on the different nosological groups (AD, ND) and their subgroups?
- What is the influence of the seasons of the year, hours of the day, sex and age?
- What is the role of pharmacokinetics?

Acknowledgments: The authors are grateful to Nedialka Petrova, head assistant in the Department of Social Medicine in MU-Plovdiv and Nedelcho Delchev, who worked in Bulgarian Academy of Sciences, Plovdiv, for their competent help in interpreting the data and statistical analysis.

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Psychological Influences Under Stress – Strategy for Prevention and Control

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Психически въздействия при стрес – стратегия за превенция и управление

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Abstract

A study has been conducted on 928 people from three professions (doctors, teachers and administration staff) and the influence of modern stresogenic factors and their psycho emotional influences on the individual.

We analyzed the effect of 106 stresogenic factors divided into 4 categories – socio-economic, professional, family environment and work environment – in three factor areas – psychological, physiological and factors of the work environment.

The effect of these stresogenic factors has been calculated using the method of J. Hristov, with the cumulative effect of the factors being studied in addition to their individual effect.

There is demand for working strategies, aimed both at the general population and individuals, which take into account the factors and risks associated with every living environment.

Резюме

Извършени са наблюдения на 928 души от три професии (лекари, учители и административни служители), като е анализирано влиянието на съвременните стресогенни фактори и техните психоемоционални въздействия върху индивида. Подчертана е необходимостта от разработване на действени стратегии за ограничаване и намаляване на стреса, имащи общо-популационна и индивидуална насоченост, които да отчитат особеностите на всяка жизнена среда и нейните рискови въздействия.

Разработен е модел за превенция и управление на стреса в който са разгледани целите, начините, подходите и мерките занеговото ограничаване и намаляване и са конкретизирани задачите на гържавните, обществени и браншови организации в тази насока.

A model for the prevention and control of stress has been developed, which reviews the aims, methods and applicable preventive measures as well as the associated tasks that national, public and private organisations need to complete to this end.

The different ways of limiting and reducing stress have been reviewed (active-passive).

There are three main strategies in worldwide practice. Every strategy has three goals: prevention, timely reaction and rehabilitation. These must be clearly aimed towards the organisations or individuals and correspond to the subject-object interaction.

In order to improve the work done to combat stress there are three types of measures used: primary, secondary and tertiary.

Key words:

stress, factors, psychosocial influences, strategies for prevention and control

Ключови думи:

стрес, фактори, психосоциални въздействия, стратегии за превенция и управление.



The changes associated with stress and its effects on both temperament and structural-psychological qualities of personality are the subject of intense scientific research. Most frequently they are associated with moral disintegration of society, the changed attitudes toward employment, others, towards oneself, one's interests and tendencies, spiritual and aesthetic values (7,8).

The opinion that the strategies for prevention and control of stress require a social policy that takes into account the varying influences of different living environments and the risk factors they pose to the health of the individual is reinforced. These strategies need to create appropriate conditions for the inclusion of organisations and individuals, so that they can effect a direct influence on both the environment and the way of life of those involved in order to stimulate adaptation to the new social requirements (3, 4, 5).

Existing social practice puts more focus on the study of stress and determining its negative effects, with not enough attention being paid to the evaluation of risk factors and the possibilities for making connections between harm done and the resulting negative health effects (1, 2).

This necessitates the establishment of both rules and an institutional frame which specify the scientific studies and prophylactic courses for combating stress (6, 7, 8).

Materials and methods

Observations have been conducted on 928 people from three professions /doctors, teachers and administration staff/ which take into account the presence and strength of different stresogenic factors and the associated changes in their psychoemotional states. On this basis and through the study of 410 literary sources, an attempt has been made to clarify the main ways of combating stress, reducing the incidence of stress-related illness and mortality, and the overall improvement of the personal health of individuals from these professions.

An evaluation of the acting legislations for prevention and control of stress, with measures of an individual and general character being suggested to improve their effectiveness.

Results and discussion

The different ways of limiting and reducing stress have been reviewed (active-passive). It has been proven that the active overcoming of stress is aimed at changing stress-generating situations with the intent of removing stresogenic factors, while the passive approach involves adapting to the situation and accepting it as unavoidable, which often leads to distress. When evaluating which course of action to take the personal qualities of the individual as well as the present material and social resources must always be taken into account.

We analyzed the effect of 106 stresogenic factors divided into 4 categories: – socio-economic, professional, family environment and work environment - in three factor areas: – psychological, physiological and factors of the work environment.

The effect of these stresogenic factors has been calculated using the method of J. Hristov, with the cumulative effect of the factors being studied in addition to their individual effect.

The complexity of stresogenic psychosocial influences requires good planning, coordination and control of preventive measures, as well their stage-by-stage implementation: determining negative factors, evaluating the corresponding risks, applying appropriate control strategies, monitoring their effectiveness, re-evaluating the risk, analysis of the necessary information, training those at risk and etc.

In practice there are two strategic approaches: standard (problem oriented) to evaluate physical risks and (emotionally oriented) for the evaluation of psychosocial risks. The evaluation of physical risks is clearer and begins with analyzing the risks and harms leading to useful information to evaluate the result. With psychosocial risks there is no unified classification of the degrees of harm and influence monitoring is complicated, as the harm is harder to perceive than physical inequality and accidents.

In worldwide practice there are three main strategies: situational (coping strategy), disposi-

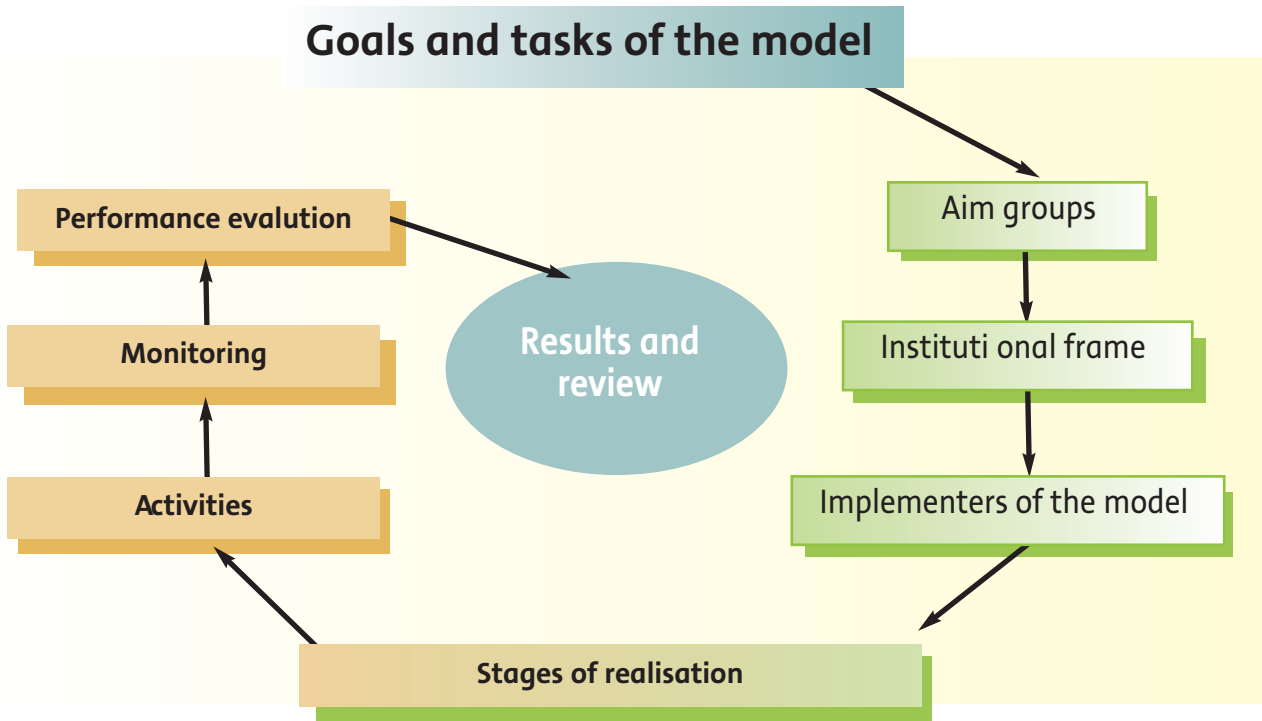


Figure 1. Structure of the model for limiting stress in doctors, teachers and administrative staff

