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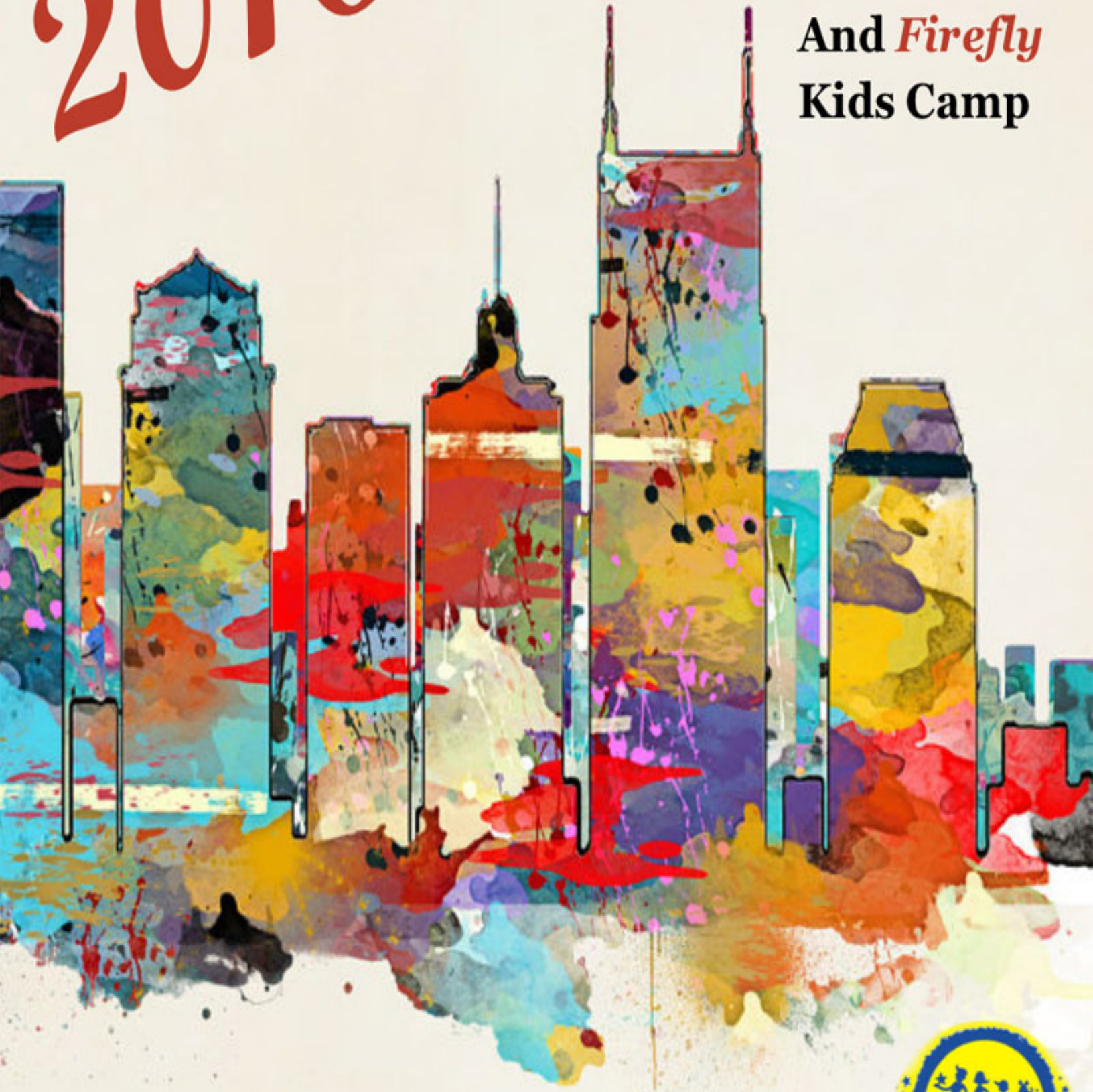

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BRIEF REVIEW

Molecular Oncology

The Research Progress of SiRNA Targeting Notch1 on Tumor Cells: A Mini Review of the State of the Art

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Abstract

Notch signaling is a highly conserved signaling pathway, playing an important role in a variety of cell differentiation, development and regulation. Notch signaling includes Notch1-4; Notch1 gene encodes Notch1 signaling that can shorten cell cycle, enhance cell proliferation, inhibit cell differentiation, and promote apoptosis. Mutation and overexpression of the Notch1 gene may induce tumorigenesis, which plays an important role in the development of tumors across a variety of signaling pathways. Currently, using RNA interference technology (RNAi) synthesizing small interference RNA (siRNA) targeting Notch1 gene (siNotch1) has become a hot topic, and clinical application of gene silencing has also obtained a certain therapeutic effect. In this paper, the application of Notch1 gene silencing in tumor progress was reviewed. **(Int J Biomed. 2016;6(3):163-166.)**.

Key Words: RNAi • Notch signaling • Notch1 gene • tumor cells.

Introduction

The Notch signaling pathway includes the Notch receptor proteins, Notch ligand proteins, transcription factors CSL (DNA binding protein), and target molecules. In mammals, four Notch genes (Notch1–4) and at least five of their ligands (Jagged1 and 2; Delta1, 3, and 4) are identified.[1] Notch1 is one of the Notch signaling pathways, by the Notch1 gene regulation. The Notch1 gene can shorten the cell cycle, quicken cell proliferation, inhibit cell differentiation and promote apoptosis.[2] The Notch1 gene, in any link in the process of T-cell development disorder, is likely to be associated with cell malignant transformation.[3]

Pancewicz J et al.[4] have found activating mutations in Notch in more than 30% of adult T-cell leukemia (ATL) patients. Activated Notch signaling contributes to ~50% of human T-cell acute lymphoblastic leukemia (T-ALL) cases through gain-of-function mutations in the Notch1 gene.[5] Therefore, Notch1 gene mutation and high expression may induce the occurrence of hematological malignancies and play an important role in a broad range of tumor occurrences and development.[6,7]

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1. The siRNA targeting the Notch1 gene in normal cells

The Notch signaling pathway has been shown to regulate angiogenesis and endothelial cell formation, and is found in numerous cell types. Increased Notch signaling in endothelial stalk cells correlates with vessel regression, whereas reduced Notch signaling in the stalk leads to formation of a new tip cell.[8]

Recent studies revealed crucial roles of the Notch system in mature T cell differentiation and activation. Notch1 plays an obligatory and selective role in T cell lineage induction. Notch1 was also reported to modulate the β selection step of thymocytes. There are a number of articles suggesting that γδ/αβ T-cell fate decisions are modulated by Notch signaling. In addition, it promotes the CD4 +, CD8 + double positive thymus cells to differentiate into single positive thymus cells.[9] It has been suggested that Notch1 signaling plays an important role in the process of mature T-lymphocytes, and Notch signaling disorders may lead to the occurrence of T-cell leukemia or lymphoma.

2. The siRNA targeting the Notch1 gene in tumor cells

2.1. Respiratory system

Hassan et al.[10] found that Notch1 has an inhibitory tumor function, especially in the context of switching off cell invasion and metastasis. Notch1 signaling in small cell lung carcinoma (SCLC) controls H69AR and SBC3 cell adhesion and epithelial mesenchymal transition (EMT). Overexpression of Notch1 in SCLC switched off EMT, cell motility and cell metastatic potential.

2.2. Digestive system

Sun et al.[11] studied the Notch1 pathway and the VEGF pathway with human gastric cancer cells (SGC7901) and found the siJagged1 affected SGC7901 cell proliferation and apoptosis by acting on Notch1 and VEGF signaling pathways.

Notch signaling pathway has been reported to play critical roles in hepatocellular carcinoma (HCC). Liu Hong showed that transfection of Notch1 small-interfering RNA (siRNA) into nude mice HCC cells resulted in cell growth inhibition and apoptosis.[12] Notch signaling was found to positively regulate cell proliferation in hepatoma HepG2 cell lines and GSI treatment inhibited tumor cell proliferation through the suppression of Notch signaling.

In recent years, studies have reported that Notch1 gene silencing has a therapeutic effect on pancreatic cancer cells,[13] which offers a new direction for the treatment of pancreatic cancer.

2.3. Urinary system

Zhang et al.[14] studied Notch1 expression in renal cell carcinoma and silence the expression of Notch1 by using siRNA and observed its effect on the proliferation of Caki- 1. To detect expression of Notch1 mRNA by RT-PCR and Notch1 protein levels by Western blot, siRNA interference transfected into RCC Caki-1 cells, transfection with Lipofectamine 2000 for 48 hours. Notch1 in renal cell carcinoma was high expressed compared with normal renal tubular epithelial cells. Specific siRNA interference significantly reduced the Caki-1 cells, mRNA and protein expression, and Caki-1 cells proliferation was significantly decreased. These results suggest that siRNA-mediated silencing of the Notch-1 gene may represent a novel target for gene therapy of renal cancer cells.

Ai Xing et al.[15] found that Notch1 gene expression increased in bladder cancer cell line BIU87 and that the interference of Notch1 gene expression had a therapeutic effect on bladder cancer cells. In addition, studies have found that the proliferation capacity of BIU87 was reduced after using siNotch1. This result may be associated with Notch1 signaling pathway suppressing and influencing the G1 stage of cell differentiation.[16]

2.4. Reproductive system

Notch1 was found to be overexpressed in prostate cancer (PCa) cells and human PCa tissue. Bin Hafeez et al.[17] showed that small interfering RNA-mediated knockdown of Notch1 in PC3 and 22Rnu1 PCa cells dramatically decreased their invasion.[17] Wang et al.[18] found that down-regulation of Notch-1 and Jagged-1 could inhibit cancer cell migration and invasion, which was in part due to down-regulation of NF-kB and its downstream target genes such as MMP-9, uPA, and VEGF. From these results, authors concluded that down-regulation of Notch-1 or Jagged-1 could potentially be an effective therapeutic approach for the inactivation MMP-9, uPA, and VEGF, which is likely to result in the inhibition of cell growth, migration, invasion and metastasis of prostate cancer.

Notch1 gene expression in cervical cancer tissue is higher than in normal cervical tissue. Some studies have found that the Notch1 gene has a significant positive correlation with tumor differentiation, and that the Ki67 gene is a sign of cell proliferation. The expression of the Notch1 gene has a positive correlation with Ki67 in cervical cancer tissue.[19] This suggests that Notch1 signaling is involved in cell proliferation in cervical cancer tissues.

2.5. Blood system

Weng et al.[5] reported that more than 50% of human T-ALLs, including tumors from all major molecular oncogenic subtypes, have activating mutations that involve the extracellular heterodimerization domain and/or the C-terminal PEST domain of Notch-1. These findings greatly expand the role of activated Notch-1 in the molecular pathogenesis of human T-ALL. Thus, siNotch1 can be used as a new method for treatment of T-ALL.

Yang et al.[20] researched the therapeutic effect of siRNA of proton-sponge-coated quantum dots (QD) on Notch1 protein over expression in T-ALL cell line. and found that the gene silencing efficiency of proton-sponge-coated QD-siRNA increased by 4-10 folds, and proton-sponge-coated QD-siRNA also decreased the content of Notch1 mRNA significantly, as compared with controls. This illustrates that proton-sponge-coated QD-siRNA can be used for treatment of T-ALL.

Weng et al.[21] studied T-ALL and found that c-MYC is a direct downstream target of Notch1 in Notch-dependent T-ALL cell lines, that contributes to the growth of T-ALL cells.. The existence of a direct link between Notch and c-MYC in T-ALL cell lines and normal thymocytes has therapeutic as well as basic implications. The c-MYC gene as a potential Notch1 target provides new ideas and a new direction for the application of siNotch1 in clinical treatment of leukemia.

Kamstrup et al.[22] studied the Notch1 gene as a potential target for cutaneous T-cell lymphoma treatments. Specific down-regulation of Notch1 by siRNA induced apoptosis in SeAx. The caspase 3/7 activity increased significantly in the Notch1 siRNA-transfected group compared with the control siRNA-transfected group 24 hours after transfection. Seventy-two hours after transfection, the apoptosis rate was up to peak. The mechanism of apoptosis involved the inhibition of NF-κB, which is the most important prosurvival pathway in cutaneous T-cell lymphoma. These data show that Notch is present in cutaneous T-cell lymphoma and that its inhibition may provide a new way to treat cutaneous T-cell lymphoma.[22]

2.6. Others

In human brain glioma cells, Notch1 gene mRNA and protein expression are significantly higher than in non-tumor cells.[23] Xu Peng et al.[24] studied Notch1 expression in the majority of 45 astrocytic gliomas with different grades and in U251MG glioma cells. Transfection of siRNA targeting Notch1 into U251 cells in vitro downregulated Notch1 expression, associated with inhibition of cell growth, arrest of cell cycle, reduction of cell invasiveness, and induction of cell apoptosis. Meanwhile, tumor growth was delayed in established subcutaneous gliomas in nude mice treated with Notch1 siRNA in vivo. These findings suggest that Notch1 plays an important oncogenic role in the development and progression of astrocytic gliomas; siNotch1 is expected to be a promising direction for the treatment of astrocytic gliomas. Other studies have reported that the Notch1 gene also plays a part in the development of breast cancer.[25,26]

3. The clinical application of RNA interference

Davis et al.[27] conducted the first in-human phase I clinical trial involving the systemic administration of siRNA to patients with solid cancers using a targeted, nanoparticle delivery system (clinical version denoted as CALAA-01). They provided the actual evidence of inducing an RNAi mechanism of action in a human from the delivered siRNA. siRNA administered systemically to a human can produce a specific gene inhibition (reduction in mRNA andprotein) by an RNAi mechanism of action.

Schultheis et al.[28] evaluated the safety, tolerability, and pharmacokinetics of Atu027 (a Liposomal siRNA inhibitor for PKN3) in a first-in-human phase I study and found that Atu027 was safe in patients with advanced solid tumors, with 41% of patients having stable disease for at least 8 weeks. It is expected to become a new method for the treatment of solid tumors.

4. Conclusion and prospect

Above all, Notch1 signaling plays an important role in the regulation of cell proliferation, differentiation, apoptosis, and invasion. Although siNotch1-related research in the field of tumor cytology has made some progress, it is mostly in the basic research stage at present; its specific mechanism is relatively limited and not comprehensive enough. Achieving its clinical application specification is also facing many difficulties and challenges, such as the problems of designing siRNA segments, effective transfection, off-target effects, delivery system, etc. At the same time, security may deserve attention. In addition, how Notch1 can be applied directly to the human body to interfere with the growth of tumor cells is a problem that has to be solved, and to do so requires further research.

In sum, further study of the mechanism by which Notch1 and siNotch1 interact with tumor cells is necessary at the molecular and gene, protein and animal model levels. For the future, a technique called RNA interference will be widely used in biomedical research. We believe that with the deepening of the research on gene silencing, siRNA targeting the Notch1 gene is expected to become the new tool for cancer treatment.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Pulmonology

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Influence of Long-Term Inhaled Glucocorticoids on the Lung Surfactant Phospholipid Levels in Rats

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Abstract

Background: Damage to lung surfactant, which is responsible for the lung local immunity, may contribute to the development of bronchial inflammation in patients with bronchial asthma. Different doses of glucocorticoids produce a stimulating or inhibiting effect on the synthesis of the surfactant protein (SP-A) mRNA. Lung surfactant disorders may negatively influence bronchial homeostasis and aggravate the condition of patients with bronchial asthma and COPD. The objective of this study was to evaluate the influence of long-term inhaled corticosteroids (ICS) on the phospholipid levels of the lung surfactant in rats.

Methods and Results: Inhalations of prednisolone hemisuccinate (PH) were given to white non-pedigree rats weighing 180-200 g at a dose of 0.3 mg/kg daily for 30 days. Already by the end of the first study period (10 days), lung surfactant phospholipid levels were found to decrease significantly from 1.35±0.060 mg to 1.02±0.045 mg ($P<0.001$). The decrease was further recorded at Day 20 and Day 30 of the inhalation period: down to 0.94±0.042 mg ($P<0.001$) and 1.04±0.047 mg ($P<0.01$), respectively. The phospholipid content continued to decrease after termination of inhalations down to 0.80±0.036 mg ($P<0.001$) and 0.63±0.028 mg ($P<0.001$) at Day 40 and 50 of the experiment. By Day 60 of the experiment (30 days after termination of PH), the phospholipid content in the lung surfactant was restored to the baseline level of 1.29±0.058 mg.

Conclusion: The content of lung surfactant was found to decrease significantly as a result of long-term ICS treatment, which may have a negative effect for chronic lung diseases. **(Int J Biomed. 2016;6(3):167-169.)**.

Key Words: inhaled corticosteroids • lung surfactant • COPD • bronchial asthma.

Introduction

The use of inhaled glucocorticosteroids (also called inhaled corticosteroids or ICS) for bronchial asthma (BA) has significantly decreased the incidence of severe cases and mortality. The use of ICS for COPD also improves the quality of life and decreases the exacerbation rate. However, a small amount of all available ICS is absorbed into the lungs, enters the blood flow and may cause complications of systemic glucocorticoids (GCs). Adverse effects of ICS, such as oral and oropharyngeal candidosis, dysphonia, and cough caused by the irritation of upper airway lead to significant discomfort in some patients. High doses of ICS (equivalent to 1000 μg of fluticasone propionate) are accompanied by complications of systemic GCs, such as pneumonia, glaucoma, cataract, suppression of adrenal function, osteoporosis and diabetes.[1]

Many lung diseases, including BA, have been shown to be accompanied by qualitative and quantitative changes in the composition and properties of the lung surfactant.[2,3] The lung surfactant obtained from the lungs of guinea pigs with a model of chronic bronchial asthma has less ability to decrease surface tension, higher content of small aggregates and decreased ratio of saturated phospholipids to surfactant proteins.[4] The influence of long-term ICS therapy on the lung surfactant has been given little attention by the researchers. Short-term use of systemic GCs in pregnant women 48 and 24 hours before delivery causes an increase of lung surfactant synthesis in the fetus; this is standard treatment for prevention of respiratory distress syndrome in newborns.[5] However, a

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stimulating effect on the synthesis of a substance is known to be often replaced by suppression of its synthesis and a decrease in levels of the end product. We assumed that a long-term use of ICS would exhaust the synthesis of the lung surfactant by type II alveolar cells and lead to a decrease of surfactant levels in the lungs.

It should be mentioned here that lung surfactant is a multicomponent natural complex comprising 7 classes of phospholipids, neutral lipids, cholesterol and its esters as well as 4 groups of surfactant proteins having a range of different functions.[2] Lung surfactant enables the breathing mechanism by effectively lowering surface tension at the air/liquid interface, protects the lungs from physical and chemical effects, has immunomodulating and anti-inflammatory properties, stimulates phagocytosis of alveolar macrophages and mucociliary clearance, and keeps small bronchi open.[2,6,7] That is why a decrease in lung surfactant levels may have negative consequences for the homeostasis of the lung tissue.

The objective of this study was to evaluate the influence of long-term ICS on the phospholipid levels of the lung surfactant in rats.

Methods

Inhalations of prednisolone hemisuccinate (PH) were given to white non-pedigree rats weighing 180-200 g at a dose of 0.3 mg/kg daily for 30 days. The rats had plastic funnels fixed in such a way that their noses and mouths were within the funnel edges. For the inhalations of PH, the ultrasound inhalator Vulkan 1 (Russia) was used for 10 minutes. The dosage was measured by the amount of PH used during the exposure time. The experiment lasted 60 days: rats received PH daily during the first 30 days, and then they were followed up for another 30 days. Every 10 days 5 rats were sacrificed, and total phospholipid content for both lungs was measured. In this way, rats were studied at Days 10, 20 and 30 (inhalation period) and at Days 40, 50 and 60 (follow-up period). In the control group, rats received inhalations of saline daily during 30 days. Rats were sacrificed in a state of deep thiopental narcosis (25 mg/kg), in compliance with the NIH Guide for the Care and Use of Laboratory Animals. After their bodies were thoroughly cleared of blood, lungs were extracted along with the trachea and were lavaged 5 times with 5 ml 36°C saline per wash. The lavage was then collected and purified from cells and debris by centrifuging at 1500×g, +4°C for 10 minutes. The supernatant was then frozen at -20°C for 1 hour and then melted at ambient temperature to form lung surfactant aggregates. The procedure was repeated three times. The resulting suspension was centrifuged at 10000×g using a Sigma 6K10 centrifuge (Germany). Lung surfactant residue was resuspended in water, and lipids were extracted using a mixture of organic solvents as suggested by Bligh and Dyer.[8] Neutral lipids were removed by means of cold (–20°C) acetone and were cleared of any trace of the acetone on a rotary evaporator (RotavaporR-114, Buche, Switzerland) under vacuum. The residue was dissolved in a chloroform and methanol mixture (2:1 ratio by volume). Lung surfactant phospholipid levels were measured by the quantity of nonorganic phosphorus,[9] with the color measured quantitatively at the wavelength 825nm on a HitachiU-3400 spectrophotometer (Japan), and phospholipid levels were calculated from the content of nonorganic phosphorus multiplied by 25 (the average ratio of phosphorus in a phospholipid molecule).