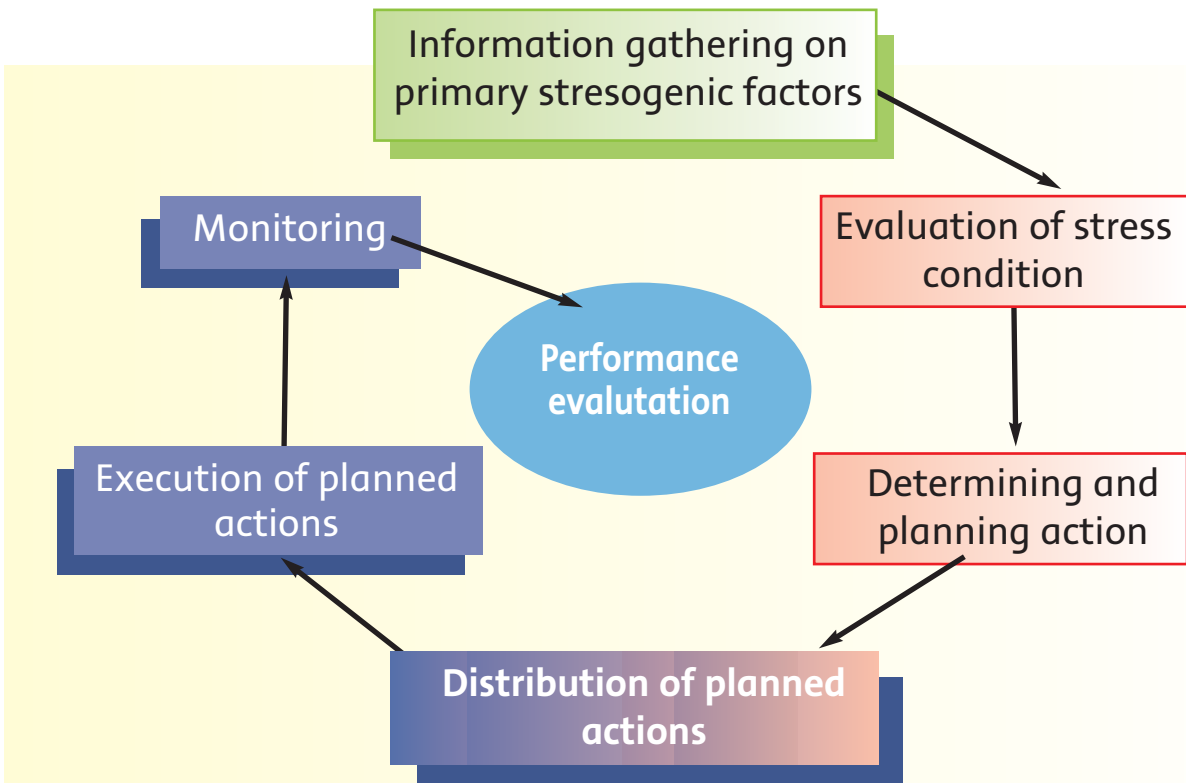


Figure 2. Algorithm for applying the model for limiting stress in doctors, teachers and administrative staff

tional (coping style), and emotionally focused coping (6, 8) which reflect the various cognitive and behavioural mechanisms for overcoming and reducing stress.

Every strategy has three goals: prevention, timely reaction and rehabilitation. These must be clearly aimed towards the organisations or individuals and correspond to the subject-object interaction.

In order to improve the work done to combat stress there are three types of measures used: primary, secondary and tertiary. It has been proven that the primary type is the least popular, because changing the nature of the labour or its overall organisation is a complicated process. Secondary measures are concerned with activating personal control of stress through different techniques (behavioural skills, special programmes, relaxation and others). Tertiary measures address individuals experiencing breakdowns or with harmful habits.

Over the course of the last decade several coping strategies have been presented, but only a small number of them have value or give an accurate assessment. Institutions which coordinate the politics, actions and programmes for overcoming stress. The necessity of public awareness of these problems and taking active decisions becomes evident.

Based on the conducted study, a model for the prevention and control of stress has been developed (fig.1)

Conclusion

Based on the conducted studies national, public and private organisations tasks have been determined for the reason of stress control and improvement of the overall health.

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Medicine – Basics and Relevant Issues

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Медицина: основа и съответни проблеми

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Abstract

Mastering the science of medicine means following two inter-related paths. The first, is the quest „to learn and know“. It involves a comprehensive study of the human body and the dynamic relationship of „life - health - illness - death“. The second, is the aim „to find the right cure“. It utilizes all acquired knowledge to intervene in the above-mentioned relationship in favor of prolonged life and health. Curing and healing a patient, however are two different concepts. Healing utilizes the science of medicine to cure the body, but also goes a few steps further to incorporate empathy (relating to the patient's specific physical and emotional state) and ethics (applying the science of medicine in a respectful and humane way) into the equation. It is well-known that the constant rise in human population places a strain on limited resources, the availability of medical specialists inclusive. Coupled with a prolonged period and the high

Резюме

Практикуването на медицина се основава на два стремежа – „да научиш и да знаеш“ и „да намериш правилното лечение“. Първият включва задълбочено изучаване на човешкото тяло и изграждане на правилно разбиране за динамичната връзка „живот – здраве – болестно състояние – смърт“. Вторият, използва наученото за да се намеси в полза на по-продължителен и качествен живот. Да лекуваш тялото и да излекуваш един пациент обаче са две корено различни неща. Същинското лечение, водещо до дългосрочно и стабилно възстановяване, използва медицината за да лекува не само физическият компонент на човешкото съществуване. То включва и практикуването на емпатия (отнасяща се до професионално вникване в индивидуалното физическо и емоционално състояние на пациента) и етика (провеждане на лечение по неоскърбителен и хуманен за пациента начин). Всеизвестно е, че нараства-

cost of educating such, as well as the competitive drive of universities to get funding, it changes the entire focus of the profession. Sadly, the impetus nowadays is either to engage in ground-breaking technologically-driven research on genetics, push-sell drugs and prosthetics, or, at the very basic level, cure the largest number of hospitalized patients to a somewhat relative state of health and, do so fast. Such prioritization creates flaws in the medical education system that divert medicine away from the art of healing towards simply the business of curing. The results for the medical students are: excessive stress, inadequate factual preparation and emotional immaturity. The results for the patients are: more and, increasingly, pharmacologically suppressed pain, longer recovery to health and a sense of diminished self-importance.

Key words:

медицина, емпатия, етика

нето на населението в световен мащаб води до недостиг на ресурси, в това число и на обучени лекари. Дългият период и високата парична стойност на тяхното обучение, както и борбата на университетите да осигурят финансови средства постепенно изменят фокуса на професията. За съжаление, в наши дни, лекарите се ориентират предимно към генетика, маркетинг за фармацевтични гиганти, протетика, или „конвейерна медицина“ (скоростно лечение на възможно най-голям брой пациенти). Подобни приоритети се отразяват и на медицинското образование. Образователните програми се преориентират от овладяване на изкуството да излекуваш един пациент към преподаване на бизнеса „да лекуваш на парче“. В резултат, сред студентите-медици се наблюдава все по-силен стрес, неадекватна подготовка и прояви на емоционална незрялост. Крайните потърпевши, разбира се, са самите пациенти. Те са подложени на фармакологично подтискане на болка, по-дълъг период на възстановяване и чувство за намалена себестойност.

Ключови думи:

medicine, empathy, ethics

What is medicine?

Medicine is old. As old as the life itself. Wherever there is a living organism, there is a dynamic state of relative well-being, normal function disturbance, healing and death. Death is not immediate and permanent. Nor is it always the final stage of existence. The key to stave it off is energy sustenance. As long as energy exists or could be swiftly reintroduced, processes such as cell acclimatization and mutation can bring back life numerous times, possibly, indefinitely. Before humans, medicine's only practitioner was nature. It would counteract the effects of climate

and chance. The aim was the continuation of life - in any shape and form, conducive to its survival. There was no imperative to sustain the greatest number and variety of organisms alive. Species would develop, mutate into new ones or perish. Life itself would not.

The human exception

Humans developed as a species and nature's dominance as the sole „life decision-maker“ was disputed. Humans, however, developed as frail organisms. Small in stature, soft both on the inside and the outside. No external

Being a genetically diverse species, not all members of humanity have the same prioritization system in life. Nor are they in agreement on how to prolong the existence of our species. Therefore, the answer to these questions would depend on the outcome of the ideological clash between universalists and singularists in the governing bodies in each state. Unfortunately, the status of this outcome is pretty unstable in time.

The „Modern“ Medical School of Thought

The observance of medical traditions is another obvious loser. The major division, of course, would be in the domain of „religion“, especially through its major varieties – Christianity, Islam and Hinduism, each of which has a slightly different take on life, medicine inclusive. However, not all news is bad news. Ever developing Schools of Medical Thought, transgressing religious contention appeared and are now accepted by more than one state. By far, the predominant, albeit not the most tolerant and inclusive (this place is reserved for Buddhism), is the evolutionized version of the Ancient Greek School of Thought. What is considered as „modern“ human medicine, builds on it.

According to its precepts, mastering the art of medicine means following three inter-related paths. The first, is the quest „to learn and know“. It requires a comprehensive study of the human body and a scientifically-based understanding of the relationship „life - health - illness - death“. The second, is the aim „to find the right cure“. It utilizes medical knowledge to intervene in favor of prolonged life and „better quality“ of it. The third one, relates to personal integrity and moral conduct. Its guiding principles are – „always give your best“, „do no harm“ and „be ethical“.

The first two paths are the major accent. They are structural, logic-based and explicit in their purpose. As such, they concentrate on students' efforts to develop themselves as successful medical specialists. The safeguard of the human species is a byproduct.

The last path is complementary. It is based on the „good will“ of each physician and is, therefore, more discretionary in nature. Full of

artificial constructs, it is intended to combat human instincts in the name of „the greater good of the greatest number“. It does so by appealing to emotional intelligence.

Issues

With that in mind, one major downside of this School of Medical Thought is the selection process of the would-be physicians. The vast amount of data that needs to be assimilated in medical school has favored an entry exam system testing factual knowledge in exact sciences only.

Medicine however, is about inter-related data. The ability of students to structure this data into useful, easy-to-extrapolate from knowledge systems remains unchecked.

There is no reference as to ethics, philosophy, empathy or effective communication skills either. Without an adequate network of checks and balances over human instincts, the power of knowledge over life and its continuation that physicians are entrusted with could be easily abused.

The problem is exacerbated by the fact that medicine offers high social reputation and, not rarely, significant material returns. Who guarantees that the most suitable candidates are the ones that are being selected?

There is some consolation, of course, in the fact that, upon graduation, all medical practitioners must take what is considered to be the „highest“ and „longest observed“ oath of acceptable and predictable conduct. Who vouches on its proper observance and by what standards? The peers? Humanity? God?

Also, without evidence of emotional intelligence, is this oath enough to ensure quality of service?

The future

Current debate on these issues remains inadequate. Within the medical community, there are some polemics about „missing elements“ in the practice of medicine or the dehumanization of the profession, but they all fall under the category of „empty talk and no action.“

Policy makers are busy with hospital management and medical insurance. No one else pays any attention. There is simply not enough steam to trigger necessary change. But, there is hope.

One way to work with the present system and still move forward is to examine what lies in between the medical school selection process and the taking of the Hippocratic oath - namely, medical education itself. Can we, the established medical professionals and pedagogues, do something to influence outcomes?

After a thorough check on life philosophy, ethics, the structural and emotional status of each medical student at the beginning of their studies, there is a battery of measures that could boost results:

- more comprehensive study of human bio-ethics

- consistent measurement and follow-up on emotional development
- involvement in non-profit social work as part of compulsory medical education
- non-monetary incentives to participate in scientific research, while still studying
- rotational practice in all major clinics

In other words, the focus of medical education itself must shift from the present, impersonal view of future physicians as well informed, life-and-death decision-makers to a broader, more comprehensive perspective.

From the onset of their studies, each student of medicine must be perceived as a separate, unique universe. Besides, knowledge acquisition, his/her internalization processes (emotional ones inclusive) must also be cared for, on an individual basis.

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Women with Cardiac Syndrome X- Depressive Symptoms and a Possibility for Their Assessment

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Жени с Кардиологичен синдром X- депресивни симптоми и възможност за оценката им

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Abstract

The cardiac syndrome X includes patients, mainly women, with the triad of angina pectoris, a positive exercise electrocardiogram for myocardial ischaemia and angiographically smooth coronary arteries. The most manifest clinical symptom in those patients is the chest pain. In most cases the syndrome is associated with debilitating symptomatology, increased psychological morbidity and a poor quality of life.

Aim of the current study is to make a cross sectional study of the depressive syndrome among women who fulfill criteria for cardiac syndrome X.

Material and methods: In the current study are included 40 women with fulfilled criteria for CSX all hospitalized at the University Hospital „Alexandrovska“, Clinic of Cardiology. Women are at the mean age of 57,73±9,5 years. All patients undergo standardized clinical examination, history of the disease was taken and 30 women were assessed by the ZUNG self-rating scale for depressive syndromes.

Резюме

Кардиологичният синдром X включва пациенти, основно жени, изпълняващи триадата: ангина пекторис, позитивен тест с натоварване и ангиографски коронарни артерии без промени. Клинично най-изявеният симптом при тези пациенти е гръдната болка. При повечето пациенти синдрома води до инвалидизираща симптоматика, повишена честота на психичните заболявания и влошено качество на живот.

Цел на настоящето проучване е да се направи срезово изследване на депресивния синдром сред жени, които изпълняват критериите за кардиологичен синдром X.

Материал и методи: В настоящето проучване са включени 40 жени, които изпълняват критериите за кардиологичен синдром X, които са хоспитализирани в Университетска болница „Александровска“, Клиника по кардиология. Жените са на средна възраст 57,73±9,5 години. Всички жени са преминали стандартен клиничен преглед, снета е анамнеза и 30 жени са оценени

Results and Conclusion:

In the studied group there is a high prevalence of women in menopause and respectively the mean age is $57,73 \pm 9,52$. Mean sum score from the ZSDS is $45,87 \pm 6,59$ which is the score corresponding to mild depressive syndromes. Almost half of the studied women have mild depressive symptoms, one third have moderate and 10% have severe.

Depressive syndromes are usual for women with CSX and because of the prevalence of core depressive, cognitive, anxiety and somatic symptoms is very difficult to determine psychological from somatic complains in these patients.

Key words:

cardiac syndrome X, depression, ZUNG scale

Cardio-vascular diseases are among the main causes for mortality and morbidity worldwide. The total social, economical and physiological burden of these diseases is growing in each developed or developing country (1,2).

In spite of the numerous studies, cardiac X syndrome (CSX) is still an undefined problem for diagnostics and treatment. This syndrome includes patients, mainly women, with the triad of angina pectoris, a positive exercise electrocardiogram for myocardial ischaemia and coronary arteries without angiographic changes. Different authors are trying to define the pathological mechanisms of the syndrome: myocardial ischemia, endothelial dysfunction, metabolic and hormonal factors (prevalence of women with CSX- more than 70%). The main clinical symptom in CSX is chest pain, which is usually more prolonged and difficult to treat than the typical anginal pain. Cannon et al discuss that in patients with CSX there is an impaired pain perception (3). All that heterogeneity makes the treatment approaches to those patients unclear and uncertain. This gives the right of J. C. Kaski to

със самооценъчната скала на ZUNG за депресивни симптоми.

Резултати и заключение:

В изследваната група се наблюдава преобладаване на жените в период на менопауза и съответно средната възраст е $57,73 \pm 9,52$ години. Средният сумарен скор от ZSDS е $45,87 \pm 6,59$, което отговаря на леко изразен депресивен синдром. Почти половината от изследваните жени имат леко изразени депресивни симптоми, една трета имат умерени и 10% са с тежки по степен депресивни симптоми.

Депресивните симптоми са обичайни за жени със КСХ и поради преобладаването на основни депресивни, когнитивни, страхови и соматични симптоми е затруднено разграничаването на психичните от соматичните оплаквания при тези пациенти.

Ключови думи:

кардиологичен синдром X, депресия, скала на ZUNG

summarize: „Patients with CSX represent a diagnostic and therapeutic riddle“ (4). Increased number of hospitalizations of patients with CSX lead to hyperdiagnostics, including repetitive coronary angiographies with „negative“ results (more than 2 in some patients). All this is with a great burden not only for the society but also for the psychic health of each patient.

Aim of the current study is to make a cross sectional analysis of the depressive symptoms and syndrome among women who have a repetitive exertional chest pain, ECG ST-T changes and coronary arteries with no changes from angiography- fulfilling main three criteria for cardiac syndrome X.

Material and methods

In the current study are included 40 women who fulfilled criteria for CSX all hospitalized at the University Hospital „Alexandrovska“, Clinic of Cardiology. Women are at the mean age of $57,73 \pm 9,5$ (ot do) years (fig1.). All patients undergo standardized clinical examina-

tion, history of the disease was taken and 30 women answered ZUNG self-rating depression scale (ZSDS). That scale is a 20-item self-report measure of the symptoms of depression. Patients rate each item according to how they felt during the preceding week. Item responses are ranked from 1 to 4 with higher numbers corresponding to more frequent symptoms (although several items are scored in reverse). The sum of the 20 items produces a raw score that is converted into a percentage of the depression measurable by the scale (termed the „SDS index“). Index scores are then categorized into 4 levels to offer a global clinical impression, as recommended by the instrument developers: I, within normal range, no significant psychopathology; II, presence of minimal-to-mild depression; III, presence of moderate-to-marked depression; and IV, presence of severe to extreme depression.

For all data analysis is used SPSS 16v. statistical package

Results

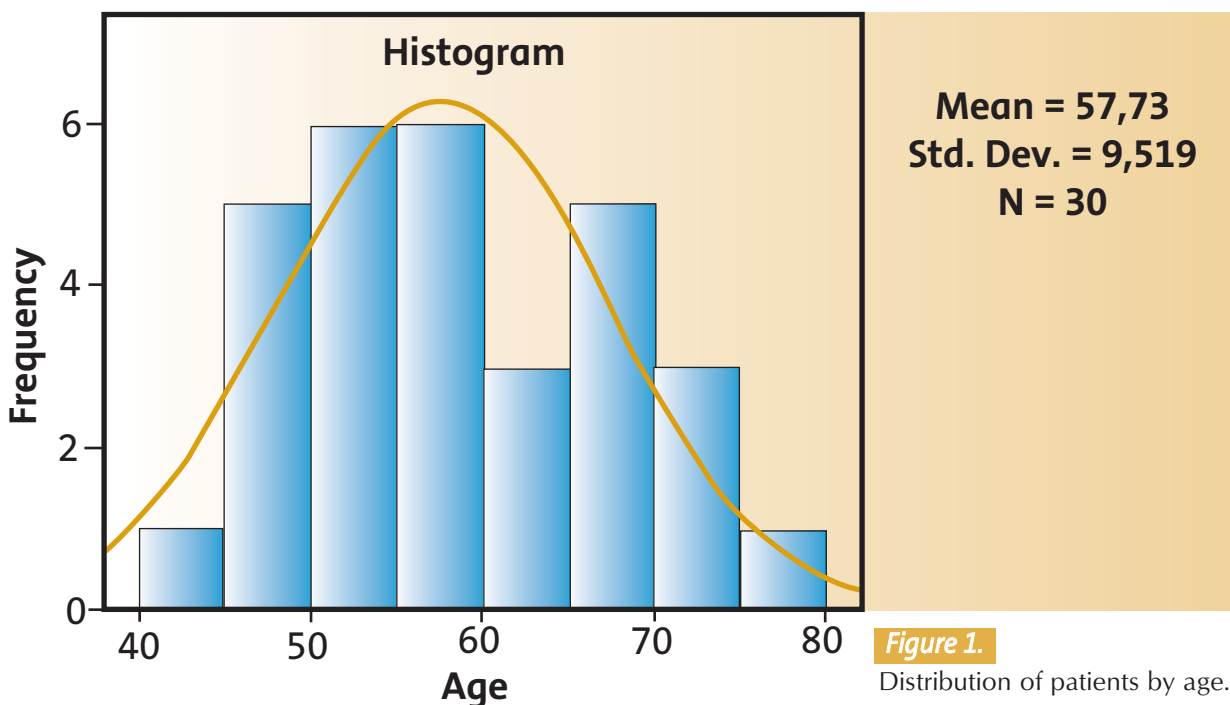
In the studied group there is a high prevalence of women in menopause and respectively the mean age is $57,73 \pm 9,52$ (fig.1, 2).

Mean sum score from the ZSDS is $45,87 \pm 6,59$ which is the score corresponding to mild depressive syndromes (Fig.3.). Almost half of the studied women have mild depressive symptoms, one third have moderate and 10% have severe (Fig.4).