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). The mean (M) and standard error of the mean (SEM) were calculated. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. A probability value of $P<0.05$ was considered statistically significant.

Results

Figure 1 shows lung surfactant phospholipid levels in the control and treatment groups at specified time points during the period of PH inhalations and the follow-up period.

The lung surfactant phospholipid content did not differ in lungs of intact rats and of rats receiving saline inhalations amounting to a total of 1.35±0.060 mg for both lungs (the zero point on Figure 1).

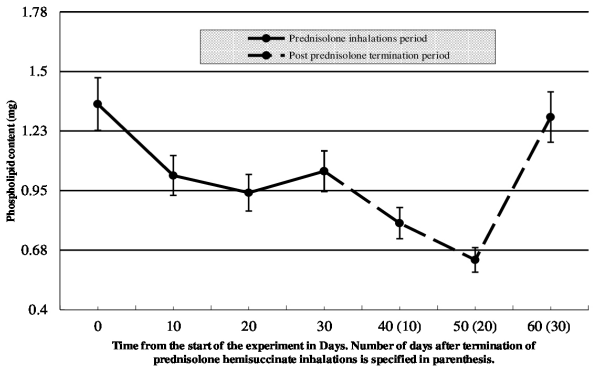

Fig. 1. *Dynamics of lung surfactant phospholipid levels during PH inhalations*

Inhalations of PH were given to rats at a dose of 0.3 mg/kg daily for 30 days with a subsequent 30 day follow-up period. Solid line shows the course of PH inhalations, dotted line shows the period after termination of PH. Each point shows the total phospholipid content for both lungs, the average across five animals. The zero point (0) shows the content of phospholipids in control.

Already by the end of the first study period (10 days), lung surfactant phospholipid levels were found to decrease significantly from 1.35±0.060 mg to 1.02±0.045 mg ($P<0.001$). The decrease was further recorded at Day 20 and Day 30 of the inhalation period: down to 0.94±0.042 mg ($P<0.001$) and 1.04±0.047 mg ($P<0.01$), respectively. The phospholipid content continued to decrease after termination of inhalations down to 0.80±0.036 mg ($P<0.001$) and 0.63±0.028 mg ($P<0.001$) at Day 40 and 50 of the experiment. By Day 60 of the experiment (30 days after termination of PH), the phospholipid content in the lung surfactant was restored to the baseline level of 1.29±0.058 mg.

A significant decrease in the content of lung surfactant after the 30-day inhalation period with a further restoration after ICS termination is in line with reversible suppression of surfactant protein SP-A (A_1 and A_2) gene expression by dexamethasone.[10] The study has shown that low doses of dexamethasone (10 nM) increase the synthesis of the SP-A mRNA in human fetus lung tissue explants and that high doses (100 nM) suppress it.[10] In a different model, involving pulmonary adenocarcinoma cell line NCI-H441, dexamethasone suppressed the synthesis of the SP-A mRNA and SP-A levels even at a low dose (10 nM). At the higher dose of 100 nM, dexamethasone suppressed the content of SP-A down to 10% of the baseline, the degree of suppression depending both on the dose of dexamethosone and on the time of the cell exposure to GCs. This effect of inhibition of SP-A gene expression was reversible and mediated through the corticosteroid receptor.[11]

Discussion

The discovered significant decrease of surfactant levels in the rat lungs as a result of 30-day course of PH can help to explain the pathogenesis of complications caused by long-term ICS used for treatment of such chronic lung diseases as BA and COPD. Long-term ICSs are known to double the risk of severe pneumonias in COPD patients.[12] Severe pneumonias have been shown to have lung surfactant deficit, and surfactant replacement therapy is used successfully for these conditions.[13,14] Lung surfactant system impairment may be the cause of bronchial obstruction, mucosal edema and increased liquid secretion into the bronchi.[3] In this way, already existing impairments of the lung surfactant system in BA patients may be aggravated by additional suppression of its synthesis due to ICS. It should be mentioned that intratracheal administration of liposomes from egg lecithin and cholesterol to rats,[15] or multiple administration of lung surfactant to newborns, increases the synthesis of endogenous surfactant,[16] which is associated with reutilization of 80% of surfactant phospholipids when surfactant is synthesized *de novo*. We may suppose that inhalations of natural formulations of lung surfactant will stimulate the synthesis of endogenous surfactant and weaken dependency on ICS in BA patients.

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Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Left Ventricular Mass Appropriateness in Hypertensive Patients with Metabolic Syndrome

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Abstract

The objective of this study was to evaluate the frequency of inappropriate left ventricular (LV) mass (iLVM) and factors associated with iLVM in hypertensive patients with metabolic syndrome (MetS). The study included 178 male patients (mean age 45.9±10.5 yrs) with hypertension (HT) and MetS. iLVM in HT patients with MetS was associated with LV diastolic dysfunction and an intensity of vascular remodeling process. Hyperinsulinemia contributes to LVM appropriateness, which aggravates the cardiovascular remodeling with development of the significant LV diastolic dysfunction and endothelium dysfunction. (**Int J Biomed. 2016;6(3):170-173.)**.

Key Words: hypertension • metabolic syndrome • left ventricular hypertrophy • inappropriate left ventricular mass.

Abbreviations

BP, blood pressure; **DBP**, diastolic BP; **EDV**, end-diastolic volume; **LVM**, left ventricular mass; **aLVM**, appropriate LVM; **iLVM**, inappropriate LVM; **LVMI**, LVM index: **LVH**, left ventricular hypertrophy; **MetS**, metabolic syndrome; **SBP**, systolic BP.

Introduction

Left ventricular hypertrophy (LVH) is a well-known prognostic factor for cardiovascular events.[1] LVH develops as the consequence of an increase in LV mass (LVM) secondary to chronic overload. A LV anatomical adaptation that balances cardiac load is, therefore, compensatory. However, at least in arterial hypertension (HT), a number of patients exhibit levels of LVM that exceed the need to sustain cardiac workload, a condition that has been defined as inappropriate LVM (iLVM). Inappropriateness of LV mass has been reported to be an independent prognostic factor, regardless of the presence of LVH or not.[2] The inappropriate or excessive growth of LVM is associated with metabolic abnormality, systolic dysfunction, and LV concentric geometry, which is independent of the presence of hypertension.[3-5] iVMI has also been reported to be associated with diastolic dysfunction, which can be demonstrated using various transmitral blood flow parameters.[6]

The objective of this study was to evaluate the frequency of iLVM and factors associated with iLVM in HT patients with MetS.

Subjects and Methods

Study population

Participants were consecutively enrolled from among outpatients in the Hypertension Department of the Republic Centre of Cardiology and written informed consent was obtained from all study participants. Exclusion criteria included a history of myocardial infarction, angina pectoris, heart failure, stroke, chronic renal insufficiency. No subjects with clinically overt diabetes were included. All procedures were approved by the Ethic Committee of the Republic Center of Cardiology.

Anthropometric measurements

SBP and DBP were measured by using a mercury blood pressure device after the subjects had rested longer than 5 min. Body mass index (BMI) was calculated by weight (kg) divided by the squared height (m) (kg/m^2). The waist circumference (WC) was measured in the standing position, at the level of umbilicus, located midway between the lower costal margin (bottom of lower rib) and the iliac crest (top of pelvic bone).

Biochemical analysis

After 12 hrs of fasting, blood glucose (FBG), total

cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) levels were obtained. The fasting serum insulin level was measured by immunoenzyme assay (Access ultrasensitive insulin, Beckman Coulter ™). Standard glucose tolerance test was performed to all patients. Insulin resistance status was calculated by using the homeostatic model assessment-insulin resistance (HOMA-IR).[7] The calculation formula was as follows: HOMA-IR=(fasting insulin [μIU/mL]×fasting blood glucose [mM/L])/22.5. Microalbuminuria (MAU) was measured by immunoenzyme assay (RANDOX, Great Britain) and defined as an albumin urinary excretion between 20-200 mg/ml.

Definitions of the MetS

According to IDF (2005), the MetS is present when the waist circumference is increased (M: >94 cm; F: >80 cm) and at least two of the following factors are present: TG 1.7 mmol/l (150 mg/dl) or greater; low HDL-C (M < 1.03 mmol/l; F < 1.29 mmol/l); SBP greater than 130 mmHg or DBP greater than 85 mm Hg or treatment of previously diagnosed HT; increased fasting plasma glucose (>5.6 mmol/l) or previously diagnosed DM [8].

Echocardiographic measures

Echocardiography was performed using ultrasound system (En VisorC, PHILLIPS, Holland). Left ventricular dimension and wall thickness was measured from two-dimensional guided M-mode echocardiographic tracings on the parasternal long axis view. LVM was estimated by using the Penn convention. It was indexed for body surface area to estimate LVMI. The presence of LVH was defined for LVMI ≥ 125 g/m^2 [9]. LV end-diastolic and end-systolic volumes were calculated using Teichholz's formula.[10] Stroke volume was generated (mL/beat) and stroke work (SW in gram-meters/beat [g-m/beat]) was computed [11] as follows:

cuff systolic BP x stroke volume x 0.0144.

LV systolic function was estimated as systolic shortening measured at the endocardial and midwall levels.[12]

Individual LVM was estimated using formula:

Predicted LVM = 55.37+6.64 x height (m$^{2.7}$) + 0.64 x SW (g-m/beat) - 18.07 x gender

where male = 1 and female = 2.

Observed LVM (oLVM) was divided by predicted LVM (pLVM) and was expressed as a percentage (oLVM/pLVM). With this method, every individual served as a reference for him/herself.

iLVM was defined as an excess of >28% from the predicted value (ie, oLVM/pLVM >128%) and *low* LVM as a decrease of >27% from the predicted value (ie, oLVM/pLVM <73%).[13]

The following parameters were measured by pulse-wave Doppler: peak velocities of early (E) and late diastolic filling (A), deceleration time (DT), isovolumic relaxation time (IVRT). The ratio of early diastolic to late diastolic mitral inflow velocities was calculated (E/A).