The more detailed analyses demonstrates that the higher scores are for questions 4,5,6, 9,10, 13, 16 and 20th, which are respectively „I have trouble sleeping at night“, „ I eat as much as I used to“, „I still enjoy sex“, „My heart beats faster than usual“, „I get tired for no reason“, „I am restless and can't keep still“, „I find it easy to make decisions“, „I still enjoy the things I used to do“. Mean values for that questions score are respectively $2,9 \pm 0,94$, $2,54 \pm 0,92$, $2,76 \pm 1,0$, $2,71 \pm 0,94$, $3,00 \pm 0,96$, $2,59 \pm 0,78$, $2,48 \pm 1,09$ and $3,07 \pm 0,84$. After statistical analysis median score for that group of questions is 3 (Fig.5).

All this data lead to the conclusion that depressive syndrome in women with CSX could consist of core depressive, cognitive, anxiety and somatic symptoms as a result form the analysis of ZSDS.

Mean age and prevalence of menopause among the included in the study women, correspond with data from literature for hormonal disturbance as one of the main mechanisms for



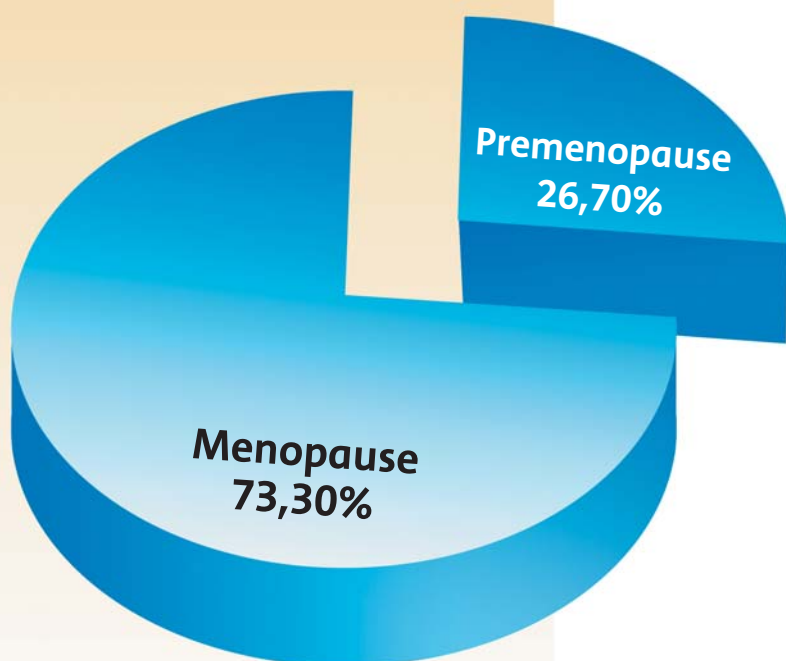


Figure 2. Women in pre-menopause and menopause.

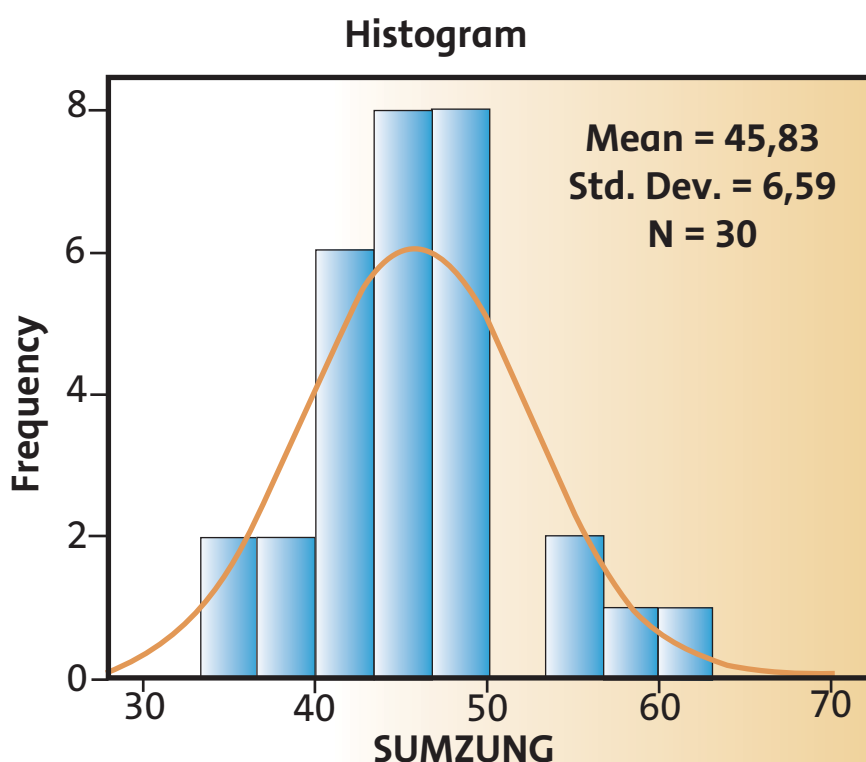


Figure 3. Distribution of patients according to the mean ZSDS sum.

occurrence of the CSX. First Kaski et al found that in women with CSX there is a significant lower levels of estradiol in comparison with the women from the healthy population (4).

No one of the studied patients have been ever diagnosed for depression or depressive symptoms. According to the WHO women suffer twice more often from depression than women (7). Worldwide there are more than 110 million people with depression or depressive symptoms (5). Because of the repetitive pain, often hospitalizations and a lot of instrumental examinations, some of which are painful and with a lot of risk (coronaroangiography for example), women with fulfilled criteria for CSX usually suffer from psychological diseases. Treatment of the pain syndrome is very uncertain and this is with a great burden to the quality of life of these patients. Depressive syndromes are usual for women with CSX and because of the prevalence of core depressive, cognitive, anxiety and somatic symptoms is very difficult to determine psychological from somatic complains in these patients. All that data correlates with some findings that in CSX there is impaired nervous system regulation and particularly depressed vagal tonus (6).

In conclusion, it is highly recommended to look for even mild depressive symptoms and to treat them aiming to benefit the quality of life of that women. ZUNG self-rating depression scale is well validated and easy to apply in the daily clinical practice even that of the general practitioners (8,9,10,11).

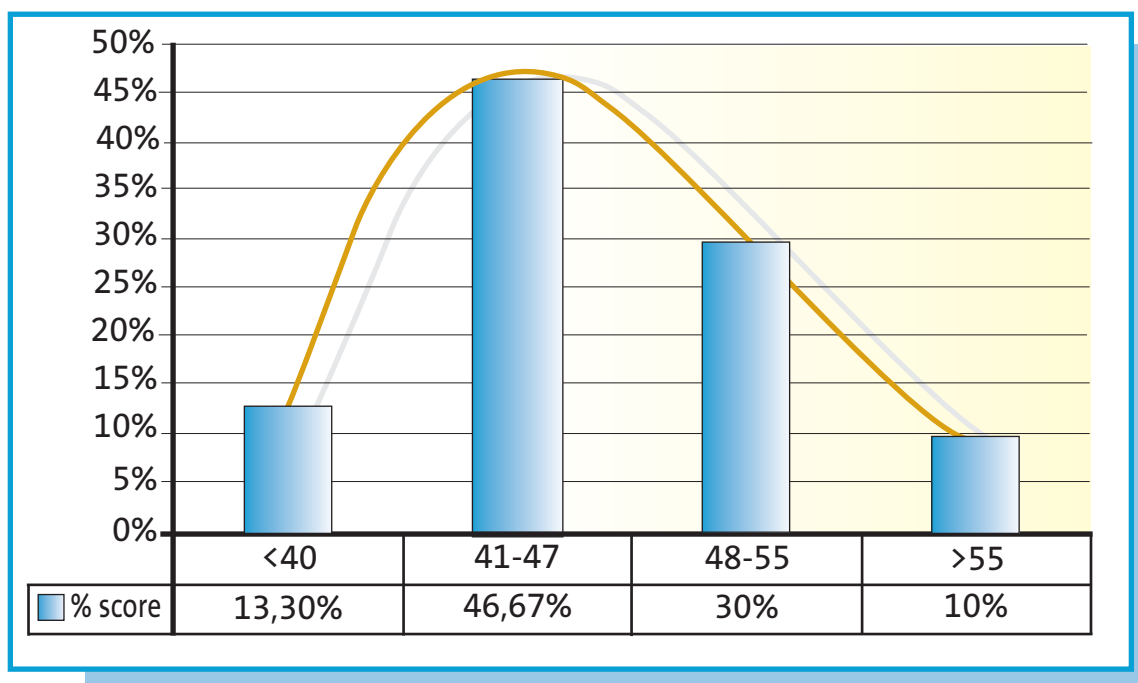


Figure 4 Women with mild, moderate and severe depressive symptoms according to the ZSDS sum.