Carotid Ultrasound Imaging

Carotid and brachial scans were obtained by high-resolution B-mode ultrasound by a 7.5 MHz linear array transducer (S4-2, PHILLIPS). Left and right common carotids were examined in antero-lateral, postero-lateral, or medio-lateral directions. Longitudinal images of the distal common carotid, in which the interfaces were very clear, were obtained. Carotid intima-medial thickness (IMT) was measured in the far wall of the common carotid artery, 1 cm proximal to the carotid bulb in a region free of plaques.

Data were stored and analyzed with the Statistica 6.0 statistical software package. All of the data are expressed as mean±SD. Characteristics of study groups are compared using Student *t* tests or nonparametric test, as appropriate. Differences among prospectively defined subgroups were analyzed by ANOVA. $P<0.05$ was considered statistically significant.

Results

The study included 178 male patients (mean age 45.9±10.5 yrs) with hypertension (HT) and MetS. Among studied patients, Stage 1 HT was identified in 36.5% patients, Stage 2 HT in 37.1% patients, and Stage 3 HT in 26.4% patients. Coronary heart disease was identified in 11.8 % patients, smoking in 33.7% patients. About 77.5% and 83.1% patients had LVH and LV diastolic dysfunction, respectively. The impaired EDV was identified in 83.1% patients. The baseline characteristics of patients are reported in Table 1.

Table 1.

Baseline characteristics of patients

Variable	Parameters
BMI, kg/m^2	31.8±3.8
Waist ratio, cm	108.6±9.8
Mean SBP, mmHg	155.9±15.7
Mean DBP, mmHg	99.7±9.5
Fasting glucose, mmol/l	5.13±0.85
Postload glucose, mmol/l	6.09±2.25
Fasting insulin, U/ml	19.49±15.51
HOMA-IR	4.49±3.89
TC, mg/dl	227.90±42.82
TG, mg/dl	229.73±146.98
HDL-C, mg/dl	40.32±8.02
LDL-C, mg/dl	141.48±36.10

At baseline, 43 (24.16%) patients had aLVM, and 135 (75.84%) patients had iLVM. It should be noted that there were no patients with iLVM with a normal LVMI. aLVM with an abnormal LVMI was identified in 6 (3.37%) patients; all patients with iLVM had abnormal LVMI. (Table 2,3).

Table 2.

Characteristics of patients according to changes in LVM appropriateness

Variable	n,%
low LVM, <73%	0
aLVM, 73-128%	43 (24.6)
iLVM, >128%	135 (75.84)
low- expressed iLVM, 128-155.9%	36 (20.22)
moderate expressed iLVM, 156-183.9%	50 (28.09)
greatly expressed iLVM, >184%	49 (27.53)

Table 3.

Characteristics of patients according to changes in LVM appropriateness and LVH

Variable	LVM appropriateness	
	aLVM	iLVM
without LVH, n(%)	37 (20.79)	0
with LVH, n(%)	6 (3.37)	135 (75.84)

An analysis of clinic, demographic, and biochemical parameters, taking into account LVM appropriateness in observed patients, has detected a strict contribution of number of factors (duration of HT, SBP, DBP) to development of iLVM. Along with iLVM, HT patients with MetS were characterized by considerable changes in the serum insulin level and HOMA-IR. This determines a probable contribution of hyperinsulinemia in the iLVM development (Table 4).

Table 4.

Comparison between groups of aLVM versus iLVM

Parameters	aLVM n=43	*P*	iLVM n=135
Age, y	41.5±9.0	*0.0015*	47.3±10.6
Duration of HTN, y	3.2±2.0	*0.0005*	5.7±4.5
BMI, kg/m^2	31.8±3.7	*NS*	31.8±3.9
Waist ratio, cm	106.8±8.2	*NS*	109.1±10.2
Mean SBP, mmHg	149.5±12.9	*0.002*	158.0±16.0
Mean DBP, mmHg	93.2±6.2	*0.000000*	101.8±9.5
Observed LVM, gr	258.3±31.6	*0.000000*	348.7±72.1
Index LVM, gr/m^2	123.5±11.7	*0.000000*	166.8±34.8
ΔD,%	6.73±6.21	*0.007*	4.12±5.27
IMT, mm	0.76±0.23	*0.000000*	0.98±0.24
MAU, mg/l	14.6±16.6	*0.03*	32.87±35.53
TC, mg/dl	225.4±38.0	*NS*	228.7±44.3
TG, mg/dl	213.4±99.5	*NS*	234.9±159.1
HDL-C, mg/dl	41.4±7.9	*NS*	39.9±8.1
LDL-C, mg/dl	139.4±39.6	*NS*	142.1±35.1
Fasting glucose, mmol/l	5.32±0.40	*NS*	5.06±0.94
Postload glucose, mmol/l	5.56±1.13	*NS*	6.35±2.60
Fasting insulin, U/ml	8.8±5.06	*0.012*	21.27±15.97
HOMA-IR	2.15±1.40	*0.031*	4.88±4.05

Further analysis according to the degree of LVM appropriateness has shown the certain peculiarities of cardiovascular remodeling process in HT patients with MetS. A greatly expressed iLVM (>184%) was associated with significantly higher SBP and DBP levels. Patients with greatly expressed iLVM had more expressed parameters of vascular remodeling (ΔD, IMT, MAU) as compared with patients with aLVM (Table 5).

Discussion

LVH detection in HT patients is very important for the risk stratification and treatment. Our study showed a high LVH prevalence among HT patients with MetS, especially iLVM. The obtained results demonstrated that the appropriateness of LVM is an independent factor determining LV diastolic dysfunction, along with age, HT duration and BP level. Some studies have demonstrated that the presence of inappropriate LVM implies a greater risk of cardiovascular events, either in the presence or in the absence of traditionally defined LVH. [13,14] Moreover, we have detected that hyperinsulinemia and insulin resistance also contributes to LVM appropriateness.

Table 5.

Parameters of system and central hemodynamic and the endothelium dysfunction markers depending on changes in LVM appropriateness

Parameters	73-128% n=43	128-155.9% n=36	156-183.9% n=50	>184% n=49	F/p
SBP, mmHg	149.6±12.9	156.7±20.3	159.4±13.9	157.6±14.6	3.5/ 0.017
DBP, mmHg	93.2±6.1	100.3±9.1	102.1±9.0	102.6±10.3	10.59/ 0.0000
SV, ml	113.2±19.4	101.3±28.2	88.2±16.3	87.3±16.3	17.03/ 0.0000
EF, %	71.0±4.0	71.5±5.4	67.8±5.5	62.8±7.9	19.96/ 0.0000
E, m/sec	0.73±0.16	0.71±0.11	0.68±0.14	0.61±0.16	5.91/ 0.0007
A, m/sec	0.67±0.15	0.64±0.13	0.67±0.17	0.69±0.33	0.41/0.7
E/A, m/sec	1.26±1.01	1.15±0.26	1.08±0.34	0.95±0.23	4.8/0.003
IVRT, msec	101.3±24.9	107.7±39.1	133.1±43.3	147.8±46.0	13.34/ 0.0000
ΔD,%	6.73±6.22	6.17±4.39	3.48±5.93	3.25±4.82	4.8/0.003
IMT, mm	0.76±0.23	0.79±0.20	1.03±0.23	1.08±0.20	24.64/ 0.0000
MAU, mg/l	14.60±6.64	15.39±5.97	22.1±14.7	25.9±16.5	8.57/ 0.0000

Conclusion

- iLVM in HT patients with MetS is associated with LV diastolic dysfunction and an intensity of vascular remodeling process.

- Hyperinsulinemia contributes to LVM appropriateness, which aggravates the cardiovascular remodeling with development of the significant LV diastolic dysfunction and endothelium dysfunction.

Competing financial interests

None.

Materials & Correspondence

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The ACE Gene Insertion/Deletion Polymorphism and Cerebrovascular Diseases in Uzbek Patients with Arterial Hypertension

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Abstract

The aim of the present study was to investigate the association between the ACE gene I/D polymorphism and the development of hypertensive encephalopathy (HE) in Uzbek patients with hypertension (HT).

***Materials and methods*:** The study included 91 male patients aged from 32 to 74 years (mean age 52.5±9.2) with HT Grade 1, 2 and 3 (ESH/ESC, 2013) *and* presence of HE. All patients were checked on office BP using Korotkov's method and ambulatory blood pressure monitoring (ABPM). Intima-media thickness (IMT) of the carotid artery was measured by a 7.5 MHz high-resolution ultrasound. Genomic DNA was isolated from peripheral blood using the Diatom™ DNA Prep 200 Kit according to the manufacturer's protocol. ACE gene I/D polymorphism genotypes were determined by PCR.

Results: Among HT patients with HE, we have identified a statistically significant predominance of ID genotype carriers (65.9%) against carriers of the II genotype (18.7%) and DD genotype (15.4%) (*P=0.000*); the frequency of I and D alleles was 51.6% and 48.4%, respectively (*P>0.05*). Comparative analysis showed a possible association between the ID genotype/D allele and HE development in HT patients, according to the general model (OR=6.36; 95%CI: 3.04 -13.31; *P=0.000*) and multiplicative model (OR=2.02; 95%CI: 1.25-3.27; *P=0.004)* of inheritance. High grades of HT were predominant in carriers of the DD genotype. IMT was significantly higher in carriers of the DD genotype than in carriers of the II and ID genotypes. The carriage of D allele was associated with the highest levels of TC, TG, and VLDL-C. Carriers of the DD genotype were characterized by higher values of daytime SBP, nighttime SBP variability and nighttime SBP load. **(Int J Biomed. 2016;6(3):174-178.)**.

Key Words: hypertension • cerebrovascular disease • ACE Gene I/D polymorphism • ambulatory blood pressure monitoring

Abbreviations

ABPM, ambulatory blood pressure monitoring; **ACE,** angiotensin-converting enzyme; **BP**, blood pressure; **DBP**, diastolic BP; **HDL-C**, high-density lipoprotein cholesterol; **IMT**, intima-media thickness; **I/D,** insertion/deletion; **LDL-C**, low-density lipoprotein cholesterol; **RAS,** renin-angiotensin system; **SBP,** systolic BP; **TC,** total cholesterol; **TG,** triglyceride; **TIA,** transient ischemic attack; **VLDL-C,** very low-density lipoprotein cholesterol.