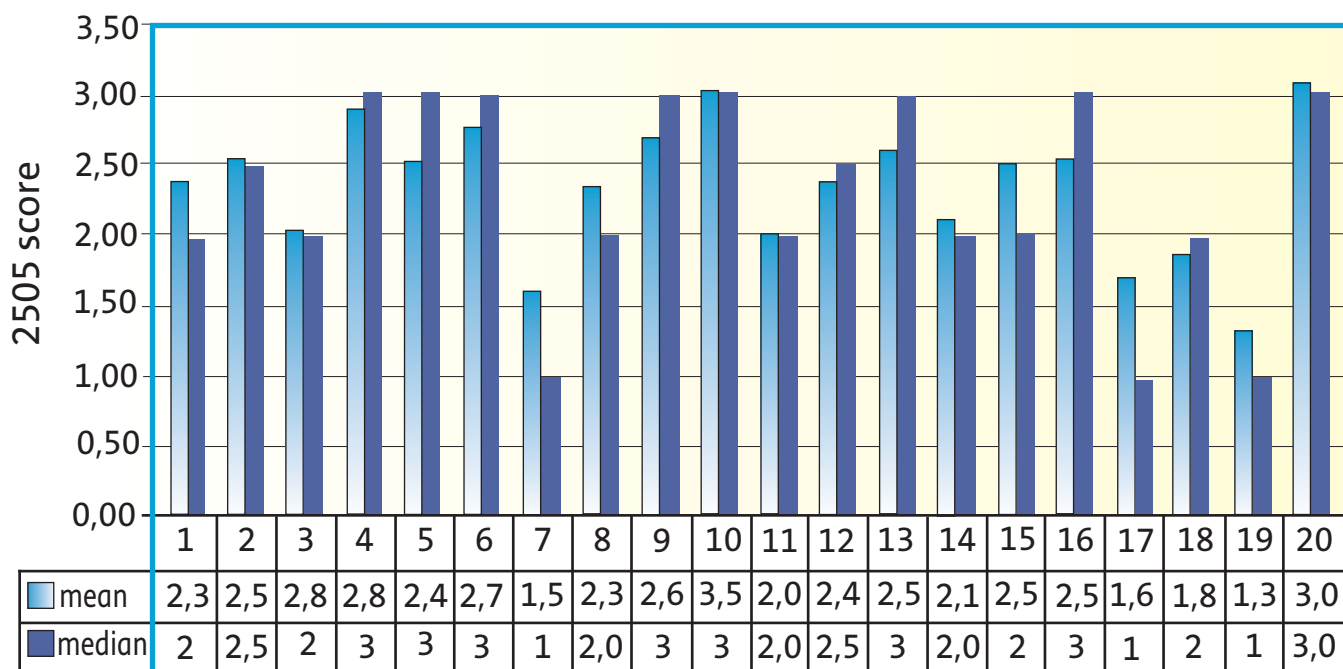


Figure 5. Mean and median score: distribution by questions from ZSDS.

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Mayer-Rokitansky-Küster-Hauser Syndrome A case report with brief review of the literature

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Синдром на Mayer-Rokitansky-Küster-Hauser: описание на случай с кратък обзор на литературата

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Abstract

The Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is characterized by congenital absence of the upper two-third of the vagina, rudimentary cornua uteri, primary amenorrhea, and morphologically normal ovaries and Fallopian tubes due to congenital Müllerian duct total aplasia in individuals with 46, XX karyotype and female phenotype. Although the incidence of MRKH syndrome is not completely clear, there are some reports which estimate the frequency of congenital absence of vagina and rudimentary uterus as 1 in 4500 female births. Several cases of this disorder have been described in Bulgaria. The present case demonstrates a woman with diminished ovarian reserve, which is not typical characteristic of the syndrome.

We report the case of a 26-year-old nulliparous woman who was referred in our centre

Резюме

Синдромът на Mayer-Rokitansky-Küster-Hauser (MRKH) се характеризира с вродена липса на горните две-трети на влагалището, рудиментарна матка, първична аменорея и морфологично нормални яйчници и Фалопиеви тръби, на базата на вродена пълна аплазия на Мюлеровия канал при индивиди с кариотип 46, XX и женски фенотип. Въпреки, че честотата на синдрома на MRKH все още не е напълно изяснена, има данни, според които вродена липса на влагалище и матка се установява при 1 на всеки 4500 новородени момичета. Няколко случая на този синдром вече са описани в България. Представеният случай показва жена с намален яйчников резерв, нехарактерен за синдрома.

26 годишна нераждала жена, насочена за изясняване по повод инфертилитет при

because of infertility, represented with no menstrual bleeding and unremarkable family history. The results of hormonal evaluation showed normal values of Gonadotropic hormones as well as Prolactin, Testosterone and 17β -estradiol. The main laboratory test finding was the low level of Anti-Müllerian Hormone (AMH) = **0,47** $\mu\text{g/L}$ (NR = 1-8 $\mu\text{g/L}$), suggesting depletion of ovarian reserve. Genetic analysis showed a normal 46, XX karyotype. The definitive diagnosis of MRKH syndrome was made via laparoscopy.

This case represents type I (isolated utero-vaginal aplasia) MRKH syndrome in the absence of any other malformations such as renal, skeletal, cardiac and auditory defects. In our case a depleted ovarian capacity was observed (low AMH), which is not a typical feature of the syndrome and is in contradiction with laparoscopic evidence of the presence of follicles and Corpus Luteum. However, it is generally known that while laparoscopy and ultrasound give a snapshot of the ovaries at the day of examination, AMH reflects ovarian reserve in perspective. As far as we know this is the first case reported of MRKH syndrome in which the level of Anti-Müllerian Hormone is low.

Key words:

Mayer-Rokitansky-Küster-Hauser syndrome, laparoscopy, Anti-Müllerian Hormone

Introduction

The Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is characterized by congenital absence of the upper two-third of the vagina, rudimentary cornua uteri, primary amenorrhea, and morphologically normal ovaries and Fallopian tubes (13, 14, 17, 19, 23) due to congenital Müllerian duct total aplasia in individuals with 46, XX karyotype and female phenotype (28).

липса на менструално кървене, водеща нормален полов живот; фамилно необременена. Бяха установени нормални серумни нива, както на гонадотропните хормони, така и на пролактина, тестостерона и 17β -естрадиола. Основната лабораторна находка беше ниското ниво на Анти-Мюлеровия хормон (AMH) = **0,47** $\mu\text{g/L}$ (Референтни стойности = 1-8 $\mu\text{g/L}$), което предполага намален яйчников резерв. Генетичният анализ показва нормален 46, XX кариотип. Окончателната диагноза на синдрома на MRKH се постави посредством лапароскопия.

Случаят представлява синдром на MRKH тип I (изолирана утеро-вагинална аплазия), при отсъствие на други малформации: бъбречни, скелетни, сърдечни и слухови. Установяването на намален яйчников резерв (нисък AMH) въпреки лапароскопските данни за наличие на фоликули и жълто тяло е нехарактерно за синдрома. Известно е, че докато лапароскопското изследване и ултразвуквата диагностика показват само състоянието на яйчниците към момента на изследването, то AMH отразява овариалния резерв в перспектива. Доколкото ни е известно, това е първият описан случай на синдрома на MRKH, при който плазмените нивата на Анти-Мюлеровия хормон са ниски.

Ключови думи:

Синдром на Mayer-Rokitansky-Küster-Hauser, лапароскопия, Анти-мюлеров хормон

This syndrome is subdivided in two types: the typical type I (isolated utero-vaginal aplasia) or Rokitansky sequence, and the atypical type II (incomplete aplasia and/or associated with other malformations such as renal, skeletal, cardiac and auditory defects) or MURCS association (Müllerian duct aplasia, Renal dysplasia, Cervical Somite anomalies); the frequency of type II being much greater (20, 27). Although the incidence of MRKH syndrome is not completely

clear (12), there are some reports which estimate the frequency of congenital absence of vagina and rudimentary uterus as 1 in 4500 female births (9, 11, 30). In women presenting with primary amenorrhea, MRKH syndrome is fairly common, being second to gonadal dysgenesis as a cause of amenorrhea (7, 29). Although the pathogenesis of Müllerian aplasia associated or not with other malformations is now well-known, on the one hand, and the spectrum of malformations encountered suggests a development field defect, involving organ systems closely related during embryogenesis, on the other hand, the etiology of MRKH syndrome is still quite unclear (12, 16, 20). It was initially considered that this syndrome is of sporadic occurrence, involving some non-genetic or environmental factors (26). Now the increasing number of familial cases supports the hypothesis of a genetic cause and although the pattern of inheritance is yet undetermined, it seems to be transmitted as an autosomal dominant trait with incomplete penetrance and variable expressivity (12, 20).

Several cases of MRKH syndrome have been described in Bulgaria (1, 2, 3). A woman with this disorder has come under our observation for nearly a year. Since each case enriches the clinical picture, we considered it reasonable to describe the patient, we have observed.

Case presentation

P. I. V. a 26-year-old nulliparous, poorly educated woman of Bulgarian origin was referred in our centre because of infertility. She had no menstrual bleeding, but she reported that every month her urine turned red, which she considered to menstrual bleeding and did not seek medical advice. She was married and had a normal sex life with normal intercourse. Her family history was unremarkable. There were no skeletal and kidney abnormalities in her family. Only her first paternal cousin was childless.

On physical examination the patient's



Table 1. Hormonal levels in the patient with MRKH syndrome

Hormone	Values	Units	Normal range
LH	2,8	IU/L	2-10
FSH	4,2	IU/L	1-10
Prolactin	460	mIU/L	< 600
Testosterone	0,7	nmol/L	0,3-3,5
TSH	0,702	μ IU/ml	0,27-4,2
Free T4	13,48	ng/l	9,3-17
Anti-Müllerian Hormone	↓ 0,47	μ g/L	1-8

height was 162 cm and her weight was 50 kg (BMI 19 kg/m²). Her pulse rate was 66/min and her blood pressure was 110/60 mmHg. No skeletal abnormalities have been detected. The external examination revealed completed puberty with normal female sexual secondary development (Fig. 1). Gynecological examination found external genitalia of nulliparous, the vagina was normal, deep, ending blindly, there were no adnexa abnormalities, and Cavum Douglasi was free.

The results of hemoglobin, hematocrit, total red and white blood cells count, platelets, erythrocyte sedimentation rate (ESR), serum creatinine, serum transaminases and hemocoagulation status as well as urinalysis and sediment were normal.

Hormonal data are presented in Table 1. Gonadotropic hormones as well as Prolactin, Testosterone and 17 β -estradiol were within normal limits. The key finding of these laboratory tests was the low level of Anti-Müllerian Hormone (AMH) = **0,47** μ g/L (NR = 1-8 μ g/L), suggesting depletion of ovarian reserve.

Genetic analysis performed on the 35 metaphase peripheral blood lymphocytes showed a normal 46, XX karyotype. An abdominal ultrasound showed normal internal organs. A thyroid ultrasound showed mild diffuse changes in the gland structure and cervical lymphadenomegaly.