Introduction

Hypertension (HT) is a well-established risk factor for cardiovascular diseases. An estimated 17.5 million people died from cardiovascular diseasesin 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke.[1] High BP multiplies the risk for stroke as much as four-fold. The risk of cerebral hemorrhage in hypertensive patients is 3.9 times higher than in non-hypertensive individuals.[2] Hypertensive encephalopathy (HE) refers to the transient migratory neurologic symptoms that are associated with the malignant hypertensive state in a hypertensive emergency. The most common cause of hypertensive encephalopathy is abrupt blood pressure elevation above cerebral autoregulation limits. HE is a life-threatening disorder characterized by severe neurological manifestations, including lowered consciousness, lethargy, confusion, blindness and seizures, in the absence of other causes. Brain MRI scans have shown a pattern of typically posterior (occipital greater than frontal) brain edema

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that is reversible. This usually is termed reversible posterior leukoencephalopathy or posterior reversible encephalopathy syndrome (PRES).[3]

Currently, increasing attention is being paid to genetic factors in the development of cerebrovascular diseases (CDs), and the value of various RAS genes has been investigated in many studies. However, ACE gene I/D polymorphism has not been studied in hypertensive patients with chronic CDs of Uzbek nationality.

The aim of the present study was to investigate the association between ACE gene I/D polymorphism and HE development in Uzbek patients with HT.

Materials and methods

The study included 91 male patients aged from 32 to 74 years (mean age 52.5±9.2) with HT Grade 1, 2 and 3 (ESH/ESC, 2013)[4] and presence of HE. Average duration of HT was 9.2±7.5 years. HE Stages 1 and 2 were detected in 34.3% and 48.1% of cases; HE with TIA was detected in 17.6% of patients. The control group included 60 healthy, age-matched male volunteers. The study was approved by the Republican Specialized Center of Cardiology Ethics Committee and conducted in accordance with the ethical principles stated in the Declaration of Helsinki. Written informed consent was obtained from each patient.

Exclusion criteria were: symptomatic HT; acute coronary syndrome, stable angina pectoris Class III-IV, chronic heart failure (NYHA FC>II), cardiac arrhythmia, history of stroke and myocardial infarction within previous 12 months, renal impairment, diabetes mellitus, severe co-morbidities.

Body mass index (BMI) was calculated by weight (kg) divided by the squared height (m) (kg/m^2). Office BP was determined in the reclining position using Korotkov's method and a mercury sphygmomanometer after 5-minute rest and an average of three readings.

ABPM was carried out on the non-dominant arm using TONOPORT V-General Electric (Germany). The device was set to obtain BP readings at 15 min intervals during the day (7am–11pm) and at 30 min intervals during the night (11pm–7am). The recording was then analyzed to obtain 24h, daytime and nighttime average SBP, DBP and heart rates. Nocturnal dipping was defined as a reduction in average SBP and DBP at night greater than 10% compared with average daytime values .[5]

Blood levels of TC, HDL-C and TG were determined using an A-25 Biosystems Autoanalyzer (DAYTONA) and the "RENDOX" sets. The atherogenic coefficient was defined by the following formula: AC= (TG- HDL-C)/HDL-C (RU).

IMT of the carotid artery was measured by a 7.5MHz high-resolution ultrasound (EnVisorC®).

Genomic DNA was isolated from peripheral blood using the Diatom™ DNA Prep 200 Kit according to the manufacturer's protocol. ACE gene I/D polymorphism genotypes were determined by PCR.[6] Reactions were performed with 10 pmol of each primer:

F: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'

R: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'.

PCR products were analyzed on 2% agarose gels after staining with ethidium bromide and were visualized using a UV transilluminator. Two alleles were identified: a 490-bp fragment *I* (with the insertion) and a 190-bp fragment *D* (without the insertion). In heterozygous samples, two bands (490 bp and 190 bp) were detected. To avoid mistyping of heterozygotes (ID) DNA samples identified as a DD genotype were subsequently amplified with second set of primers designed for the insertion specific allele.

Depending on carriage of the I/D polymorphic marker, all patients were divided into three groups: Group 1 included 17 patients (II genotype carriers), Group 2 included 60 patients (ID genotype carriers), and Group 3 included 14 patients (DD genotype carriers).

Statistical analysis was performed using StatSoft Statistica v6.0. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. The genotype frequency distribution for each variant was separately tested for Hardy-Weinberg equilibrium (HWE) with a chi-square test in the patient and control groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Multiple comparisons were performed with one-way ANOVA and post-hoc *T*ukey HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results

In the group as a whole, the mean sitting office DBP and SBP were 170.5±24.6 mmHg and 100.4±10.4 mmHg, respectively. The average BMI was 28.9±4.1 kg/m^2; 54.5% of patients were overweight and 30.8% of patients suffered from first or second degree obesity (BMI >30 kg/m^2). Among HT patients with HE, we have identified a statistically significant predominance of ID genotype carriers (65.9%) against carriers of the II genotype (18.7%) and DD genotype (15.4%) (*P=0.000*); the frequency of I and D alleles was 51.6% and 48.4%, respectively (P>0.05). In control group, the frequency of II, ID and DD genotypes was 56.7%, 23.3% and 20%, respectively (*P=0.000*); the frequency of I and D alleles was 68.3% and 31.7%, respectively (*P=0.000*) (Fig.1.). In both groups, genotypes were in Hardy-Weinberg equilibrium.

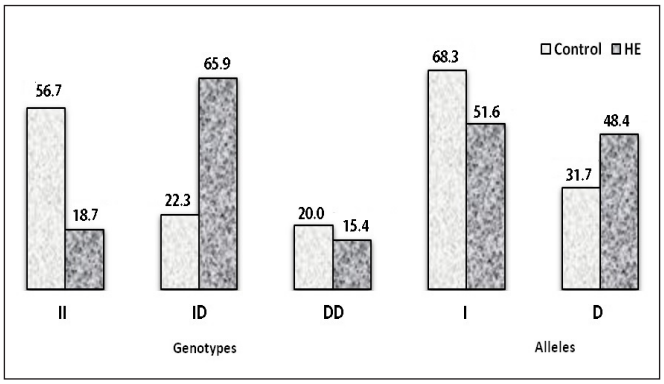

Fig.1. *The frequency (%) of genotypes and alleles of the ACE gene I/D polymorphism in Uzbek patients with HE.*

Comparative analysis showed a possible association between the ID genotype/D allele and HE development in HT patients, according to the general model (OR=6.36; 95%CI:3.04 -13.31; *P=0.000*) and multiplicative model (OR=2.02; 95%CI:1.25 -3.27; *P=0.004*) of inheritance. No significant relation was observed between the I/D polymorphism and BMI. We found an association between the office SBP and ACE gene I/D polymorphism. Carriers of the II genotype had SBP levels corresponding to Grade 1 HT; carriers of the ID and DD genotypes had SBP levels corresponding to Grade 2 HT (Table 1). We did not observe notable differences in office DBP levels between genotypes. High grades of HT were predominant in carriers of the DD genotype (Fig.2.).

Table 1.

Baseline characteristics of HE patients depending on the ACE gene I/D polymorphism

Indices	II, n=17 (1)	ID, n=60 (2)	DD, n=14 (3)	*P**	Tukey HSD test		
					P_{1-2}	P_{1-3}	P_{2-3}
Age,y	50.8±7.0	53.2±9.9	52.3±4.8	*0.607*	*0.586*	*0.885*	*0.937*
BMI,kg/m²	29.5±4.6	28.9±3.9	27.7±4.2	*0.464*	*0.854*	*0.443*	*0.585*
HT^,y	8.4±6.4	9.0±7.2	11.9±9.3	*0.360*	*0.953*	*0.395*	*0.389*
SBP,mmHg	155.9±21.3	175.8±25.0	174.3±15.0	*0.009*	*0.007*	*0.076*	*0.974*
DBP,mmHg	97.6±12.4	101.3±10.3	100.7±7.3	*0.431*	*0.398*	*0.685*	*0.979*
IMT,mm	0.85±0.24	0.99±0.30	1.25±0.28	*0.001*	*0.184*	*0.001*	*0.008*

^-*duration; P**- for ANOVA

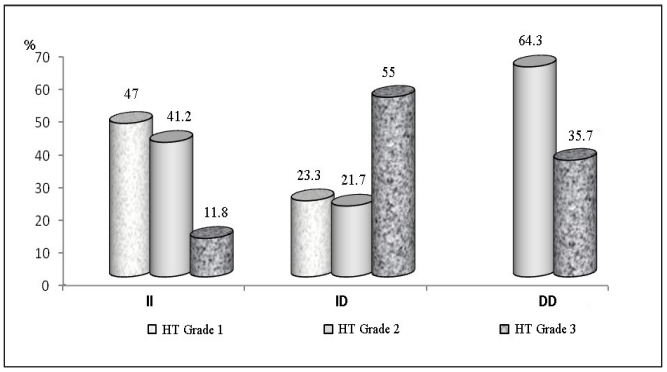

Fig.2. *The distribution of HT grades depending on the ACE gene I/D polymorphism*

IMT of the carotid artery exceeded standard measures in all patients. In the comparative aspect, IMT was significantly higher in carriers of the DD genotype than in carriers of the II and ID genotypes. Dyslipidemia was found in 62.6% patients. The carriage of D allele was associated with the highest levels of TC, TG, and VLDL-C (Table 2).

According to ABPM data, we found a significant increase in indicators of average daytime and nighttime SBP and DBP against the standard indices. Carriers of the DD genotype were characterized by higher values of daytime SBP, nighttime SBP variability and nighttime SBP load (Table 3). Of 91 patients, 60/65.9% showed a drop in SBP/DBP<10% during nighttime sleep and were categorized as non-dippers; the remaining patients were categorized as dippers (13/14.3%), over-dippers (6/6.6%), and night–peakers (12/13.2%) (p=0.000). The statistical prevalence of non-dippers was significant for all genotypes (Fig.3).

Table 2.