Diagnostic laparoscopy revealed two rudimentary uteri – one on the right side smaller in size and the other on the left side. The left Fallopian tube, running from the left rudimentary uterus, was found to be 10-12 cm in length, normal proceeding, free of adhesions, and intact

fimbriae. The left ovary was located on a typical site, with normal shape and size, free of adhesions, and the presence of follicles in various stages of development. The right Fallopian tube, running from the right rudimentary uterus, was found to be 10-11 cm in length, normal proceeding, free of adhesions, and intact fimbriae.

The right ovary was located on a typical site, with normal shape and size, free of adhesions, and the presence of Corpus Luteum. Ligamentum sacrouterina, liver, gallbladder, spleen showed no abnormalities. No surgical reconstructions had been carried out.

We also assessed her husband's fertility by semen analysis and evaluation of his hormonal levels at the same time. The result of the semen analysis showed normozoospermia and his hormonal levels were within the normal ranges as well (FSH = 3,18 mIU/ml and Inhibin B 145 ng/l (NR = 1,5-12,4 and 120-400, respectively)) which provided evidence of preserved spermatogenic capacity.

Discussion

In this paper we describe a typical case of type I (isolated utero-vaginal aplasia) MRKH syndrome in the absence of any other malformations such as renal, skeletal, cardiac and auditory defects (20, 27) which is not the first described in Bulgaria (1, 2, 3). In the case presented, clinical suspicion of this syndrome was aroused by accident and relatively late in a prophylactic medical examination and definitively diagnosed via laparoscopy. With regard to delayed diagnosis in this case, as mentioned above, it refers to a low-educated woman, with low health literacy, who has neglected the absence of menstruation. Moreover, there is a satisfactory development of the lower segment of the vagina which allows normal sexual intercourse.

Differential diagnosis includes three conditions presenting with primary amenorrhea and normal female secondary sexual characteristics: androgen insensitivity, isolated vaginal atresia and WNT4 defects (4, 22). The most common differential diagnosis is androgen insensitivity, affected males and characterized by presence of abdominal or inguinal testes and male karyotype 46, XY (20). Another condition is isolated vaginal atresia, which occurs due to mutations in MKKS gene located on chromosome 20p12, and is found in a number of syndromes, such as Winter syndrome (involving renal, genital and middle ear malformations) and McKusick-Kaufman syndrome (involving hydrometrocolpos, postaxial polydactyly and congenital hearing malformations). Whereas the isolated vaginal atresia could be surgically corrected to make pregnancy possible, MRKH syndrome is connected with irreversible infertility (15). Finally, we should take into consideration WNT4 defects, although only two cases have been described in the literature so far. WNT4 belongs to a WNT family of genes associated with the regulation of cell and tissue growth and differentiation, and has a dominant effect. Its mutations cause a masculinization of the fetal gonads, leading to an excessive androgen production, and interfere with the primary differentiation of Müllerian ducts. Their specific clinical features are hyperandrogenism and uterine aplasia (20).

Classic clinical description of MRKH syndrome includes persons with a primary amenorrhea, normal 46, XX karyotype (5, 8, 18, 20, 24), normal ovarian function, no signs of androgen excess (10, 25), and normal secondary female sexual characteristics (20), whereas in our case a depleted ovarian capacity was observed (low AMH), in spite of the presence of follicles and Corpus Luteum. Whereas gonadal dysgenesis associated with Mayer-Rokitansky-Küster-Hauser syndrome, considered to be coincidental, has been found (6), as well as abnormally high plasma levels of AMH in patients with the same condition have been described (21); as far as we know this is the first case of MRKH syndrome in which the level of Anti-Müllerian Hormone is low. While laparoscopy and ultrasound give a snapshot of the ovaries at the day of examina-

tion, AMH reflects ovarian reserve in perspective. These data indicate that infertility is the most significant issue of the disorder and it is less likely ova from the patient for *in vitro* fertilization to be received. The improvement of medical technologies allows, in many countries, women to appeal for *in vitro* fertilization and surrogate pregnancy (4). In our case the use of donor ova should be considered.

Consent

Informed consent was obtained from the patient for publication of this case report and accompanying images at the time of the diagnosis.

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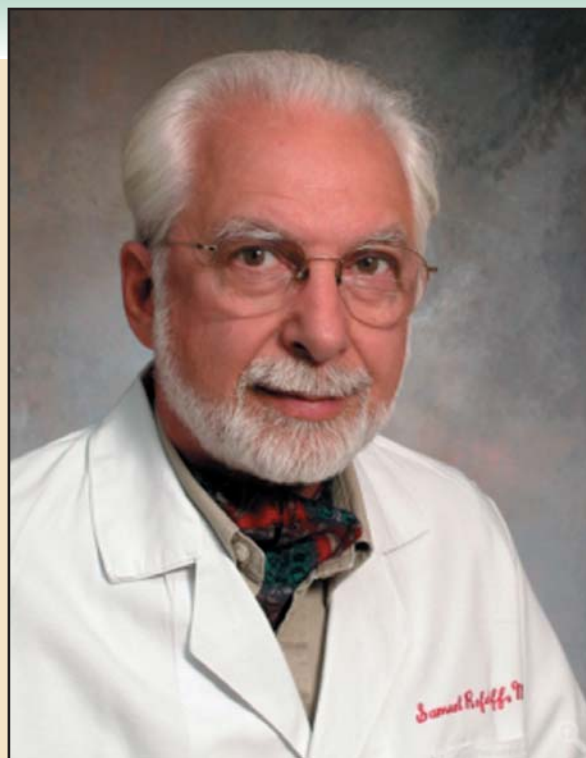
Academician Professor Dr Samuel Refetoff, a new member of the Bulgarian Academy of Sciences and Arts

Professor Samuel Refetoff from the University of Chicago was nominated as Academician to the Bulgarian Academy of Sciences and Arts (BASA) in September 2010. The official announcement of his admission as a BASA foreign member took place in May 2011 during his visit in Sofia organized by the Bulgarian Fulbright Commission for educational exchange.

Professor Samuel Refetoff was born on July 11, 1937, in one of the most cultural centers of Bulgaria, the city of Russe. His education and post-graduate training in medicine has been held in universities of Montreal (Canada), Los Angeles and Boston (USA). The academic career of Prof. Refetoff started in Harvard Medical School in 1968 following the University of Chicago where he was elected as professor of medicine (1977), later as professor of pediatrics. Since 2001 he has been professor in the Committees on Genetics and Molecular Medicine, Director of the endocrinology training program to the same university.

Prof. S. Refetoff is prize-winner of many awards as well as honorary degrees. His professional memberships include more than 15 medical associations and institutes in the USA and Europe. He is also a member of many scientific journals, including „Bulgarian Medicine“ (BASA) and „Endocrinologia“ (Edition of the Bulgarian Society of Endocrinology).

The research of Prof. S. Refetoff has been funded without interruption since 1971 up to date mainly by the



National Institutions of Health (USA) as well as the University of Chicago. He discovered the inherited syndrome of resistance to thyroid hormone („Refetoff Syndrome“) and elucidated its genetic and molecular base. This was the subject of his plenary lecture at the Scientific Meeting organized by BASA on May 19, 2011 in Sofia. In the same event he was congratulated by the Academy President, Academician Prof. Dr. Grigor Velev who gave him the acknowledgement as an outstanding foreign member of BASA. He also expressed the highest appreciation for the world-recognized contributions of Professor Samuel Refetoff in fundamental and clinical endocrinology.

One anniversary – two heroes

This year Academician Prof. Dr Miladin Apostolov, and the foreign BASA member Academician Prof. Dr Spiros Marketos, both born in 1931, are getting on for the dignity age of 80! Both of them are historians of Medicine and are doyens of the History and Philosophy of Medicine in Bulgaria and in Greece. Both of them are supporters of the idea for the unification of the Balkan historians of Medicine.

They share in Hippokrates' philosophy by teaching Hippokratism and Neohippokratism.

They both are pillars of the historic-medical science in Bulgaria and in Greece, in the Balkan Peninsula and in Europe.

The irksome biographical data describe – individually or in common – their rich professional, scientific, intellectual, teacher's and publication activities.

Prof. Miladin Apostolov comes from the village Komoshtitza, near Lom town, Bulgaria, and Prof. Spiros Marketos – from Athens, Greece. But both of them have graduated Medicine – the science that gives knowledge about human beings at the most - and they elicit from the profundity of the civilization and to nowadays the development of Medicine and its prominent creators in their historical, philosophic, social and ethical aspects. Prof. M. Apostolov is more socially engaged, while Prof. Sp. Marketos is mostly a theoretician.



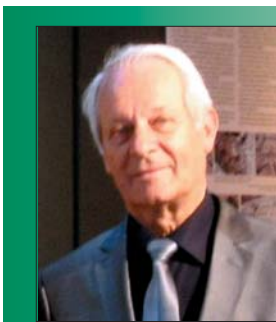
**Spyros
Marketos**

The philosophy roots and humanistic sources of Hippokratic medicine are among the favourite research topics of Prof. Marketos – he has a lot of publications and congress reports about Medicine as science and art in Ancient Greece, on Hippokratic ethics in modern medicine, that historically and culturally reveals how necessary it is for the overcoming the biomedical problems of today's medicine, etc. Some of his recent books are Hellenic Medicine (1991), History

of Medicine (1996), History of Medicine of the 20th century (2001). Instead of his submission to Hippokrates, Prof. Marketos shares the conception that the most important physician-philosopher at all times is Galen, who combined Medicine with Philosophy. Prof. Marketos studied the works (Timaeus, Laws, Republic, Phaedo, Gordias) and philosophy of Plato – who is one of the brilliant historical figures, and, probably, the most important proponent of the „active euthanasia“ („physician-assisted suicide“) in the Ancient times.