Blood lipid spectrum of HE patients depending on the ACE gene I/D polymorphism

Indices^	II, n=17 (1)	ID, n=60 (2)	DD, n=14 (3)	*P**	Tukey HSD test		
					P_{1-2}	P_{1-3}	P_{2-3}
TC	208.2±36.1	256.3±41.3	215.8±28.4	*0.000*	*0.000*	*0.850*	*0.002*
TG	142.5±58.7	197.0±89.8	212.2±75.4	*0.034*	*0.049*	*0.057*	*0.811*
HDL-C	42.4±6.2	39.3±7.8	39.1±7.0	*0.294*	*0.286*	*0.437*	*0.996*
VLDL-C	28.6±11.8	40.4±18.6	41.4±15.8	*0.038*	*0.037*	*0.102*	*0.979*
LDL-C	137.2±30.8	139.1±37.3	134.9±27.6	*0.915*	*0.979*	*0.982*	*0.913*
AC, RU	4.0±1.0	4.8±1.7	4.6±0.8	*0.153*	*0.129*	*0.506*	*0.893*

^- TC, TG, HDL-C VLDL-C and VLDL-C in mg/dL

*P**- for ANOVA

Table 3.

ABPM indices in HE patients depending on the ACE gene I/D polymorphism

Indices	II, n=17 (1)	ID, n=60 (2)	DD, n=14 (3)	*P**	Tukey HSD test		
					P_{1-2}	P_{1-3}	P_{2-3}
	Daytime BP, mmHg						
SBP	152.3±10.6	158.2±14.7	167.7±15.2	*0.012*	*0.286*	*0.009*	*0.066*
DBP	92.8±9.7	94.1±10.1	97.3±9.1	*0.430*	*0.882*	*0.421*	*0.522*
	Nighttime BP, mm.Hg						
SBP	136.4±12.6	143.1±17.7	145.3±12.0	*0.240*	*0.290*	*0.282*	*0.890*
DBP	78.4±6.0	81.8±7.6	82.6±7.5	*0.190*	*0.214*	*0.255*	*0.928*
	Daytime BP variability, mmHg						
SBP	12.9±2.7	13.8±4.4	15.3±3.2	*0.247*	*0.689*	*0.222*	*0.416*
DBP	11.7±3.1	13.5±4.5	14.4±5.7	*0.216*	*0.315*	*0.223*	*0.778*
	Nighttime BP variability, mmHg						
SBP	10.7±3.1	12.9±3.8	13.9±4.2	*0.045*	*0.088*	*0.052*	*0.642*
DBP	9.6±2.5	11.3±3.9	12.7±4.4	*0.075*	*0.234*	*0.064*	*0.426*
	Nocturnal BP fall, %						
SBP	5.7±3.9	4.9±5.7	3.4±4.5	*0.471*	*0.844*	*0.448*	*0.602*
DBP	7.8±3.0	6.3±6.8	4.2±7.6	*0.302*	*0.672*	*0.271*	*0.515*
	Daytime BP load, %						
SBP	34.8±24.6	51.1±31.0	59.8±32.8	*0.060*	*0.128*	*0.062*	*0.598*
DBP	40.5±30.5	48.2±30.2	49.0±29.0	*0.624*	*0.622*	*0.714*	*0.995*
	Nighttime BP load, %						
SBP	48.6±30.4	61.5±30.7	74.5±23.0	*0.058*	*0.258*	*0.046*	*0.306*
DBP	56.0±31.4	62.8±29.6	66.4±21.9	*0.579*	*0.670*	*0.582*	*0.908*

*P** - for ANOVA

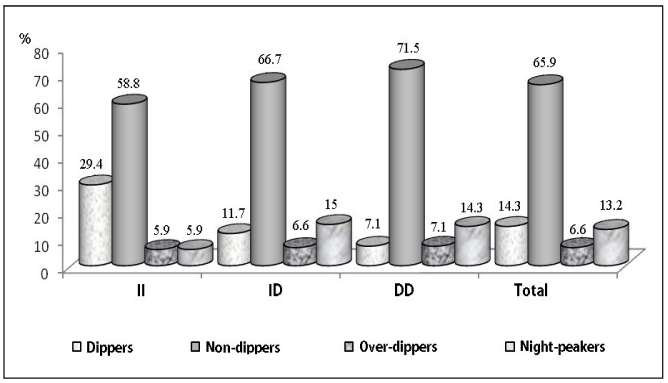

Fig.3 *Circadian Variation of Blood Pressure depending on the ACE gene I/D polymorphism*

Discussion

The results of our study revealed a significant association between the ID genotype and the D allele of ACE gene I/D polymorphism with a high risk of HE development in HT patients of Uzbek nationality.

A strong association between the DD genotype and the D allele with ischemic stroke (IS) was revealed in a number of studies,[7-10] while others showed no such association.[11-14] Siera et al.[15] investigated a possible association between 3 different genetic polymorphisms of RAS and the presence of cerebral white matter lesions (WML) in 60 never-treated essential hypertensive patients (36 men, 24 women), aged 50 to 60 years, without clinical evidence of target organ damage. All patients underwent brain magnetic resonance imaging to establish the presence or absence of WML. The I/D ACE gene, the M235T angiotensinogen (AGT) gene, and the A1166C angiotensin II type 1 receptor gene polymorphisms were determined by standard polymerase chain reaction. Twenty-five hypertensive patients (41.6%) were found to have WML on brain MRI. Significant association with the presence of WML was found only for ACE gene I/D polymorphism. The frequency of the DD genotype in patients with WML (64%) was significantly higher than that observed in patients without WML (28.6%; *P=0.022*). The DD genotype OR for the presence of WML was 4.44 (95%CI: 1.48-13.3). Likewise, the proportion of the D allele in patients with WML (74%) was significantly higher (*P=0.014*) than that observed in patients without WML (51.4%). Authors concluded that the presence of the DD genotype and/or the D allele of the ACE gene may be a predisposing factor for developing WML in essential hypertensive patients.

Presumably, these observed variations could be attributed to the differences in ethnic composition of study subjects and study design.[16] A recent meta-analysis revealed the D allele as a low-penetrance susceptibility marker for IS.[17] It is possible that ACE gene I/D polymorphism can modulate cerebrovascular risk only in combination with other risk factors, such as HE, hyperlipidemia, smoking, diabetes, and systemic atherosclerosis. In our study, we found a significant association between the ID allele and pronounced disorders in BP profile, high levels of TC, TG, VLDL-C, and IMT values. The association between the DD genotype and a high degree of LVH and endothelial dysfunction in Uzbek HT patients was shown previously.[18-20] Some studies[19,21,22] have shown that the DD genotype is a potential risk factor for the development of carotid atherosclerosis with hemodynamically significant stenosis.

In conclusion, the carriage of the ID genotype and/or the D allele of the ACE gene may be a predisposing factor for developing HE in HT patients of Uzbek nationality. Among HT patients with HE, the presence of the ID and DD genotypes has been associated with a high degree of HT and IMT value in comparison with the II genotype. We found significant association between the DD genotype/D allele and pronounced disorders in daily BP profile, high levels of TC, TG, and VLDL-C. However, because of the small sample size, these results require confirmation in a larger study.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Cardiology

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Dynamic Networks of Human Homeostatic Control in Norm (Part 2)

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Abstract

We undertook this research to study the relationships between elements of the vascular system in individuals without clinical signs of pathology (in "norm"). Indicators of hemodynamics obtained by catheterization in various vascular areas (RA, RV, LV, PT, Ao, SS, RHV (FHVP and HVWP), CS) were the pressure levels (mmHg). During the correlation analysis, the significant ($P<0.05$) relation signs (+, 0, -) without regard to their power were considered.

The obtained results allow us to draw following conclusions:

- Capacitive venous vessels of the liver are a communication channel between the evolutionarily younger (lungs) and older (organs of the splanchnic pool) functional systems of the human body.
- Hepatic venous circulation is a field of interference of the information and biochemical relationships between the body and the external environment through the venous outflow from GIT (the zone preceding the liver) and arterial flow - CHA (an indicator of aortic hemodynamics and gas exchange in lungs).
- The result of marked interactions is an integrated bio-informatic flow of venous outflow from the liver (whose "thesaurus" includes the information volumes of organs preceding the liver), which has uniform hemodynamic and biochemical parameters and interacts with the blood flow of RA and all phases of CC and hemodynamics in IVC/SVC pools and CS.
- The intersystem relationship in the cardio-hepato-pulmonary complex of the human body is the highest biology hierarchical level of the homeostatic relationships in the human–external-environment system. **(Int J Biomed. 2016;6(3):179-183.)**.

Key Words: human homeostasis control matrix • hydrodynamic balance • hepatic venous outflow • coronary sinus

Abbreviations

Ao, aorta**; CC,** cardiac cycle**; CS,** coronary sinus**; CHA,** common hepatic artery *(a. hepatica communis)*; **CO,** cardiac output**; CT,** coeliac trunk *(truncus coeliacus)*; **DP, d**iastolic pressure; **EDP,** end-diastolic pressure; **FHVP,** free hepatic venous pressure; **GIT,** gastrointestinal tract; **HVWP,** hepatic venous wedge pressure; **HPV,** hepatic portal vein *(v. portae hepatis)*; **IJV,** internal jugular vein *(v. jugularis interna)*; **IVC**, inferior vena cava *(v. cava inferior)*; **LV,** left ventricle *(ventriculus sinister)*; **LA,** left atrium *(atrium sinistrum)*; **M,** maximum pressure; **MP,** medium pressure; **N,** Norm; **PP,** pulse pressure; **PT,** pulmonary trunk *(truncus pulmonalis)*; **RHV,** right hepatic vein *(v. hepatica dextra)*; **RA,** right atrium *(atrium dextrum)*; **RV,** right ventricle *(ventriculus dexter)*; **RHA/LHA,** right/left hepatic artery; **RLL/LLL,** right/left lobe of the liver; **SS,** sigmoid sinuses *(sinus sigmoideus)*; **SinP,** sigmoid sinus pressure; **SVC,** superior vena cava *(v. cava superior)*; **SP,** systolic pressure; **SVP,** hepatic sinusoidal pressure; **SA,** splenic artery; **SV,** stroke volume.

Materials and Methods

All of the research was conducted in the same laboratory and with the help of the same methods and equipments in the supine position (in the perpendicular position to the vector of gravity when its influence in all parts of the venous system is equal). The catheterization was performed by percutaneous puncture of the right femoral artery and the right femoral vein under local anesthesia, using the Seldinger technique. A vein dilator was used during the vein puncture. The catheter reached the aforesaid parts of the cardiovascular system. The arterial pressure values were recorded for the thoracic aorta.

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The studies were monitored by using radiography equipment. The detailed description of studied groups were presented in Part 1 of this publication. [1]

Discussion

Capacitive liver vessels, being negatively correlated (Table 1) with the hemodynamic processes described previously ("single units": RA-RV and RV-PT; vascular flow to RA; hemodynamics of CS except PP),[2-10], are pathways of outflow from organs of the splanchnic pool (SP) and participate in the generation of pulsating turbulent flow (the work of the right heart chambers). In "norm," they provide a laminar permanent outflow during CC in the zone of hydrostatic indifferent points and regulate inflow (CHA and HPV) according to outflow (RHV) due to their anatomical and functional features:

1. Coincidence of the axes of the mouths of RHV and CS; lack of influence of the venous damping of IVC in the study block: a fixed capacity (RHV) – a variable capacity (RA).

2. The asymmetrically located intrahepatic part of branches of RHV depends on the surrounding tissues with fixing throughout to the encapsulated stroma of the liver, whose liquid medium is incompressible. Changing the configuration of the lumen and volume of RHV can only take place due to opposite changes in the surrounding vessels and tissues, which is analogous to the Monro–Kellie doctrine for the skull.

3. The structure of RHV is such that does not prevent

a) the retrograde pressure transmission (This effect is enhanced by the absence of the influence of the previous damper IVC.), or

b)the retrograde transmission of wave impulses and other central effects (cardiac activity).

4. An interaction of intrahepatic vessels of inflow (CHA and HPV) and outflow (RHV) occurring in mode of serried fingers (positive correlations with the Ao pulse pressure, see Table), leads to the fact that changes in pressure in RVH regulates the blood inflow to the liver parenchyma. Thus, an increase in the pressure in RHV leads to compression of intraorganic blood vessels of HPV and CHA, restricting blood flow to the liver. A decrease in the pressure in RHV is associated with the opposite effect. This pattern is more characteristic of RLL, where branches of RHA and HPV run parallel; the architectonics of intraorganic branches of LHA and HPV in LLL do not match. Thus, the regulation of inflow in the liver is dependent on the conditions of outflow. At the same time, there is an extrahepatic portion of magistral veins, which carries all the properties of the veins (changes in configuration and volume under changes in transmural pressure) in which the serried fingers mechanism does not work.

5. The lack of the effect of orthostasis on the intrahepatic part of IVC with fixing by fibrous rings at the entrance to and exit from the liver determines the hemodynamic stability of the outflow pathways by RHV.

6. Branches of RHV fall into the ampoule-shaped extension of the intrahepatic segment of IVC near its mouth, passing through a hole in the tendinous part of the diaphragm at an acute angle (35-40º), creating favorable conditions for blood circulation and drainage from RLL.

7. The mouth of IVC is provided with the closing apparatus, the myocardial sphincter, which performs the function of the fifth heart valve and the Eustachian valve of IVC; this sphincter is a structural and functional element of the heart. It takes part in preventing excessive blood regurgitation in IVC, protecting the liver, as the biochemical laboratory, against the stress effect of the regurgitation from RA, regulating the blood inflow to RV and preventing the chronic volume overload of RA. [11-13]

8. Heart chambers RA, RV, "united systole", CS) influence the hemodynamics in RHV; hemodynamic changes in these chambers affect the hepatic venous circulation and as a consequence, indirectly, the portal circulation (Table 1).

In norm, the anatomical structure of inflow and outflow pathways of the liver is built on the principle of minimizing the pressure fluctuations inside the liver parenchyma and constancy of liver functions. This structure aims to prevent the impact of the stress effect of the regurgitation from RA. This is achieved by hemodynamic and biochemical damping of arterial blood flow by SP organs, which precede the liver and form the blood flow in HPV.

Evolutionary deterministic branching of magistral vessels arising from the aorta and moving to each organ and functional element (FE), promotes the hemodynamic damping of arterial flow. Therefore, CT arises from the abdominal aorta at a right angle; RHA and SA arise from CT at an acute angle. The result is splitting of the spherical pulsating aortal flow, which is formed by LV, into a number of streams, which have different pressure and impulse and are specific to each organ of SP.

The difference in branching of the magistral blood vessels from Ao and the intensity of the determined response may be different for each organ and FE, as well as the degree of withdrawing information from the pulsating spheroid of the Ao pulse pressure. Laminar flow velocity in the cross section varies from zero at the wall of vessel to a maximum speed along the vessel's axis, having parabolic shape. With increasing the difference in pressure and the Reynolds number, parabola becomes increasingly elongate. Those, the stricture of the laminar flow has an elongated telescopic design, in which elements move with different speeds, where the structured layers are the independent variables, between which, there is an energy dissipation due to friction.[14] Thus, the angular differences in the ramifications of the vessels lead to a difference in the pressure levels (information) withdrawn from the spheroid of Ao, which causes the difference in information obtained by various SP organs. In norm, the spherical dissipative wave structure of the pulsating flow generated by LV in Ao reaches all points of the microcirculatory bed in the human body before aortic valve closure. The impulse of the pulse wave adapted for each organ with the participation of its own arterial system, outpacing the formation of the bio-informatic flow (BIF) formed by CC, prepares the exchange structures of the organ to receive this BIF. Vessels of inflow are in a single fascial bed with vessels of outflow; thus, the impulse of the pulse wave also affects the information flow of

the outflow pathway, sharing the information and performing the feedback functions. Along with the fact that each organ of SP obtains identical biochemical composition of the blood, the nature of branching of magistral vessels from Ao (which is specific to each organ) provides the targeted obtaining of the fixed front portion of the spheroid of the stroke pulse wave and forms the information flow individually for each organ. Thus, the biochemical composition of arterial blood is the same for all SP organs, but the impulse of the pulse wave is different and specific for each organ of SP. The arterial "knee" is a damping chamber, which creates a laminar flow providing the stable functioning of the liver in the norm.

The blood of CHA, which passes through a metabolic filter of lungs, has contact with the external environment through gas exchange in the lungs and reflects the functioning of small and large circulatory circles. The biochemical composition of the blood flowing from each organ of SP into HPV depends on the biochemical composition of arterial blood, the functioning of this organ and the functioning of other SP organs.

We identify the following groups of organs:

1. Organs of GIT (stomach, intestines). The biochemical composition of the blood flowing from these organs will depend on the biochemical composition of arterial blood, food, pancreatic juice and bile (ie, the influence of other organs of SP), microbial flora, the composition of intestinal gas, etc. Thus, a) these organs are associated with the external environment through the lungs and food; b) biochemical composition of the blood flowing from them depends on many factors and therefore is variable.

2. The spleen is an exclusive organ of SP, which is associated with environmental factors only through arterial blood, is not subjected to the influence of other SP organs, and affects only the liver.

3. The pancreas is an organ that occupies a special position among other SP organs. The shortest way communication with the external environment is realized through arterial blood. At the same time, the pancreas affects the functioning of other SP organs through pancreatic juice secreted in the intestinal lumen and active substances (hormones, enzymes, etc.) released into the blood flowing to HPV.

Biochemical damping of the blood flowing to the liver is provided by five BIFs:

- arterial blood of CHA, which passes through the metabolic filter of lungs and has contact with the external environment through gas exchange, reflecting the functioning of small and large circulatory circles;
- venous blood, which flows from the spleen;
- venous blood, which flows from the pancreas;
- venous blood, which flows from GIT; and
- lymph, which flows through the lymphatic pathways of SP organs.

Among these, CHA is the shortest way to deliver information about the activities of organs of the human body and their interaction with the environment through the most fast-acting factors - gases. Other SP organs also obtain the same information.

A feature of the biochemical composition of the blood coming from GIT is an independent relationship with the external environment through food. This relationship is not constant; it is less fast-acting and can be offset for many substances.

Communication between the spleen and the external and internal environments depends on the arterial blood. Thus, biochemical composition of the blood of HPV depends on the biochemical composition of arterial blood, food consumption, pancreatic juice and bile secreted in GIT lumen (the influence of other organs of SP), microbial flora, the intestinal gas composition, etc.

Of particular importance is the distribution of immiscible blood streams in HPV, which are formed by different SP organs. HPV has no valves, so there is a possible laminar flow in opposite directions.[11,15] Because of the hydrodynamic damping that provides the laminar flow, in norm, two blood streams can be distinguished in the HPV system:

a) an upper stream extending away from the spleen (SV) is directed to LLL without mixing with other streams;

b) a lower stream (from other SP organs) goes to RLL.

The hydrodynamic damping of blood pressure by the spleen as blood enters it selectively promotes the flow of information about the biochemical composition of the formed blood elements and plasma. To LLL, which regulates the most subtle abnormalities in the biochemical processes, the blood flows from the spleen, pancreas, stomach, and the left half of the colon. To RLL, which is the "working" portion of the liver and depends on the information coming from LLL, the blood flows from the small intestine, where basic digestion and absorption of nutrients are carried out, and from the right half of the colon.

Perhaps, the spleen is not only a hydrodynamic damper in the human body, but also an organ which duplicates the management of biochemical processes in the human body through its impact on the metabolic processes in LLL. The independence of the information flow coming from SV and CHA is one of the conditions for the functioning of SP organs as a biochemical damper that, in particular, is manifested in liver cirrhosis, hypertension, and peritonitis.

Thus, the liver has three BIFs, which integrate the communication between the right and left liver lobes:

1. By RHA/LHA (in LLL and RLL) through arterial blood carrying the information regarding the interaction between the liquid environment of the human body and the external gas environment in the lungs.

2. By HPV (in LLL) from the spleen (by SV).

3. By HPV (in RLL) from SP organs carrying the information regarding the interaction between the liquid environment of the human body and the external environment through factors coming from GIT.

Thus, HPV is an exclusive vessel through which the integrated information flow (IIF) combining the two types of BIFs moves to the liver:

1. BIFs that mainly carry the information (regarding the interaction with external gas environment) in LLL, which is damped by the spleen (the organ of "stress situations") regulating the most subtle deviations in biochemistry.

2. BIFs that carry the information (regarding the interaction between the liquid environment of the human body

Table 1.