Prof. Marketos investigates some contemporary Greek scientist, as well – the pioneer in neurophysiology Dr George C. Cotzias; the promoter of the neo-Hellenic history of medicine Prof. Nikolas C. Louros; the most prominent neurosurgeon Prof. Petros S. Kokkalis. Prof. Marketos explored the great medical figure of Dionysios Pyrros and his outstanding scientific activities in the field of Balkan medicine – Pirros' treatise „Practical medicine“ was the first textbook on medicine translated in Bulgaria (1854).

At present Prof. Spiros Marketos is Honorary president of the International Hippocratic fund – IHFK (Greece), president of the Pan-Hellenic association of history, sociology and philosophy of medicine, foreign member-academician of BASA – Bulgaria (2009), Doctor Honoris Causa of the Odessa state medical university – Ukraine (2000), past president of the World Hippocratic society of Kos, fellow of many national and international scientific societies and of some editorial and advisory boards in Greece and elsewhere. He has a lot of prizes, and was given the Bulgarian awards Purple Heart and Golden Hippocratic Oath.



**Miladin
Apostolov**

As for Prof. Miladin Apostolov, he is a very productive author, editor and co-author, too. Let me note down some of his scientific works: the first Bulgarian textbook on History of Medicine (1977, 1981, 1984, 1992, 1997), the first and only Reader on history of Medicine (1995), Hippokrates, Hippokratism, Neohippokratism (1972), History of Medicine and nursery (1998, 2001), Schools in Bulgarian Medicine (1987, 1995), History of Medicine and social matters [in Bulgaria] (1994), Dimensions of Bulgarian Medicine (2001), History of surgery in Bulgaria (2007), Historical, social and ethical dimensions of Medicine (2000), Medical Faculty – Sofia.1918-1993 (1993), Medical relations between Iberian state and Balkan Peninsula states in the Middle Ages [in French] (1981). It's interesting to mention, that Prof. Apostolov shows a capacity for a novelist, as well. He wrote novelized biographies of Academician Metody Popov (1981) and of Yane Sandansky (1971). But his masterpiece is the historical novel Grand Duchess Helen-Olga, edition of the Bulgarian Writer Publ. (2009, 2011). He revived the Bulgarian Asklepios journal and since 1992 is its editor-in-chief.

As a scientist of dynamic nature Prof. Apostolov has organized several international scientific expeditions on History of Medicine – Hippokrates (Kos island, 1972), Ioan Petriciy (Tbilisi, Baku, Yerevan, 1983), Amatus Lusitanus (Spain, Portugal, 1986), and in Bulgaria – Pavlikeny town, Kabile-Yambol, Varna, etc.

Prof. M. Apostolov is the „father“ of some awards – Golden Hippokrates – for excellent mark and scientific research of medical students; Purple Heart – for mercy and charity; BAHPM's Golden Hippocratic Oath – for high professionalism in medicine and Hippocratic attitude to the patients.

Prof. Miladin Apostolov is president of the Bulgarian society of history of medicine (since 1983), Honorary member and Doctor Honoris Causa of Panhellenic society of history of medicine; member-academician of BASA and its vice-president; Honorary academician at the Petrovskaya academy of sciences and arts (PASA); Honorary member of the Panhellenic association on history,

sociology and philosophy of medicine and of the Russian confederation of the historians of medicine; member of the Union of Bulgarian writers; fellow of many foreign scientific societies. Prof. Apostolov is a twice holder of the St. St. Cyril and Methodius order for science and education and knight of the highest Bulgarian order Stara Planina, 1st degree.

In 2005 the distinguished professors Spiros Marketos and Miladin Apostolov together established the Balkan association on history and philosophy of medicine (BAHPM) and as co-founders and co-presidents they signed its documents – the Varna declaration, its Statute and Protocol. The Asklepios journal became BAHPM's official edition and both of them – its co-editors-in-chief.

It's impossible to give a complete picture of the so rich and diverse scientific, teaching, publication and public activities of Prof. Dr Spiros Marketos and Prof. Dr Miladin Apostolov in several pages only. That's why let me stop and say:

**Thank you,
congratulations
and be healthy,
dear Professors!!!**

Assoc. Prof. Dr
Marussyia Petkova, MD, PhD
Bulgarian Academy of Sciences
and Arts (BASA) – Sofia

Clinical Toxicology in Bulgaria

Клинична токсикология в България. АВСТ, София, 2010.



The Bulgarian Clinical Toxicology Association issued a collection of articles on the development of the Clinical toxicology in Bulgaria, founded as a separate medical discipline in 1963. The articles cover the civil and military Clinical toxicology net in Bulgaria – N. I. Pirogov University Hospital and Military medical academy (Sofia), the toxicology clinics in Plovdiv and Pleven, Naval hospital (Varna) and the regional centre for acute intoxications (Dobrich).

Assoc. Prof. and BASA member-observer Dr Eugenia Barzashka describes the short history of the Clinical toxicology care in Pleven region. A sector has been established in 1990, and a ward – in 1995. At present the pediatric sector operates with 5 beds, and this one for the adults – with 10 beds. The activities of the oldest clinical toxicology unit in Bulgaria is presented historically and in detail by Assoc. Prof. and BASA correspond-

ing-member Dr Aneta Hubenova – chief of the Toxicology clinic and national adviser and regional consultant for Sofia in Clinical toxicology at the Ministry of Health. The idea for the opening of a toxicology unit in N. I. Pirogov hospital belongs to Prof. Dr Alexander Monov – the most distinguished Bulgarian toxicologist – who conducted the Toxicology clinic for 28 years (1963 - 1991). Step by step new departments and sectors appear and today the well described structure of the clinic includes pediatric department, ward for adults, sector for treating any kind of dependences, chemistry-toxicological laboratory, toxicology information, psychiatric and rehabilitation sectors. Thanks to the modern equipment the Toxicology clinic provides therapeutic, diagnostic, consulting, methodological, teaching, medical information and scientific research activities at higher medical levels and meets the needs all over the country.

10 Principles of the Medic and the Manager

Воденичаров, Ц. 10-те принципа на медика и мениджъра. С., Симелпрес, 2010.



The author is well known as a scientist, teacher, manager and creator among the medical community. After decades of gaining experience and knowledge, broadening of philosophic, ethic, cultural and professional outlook and ideas, efforts for intellectual self-perfection, it comes the time to take the pencil and to lay down on the white sheet the clarified cogitations. In his last book Prof. Dr Tzekomir Vodenicharov – academican at BASA – shows his ten principles, leading to success and recommends them to the medical doctors and health managers: Have an immortal aim; Don't believe, but analyse; Think associatively and integrally; Follow the great, but take care for the details; Be useful; Plan out; Forecast; Organize; Dream; Act. Principle by principle he describes the mode of behaviour to reach the wanted level of professional, managerial and material success. To illustrate his recommendations Prof. Vodenicharov uses a lot of examples, coming from history, philosophy, world literature, culture and his own life. In unison with the philosophic character of the text the somehow abstract illustrations, made by Anatoly Alexiev, follow its intellectual ringing. The narration is interesting, the book is written in nice and rich Bulgarian language and really it's a pleasure to read it.

Looking for the balance in our life, let us follow Prof. Vodenicharov's testaments: Be person of yourself, Share your knowledge to reach immortality.

Assoc. Prof. Dr
Marussya Petkova, MD, PhD
Bulgarian Academy of Sciences
and Arts (BASA) – Sofia

IN MEMORIAM

Last goodbye to Academician Dr Peter Konstantinov

On 12th of June 2011, as a result of a painful illness Prof. Dr Peter Konstantinov – full member of BASA, sharing its ideas, mission and activities, patriot, zealot for everything Bulgarian, distinguished intellectual and prominent public personality, passed away.

Peter Konstantinov Stoyanov was born on 01.08.1929 in the town of Kazanluk. He was master in Medicine (1952) and Political economy (1966), and specialist in Internal Medicine, Cardiology and Rheumatology. He is the author of more than 140 scientific publications and had active medical practice for 45 years. But the Bulgarian communities all over the world knew Dr Konstantinov mainly as an interesting writer, novelist and publicist, and as a socially active person – one of the founders and president since 1989 of the National association „Mati Bolgaria“ - the first independent organization in Bulgaria after 1989, that, thanks to Dr Konstantinov, worked intensively for saving of the Bulgarian traditions, culture and historical memory among the Bulgarians all over the world.

As a publicist in the field of Political economy Dr Konstantinov wrote the books „History of Bulgaria with her concealed historical facts“, „For and against the Bulgaria national interests“, „Social and economic problems of the sciento-technical revolution“, etc. For the lovers of literature and art, prof. Konstantinov is a remarkable narrator and eloquent writer, prominent intellectual with mighty Renaissance spirit. An ardent admirer of fine arts he wrote some art itinerary books – „The treasures of the world“, „The treasures of Europe“, „The treasures of Bulgaria“, etc. All his works are written in vivid, beautiful and rich Bulgarian language.

Besides, in 1985 he endowed the town „Iskra“ library in Kazanluk with 1023 volumes of fiction and reference books, and some years later he gifted 100 canvases by distinguished Bulgarian artists of his own to the Kazanluk Picture-gallery.

For his literary creation Dr Peter Konstantinov received two significant awards – the „Dimitar Dimov“ literature prize, given to physicians (2006) and in 2009 he was elected as Honorary Citizen of Kazanluk town.

Deep obeisance to his luminous memory and may his spirit live and rest in peace for ever!

Editorial Board of Bulgarian medicine journal

Author's guidelines

The Bulgarian Medicine Journal, official edition of the Bulgarian Academy of Science and Arts, Science Division, Research Center for Medicine and Health Care is published in 4 issues per year. It accepts for publication reviews, original research articles, case reports, short communications, opinions on new medical books, letters to the editor and announcements for scientific events (congresses, symposia, etc) in all fields of fundamental and clinical medicine. The journal is published in English with exceptional reviews on significant topics in Bulgarian. The detailed abstracts and the titles of the articles, the names of the authors and institutions as well as the legends of the illustrations (figures and tables) are printed in Bulgarian and English.

The manuscripts should be submitted in two printed copies, on standard A4 sheets (21/30 cm), double spaced, 60 characters per line, and 30 lines per standard page.

The size of each paper should not exceed 10 pages (up to 5 000 words) for original research articles, 12 pages for reviews (7 500 words), upto 4 pages for case reports, 2 pages for short communications, 4 pages for discussions or correspondence on scientific events or medical books. The references and illustrations are included in this size (two 9x13 cm figures, photographs, tables or diagrams are considered as one standard page).

The abstracts are not included in the size of the paper and should be submitted on a separate page with 3 to 5 key words at the end of the abstract. They should reflect the most essential topics of the article, including the objectives and hypothesis of the research work, the procedures, the main findings and the principal conclusions. The abstracts should not exceed one standard typewritten page of 200 words.

The basic structure of the manuscripts should meet the following requirements:

Title page

The title of the article, forename, middle initials (if any) and family name of each author; institutional affiliation; name of department(s) and institutions to which the work should be attributed, address and fax number of the corresponding author.

Text of the article

The original research reports should have the following structure: introduction (states the aim, summarize the rationale for the study), subjects and materials, methods (procedure and apparatus in sufficient detail, statistical methods), results, discussion, conclusions (should be linked with the aims of the study, but unqualified statements not completely supported by research data should be avoided). These requirements are not valid for the other types of manuscripts. Only officially recognized abbreviations should be used, all others should be explained in the text. Units should be used according to the International System of Units (S. I. units). Numbers to bibliographical references should be used according to their enumeration in the reference list.

Illustrations

The figures, diagrams, schemes, photos should be submitted in a separate file with: consecutive number (in Arabic figures); titles of the article and name of the first author. The explanatory text accompanying the figures should be presented along with the respective number of the figure in the main text body with space left for insertion of the figure.

References

The references should be presented on a separate page at the end of the manuscript. It is recommended that the number of references should not exceed 20 titles for the original articles and 40 titles for the reviews; 70% of them should be published in the last 5 years. References should be listed in alphabetical order, English first, followed by the Bulgarian ones in the respective alphabetic order. The number of the reference should be followed by the family name of the first author and then his/her initials, names of the second and other authors should start with the initials followed by the family names. The full title of the cited article should be written, followed by the name of the journal where it has been published (or its generally accepted abbreviation), volume, year, issue, first and last page. Chapters of books should be cited in the same way, the full name of the chapter first, followed by "In:" full title of the book, editors, publisher, town, year, first and final page number of the cited chapter.

Examples:

Reference to a journal article:

1. **McLachan, S., M. F. Prumel, B. Rapoport.** Cell Mediated or Humoral Immunity in Graves' Ophthalmopathy? *J. Clin. Endocrinol. Metab.*, 78, 1994, 5, 1070-1074.

Reference to a book chapter:

2. Delange, F. Endemic Cretenism. In: *The Thyroid* (Eds. L. Braveman and R. Utiger). Lippincott Co, Philadelphia, 1991, 942-955.

Submission of manuscripts

The original and one copy of the complete manuscript are submitted together with a covering letter granting

the consent of all authors for the publication of the article as well as a statement that it has not been published previously elsewhere and signed by the first author. The procedure should be complemented via electronic submission. Manuscripts of articles accepted for publication will not be returned to the authors.

Peer-review process: following the international standards in the field, the Editorial board has adopted double-blind peer-review policy assigned to independent referees. Following acceptance for publication the authors are charged 5 euros per page.

Ethical regulations: reports with experiments on human subjects should specify whether the procedures were conducted in accordance with the ethical norms of the responsible committee on Human experimentation (local or regional) and/or with the Helsinki Declaration, as revised in 2000. Respective guidelines for animal experimentation should be considered.

Address for sending of manuscripts and other editorial correspondence

Prof. Dr Philip Kumanov Editor-in-chief, University Hospital of Endocrinology, 1431 Sofia, Zdrave str. 2, or electronic address: phkumanov@lycos.com

With copy for the scientific secretary – Assoc. Prof. Drozdostoj Stoyanov: stojanovpisevski@gmail.com

Изисквания към авторите

Списание „Българска медицина“, издание на Българската Академия на Науките и Изкуствата, Отделение за наука, Научен Център по Медицина и Здраветопазване излиза в четири книжки годишно. В него се отпечатват оригинални научни статии, казуистични съобщения, обзори, рецензии и съобщения за състояли се или предстоящи научни конгреси, симпозиуми и други материали в областта на клиничната и фундаменталната медицина. Списанието излиза на английски език с подробни резюмета на български и английски. Изключения се правят за обзорни статии по особено значими теми. Заглавията, авторските колективи, а също надписите и означенията на илюстрациите и в таблиците се отпечатват и на двата езика. Материалите, предоставени от чужди автори се поместват на английски с подробно резюме на български.

Материалите трябва да се предоставят в два еднакви печатни екземпляра на хартия формат А4 (21 x 30 см), 30 реда с 60 знака на ред при двоен интервал между редовете (стандартна машинописна страница). Оформени така, материалите могат да бъдат изпращани и като прикачени файлове по електронната поща на адреса, посочен по-долу.

Обемът на представените работи не трябва да превишава 10 стандартни страници за оригиналните статии (или 5000 думи), 12 страници (7500 думи) – за обзорните статии, 3-4 страници за казуистичните съобщения, 4 страници за информации относно научни прояви в България и в чужбина, както и за научни дискусии, 2 страници за рецензии на книги (монографии и учебници). В посочения обем се включват книгописът и всички илюстрации и таблици. В същия не се включват резюметата на български и английски, чийто обем трябва да бъде до 200 думи за всяко (25-30 машинописни реда). Резюметата се представят на отделни страници. Те трябва да отразяват конкретно работната хипотеза и целта на разработката, използваните методи, най-важните резултати и заключения. Ключовите думи (до 5), съобразени с „Medline“, трябва да се посочат в края на всяко резюме.

Структурата на статиите трябва да отговаря на следните изисквания:

Титулна страница

а) заглавие, имена на авторите (собствено име и фамилия), название на научната организация или лечебното заведение, в което те работят. При повече от едно заведение имената на същите и на съответните автори се маркират с цифри или звездички;

б) същите данни на английски език се изписват през българския текст.

Забележка: при статии от чужди автори българският текст следва английския.

Основен текст на статията

Оригиналните статии задължително трябва да имат следната структура: увод, материали методи, собствени резултати, обсъждане, заключение или извод.

Методиките следва да бъдат подробно описани (включително видът и фирмата производител на използваните реактиви и апаратура). Същото се отнася и за статистическите методи.

Тези изисквания не важат за обзорите и другите видове публикации. В текста се допускат само официално приетите международни съкращения; при използване на други съкращения те трябва да бъдат изрично посочени в текста. За мерните единици е задължителна международната система SI. Цитатите вътре в текста е препоръчително да бъдат отбелязвани само с номерата им в книгописа.

Илюстрации и таблици

Илюстрациите към текста (с необходимия добър контраст и качество), се представят на отделни листове (без обяснителен текст), в оригинал и две копия за всяка от тях. Текстът към фигурите със съответната им номерация (на български и на английски език) се отбелязва вътре в основното текстурално тяло на статията под съответния номер на мястото, където трябва да се разположи при предпечатната подготовка. Таблиците се представят с готово написани обяснителни текстове на български и на английски, които са разположени над тях; номерацията им е отделна (също с арабски цифри).

Използвана литература:

Книгописът се представя на отделна страница. Броят на цитираните източници е препоръчително да не надхвърля 20 (за обзорите до 40), като желателно е повечето от тях да бъдат от последните 5 години. Подреждането става по азбучен ред на фамилното име на първия автор (първо на кирилица, после на латиница), като след поредния номер се отбелязва фамилното име на първия автор, след това инициалите му; всички останали автори се посочват с инициалите, последвани от фамилното име (в обратен ред) до третия автор, последвани от съкращението et al. Следва цялото заглавие на цитираната статия, след него – названието на списанието (или общоприетото му съкращение), том, година, евентуално брой на книжката, началната и крайната страница. Глави (раздели) от книги се изписват по аналогичен начин, като след автора и заглавието на главата (раздела) се отбелязват пълното заглавие на книгата, имената на редакторите (в скоби), издателството, градът и годината на издаване, началната и крайната страница на раздела.

Примери: Статия от списание:

1. **McLachlan, S., M. F. Prumel, B. Rapoport.** Cell Mediated or Humoral Immunity in Graves' Ophthalmopathy? *J. Clin. Endocrinol. Metab.*, 78, 1994, 5, 1070-1074.

Глава (раздел) от книга:

2. **Delange, F. Endemic Cretenism.** In: The Thyroid (Eds. L. Braveman and R. Utiger). *Lippincott Co*, Philadelphia, 1991, 942-955.

Адрес за кореспонденция с авторите

Той се дава в края на всяка статия и съдържа всички необходими данни (вкл. електронна поща) на български език за един от авторите, който отговаря за кореспонденцията.

Всички ръкописи трябва да се изпращат с придружително писмо, подписани от авторите, с което потвърждават съгласието си за отпечатване в сп. „Българска медицина“. В писмото трябва да бъде отбелязано, че материалът не е бил отпечатван в други научни списания у нас и в чужбина. Ръкописи не се връщат.

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ISSN 1314-3387

Bulgarian *medicine*



Journal of
**Bulgarian Academy of
Sciences and Arts**

Founding Editor
Prof. Dr Grigor VELEV

Списание на
**Българска Академия на
Науките и Изкуствата**

Основател и първи главен редактор
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