The extended summary matrix table of synergistic relationships for parameters of metabolism and hemodynamics

Variable		Ao				RA					RV		LV				SinP				PT				RHV				HVWP			
		SP	DP	PP	MP	A	X	V	Y	MP	EDP	MP	SP	PP	CO	SV	SP	DP	PP	MP	SP	DP	PP	MP	SP	DP	PP	MP	SP	DP	PP	MP
SinP	SP				+	-	+			+		+	-	-	-		x	x	x	x					-	-	+	-				
	DP		+		+		+			+		+	-	-	-		x	x	x	x												
	PP		-				-				+						x	x	x	x					-	-		-				
	MP				+		+		+	+	-	+	-	-	-	-	x	x	x	x					-	-	+	-				
HVWP	SP		-	+	-	-	-	-	-	-	-	+	x	x	x	x	-	-	-	-				-	+	+	+	+	x	x	x	x
	DP		-	+	-	-	-	-	-	-	-	+	x	x	x	x	-	-	-	-				-	+	+		+	x	x	x	x
	PP							-	-		+	+	x	x	x	x	-	-	-	-				+		-			x	x	x	x
	MP		-	+	-	-	-	-	-	-	-	+	x	x	x	x	-	-	-	-				-	+	+		+	x	x	x	x
PT	SP	+	+		+	+	+	+	+	+	+		x	x	x	x	+	+		+	x	x	x	x								
	DP		+		+						+		x	x	x	x			-		x	x	x	x								
	PP	+			+	+				+			x	x	x	x	+	+	+	+	x	x	x	x	+	+		+				
	MP	+	+		+	+	+	+	+	+	+	+	x	x	x	x		+	-		x	x	x	x								
CS	SP	+	+	-	+	+	+	+	+	+	+	-	x	x	x	x	+	+	-	+	+	+	+	+	-	-	+	-	-	-		-
	DP		+	-	+	+	+	+	+	+	+	-	x	x	x	x	-	-	+	-		+	-				+				+	
	PP		+	-					+			-	x	x	x	x	+	+	-	+	+		+	+	-	-		-	-	-	-	-
	MP	+	+	-	+	+	+	+	+	+	+	-	x	x	x	x	-	-	+	-	+	+			-	-	+	-	-	-	+	-

and the external environment through factors coming from GIT) from GIT in RLL.

Normally, each IIF entering the channels for the information collection from this system is not dominant (individually) in regulating the function of organs and the whole organism. We believe that the total humoral information delivered by HPV in the liver (in norm, 75% of blood volume) and dependent on the factors of the internal and external environments is subjected to significant correction and damping by other SP organs. The dependence of the liver on the overall characteristics of HPV weakens the influence of any single BIF, including the BIF moving by CHA. That is, biochemical regulation of the liver function is associative, which increases the stability of homeostasis and homeokinesis of the human body. Small and large circulatory circles are the interconnected systems with a partial cross-duplication of functions. In lungs (the pulmonary circulation), the metabolic and non-gas-exchange functions that belong to the systemic circulation (large circulatory circle) are partially represented.

Conclusion

The obtained results allow us to draw following conclusions:

- Capacitive venous vessels of the liver are a communication channel between the evolutionarily younger (lungs) and older (SP organs) functional systems of the human body.

- Hepatic venous circulation is a field of interference of the information and biochemical relationships between the body and the external environment through the venous outflow from GIT (the zone preceding the liver) and arterial flow - CHA (an indicator of aortic hemodynamics and gas exchange in lungs).

- The result of marked interactions is an integrated BIF of venous outflow from the liver (whose "thesaurus" includes the information volumes of organs preceding the liver), which has uniform hemodynamic and biochemical parameters and interacts with the blood flow of RA and all phases of CC and hemodynamics in IVC/SVC pools and CS.

- The intersystem relationship in the cardio-hepato-pulmonary complex of the human body is the highest biology hierarchical level of the homeostatic relationships in the human–external-environment system.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Human Homeostatic Control Matrix in Norm

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Abstract

We undertook our research to study and systemize the relationship between hemodynamics and biochemical parameters of arterial and venous blood in healthy people. Hemodynamic and biochemical characteristics were obtained through a probe by using catheterization in various vascular areas (aorta, brain, heart, lungs, and liver). Correlation and factor analyses were conducted to study the relationship between the obtained characteristics of the regional and systemic blood flow. Due to the nature of the correlation analysis, the significant ($p<0.05$) relation signs (+, 0, -) without regard to their power were considered.

The obtained results suggested that there are sets of both intra-organ and system regulatory relationships between metabolic and hemodynamic characteristics. The complex of relationships among the studied parameters makes it possible to maintain the homeostatic equilibrium in the body. The psychophysiological control system includes the subsystems we described: 1) the cardiac-hepatic-pulmonary complex having properties of the metabolic and hemodynamic information field providing biological stability of the homeostasis; any significant imbalance of its elements triggers afferent information flows actualizing an afferent synthesis; 2) the mind forming gradient patterns of targeted behavior to eliminate metabolic imbalance, to achieve goals both as coded biological parameters and as the highest forms of behavior, to reach the ultimate goal: parametric, homeostatic equilibrium in the "biosphere" of the human body. By using the results of our research and the complex of dynamic relationships in human homeostasis, we built a homeostatic control matrix (HCM). (**Int J Biomed. 2016;6(3):184-189.**)

Key Words: homeostasis • control matrix • coronary sinus • cerebral blood flow • hepatic blood flow.

Abbreviations

Ao - aorta; **AVDO2**, arterial venous difference O2; **BIF**, bioinformatic flow; **BUs**, behavioral units; **CC**, cardiac cycle; **CHA**, common hepatic artery; **CS**, coronary sinus; **CO**, cardiac output; **DP**, diastolic blood pressure; **Er**, erythrocyte; **EDP**, end-diastolic pressure; **F-n**, fibrinogen; **GIT**, gastrointestinal tract; **HPV**, hepatic portal vein; **HVWP**, hepatic venous wedge pressure; **IJV**, internal jugular vein; **IG**, image of the goal; **LV**, left ventricle; **MP**, mean blood pressure; **N**, norm; **Pl**, plasma; **P-n**, total protein; **PP**, pulse pressure; **PT**, pulmonary trunk; **RA**, right atrium; **RV**, right ventricle; **RHV**, right hepatic vein; **Sin P**, sigmoid sinus pressure; **SS**, sigmoid sinus; **SP**, systolic pressure; **SVC**, superior vena cava; **SAH**, stable arterial hypertension; **SV**, stroke volume.

Objective

The aim of the research was to systematize findings obtained during the analysis of the regional and systemic relationship between biochemical and hemodynamic characteristics; to build a principal diagram of stable relationships between characteristics of metabolism, gas exchange, and hemodynamics; and to build a summarized matrix for relationships typical of homeostatic control in healthy humans.

Materials and Methods

All of the research through which we received the discussed results was conducted in the same laboratory and with the help of the same methods. The patients were in the

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supine position (in the perpendicular position to the vector of gravity when its influence in all parts of the venous system is equal). The data obtained by using catheterization performed with a probe show the blood pressure levels and biochemical parameters of the outflow from the studied organ (the wedging made it possible to obtain exchange and hemodynamic data directly from the target organs: the brain and the liver). We performed the catheterization of LV, Ao, IJV, SS, CS, SVC, RHV, RA, RV, and PT; during the procedure the pressure levels were recorded and blood samples were collected. The catheterization was performed by percutaneous puncture of the right femoral artery and the right femoral vein under local anesthesia, using the Seldinger technique. A vein dilator was used during the vein puncture. The catheter reached the aforesaid parts of the cardiovascular system. The arterial pressure values were recorded for the thoracic aorta. The studies were monitored by using radiography equipment.

The results for assessment were obtained from the studies conducted on apparently healthy people who were thoroughly examined in hospital before complex reconstructive maxillofacial surgery (these data have been represented in our early research papers, starting from 1976).The total number of the examined people was 60 (48 men and 12 women). The age of 52 of those people ranged from 20 to 45, and 8 people were over 45 years old. In 18 (13 males and 5 females aged from 21 to 42 years) people, out of necessity, catheterization of CS (for estimation of biochemical and hemodynamic parameters in 6 persons) and right HV (for biochemical parameters in 11 people and hemodynamic parameters in 14 people) was conducted.

Discussion

When assessing the results, we took into consideration some principles of the general system theory, namely, regulation theory.

Homeostatic regulation is seen as a dynamic state, with changing adaptive oscillation of the parameters (regulatory subsystems) and maintenance of a flowing balance in the internal environment despite external changes. When external conditions change, the open self-regulating homeostatic system finds a multitude of functional stability parameters to restore the stability of the system. The range of physiological changes, within which the optimum life activity for the present environment persists, is seen as normal. To define boundaries of the normal range we used the confidence interval boundaries set for each parameter and value; these boundaries define the range within which the probability of the relevant parameter value is 95%. We have investigated the values of the following parameters: content levels of plasma electrolytes (K, Na) and red blood cells (Er); blood gases (pO_2, pCO_2); acid-base composition (pH, SB); blood proteins: hemoglobin (Hb), F-n, total protein (P-n); and arterial venous difference ($AVDO_2$) in some segments.

We have investigated the following parameters of central and systemic hemodynamics (expressed in mmHg): SP, DP, PP, MP; EDP for LV and RV, and A/ X/ V/Y waves for RA.

To analyze the cerebral blood flow we opted for the hemodynamic pathway: LV—Ao—SS—IJV—SVC—RA. In addition to other relationships, we found (see HCM) that the active process of LV and Ao (systolic and pulse pressure) had no correlation relationships with the SinP pulse pressure, thus demonstrating absence of any relationships. The LV systolic pressure correlates negatively with the Sin P systolic, diastolic and mean pressure. Therefore, tin norm, Sin P development does not depend on the Ao pulse pressure. The negative correlation between LV and the SinP levels can be explained only by the structure that is located between the cranial cavity and extracranial vessels; the aortic flow makes the opening change. We assume that the above structure is represented by carotid siphons, which damp pressure values to the value equal to Ao mean pressure, which indicates that the integrated hydrodynamic system has moved into a stage of diastolic evolution (from MP to DP): Ao–cranial inlet vessels—Sin P. The positive correlations between the PT pressure levels, the RA pressure, and the Sin P indicate an existing relationship between the venous part of the cerebral blood flow and the hemodynamics of RA and PT, which create a pressure gradient of the blood flow through pulmonary capillaries (ie, boundaries with the external (gaseous) environment, where changes in the gradient and the gas composition of venous blood take place). The obtained data prove that rheological properties of blood and viscosity can be seen as a primarily regulated homeostasis parameter. We think that the integrated hydrodynamic space is one of the regulators of cerebral gas exchange, where the main factor is the minimum pressure along the path of the cerebral outflow – in RA.[1]

When comparing the respective values in healthy people and those suffering from SAH, we revealed their compliance with the averages values of the entire research range. Thus, we assumed that there were differences in interaction of biochemical substances and relationships between hemodynamic processes in healthy people compared to SAH patients.[2] We compared the respective sets of correlation relationships between hemodynamic and biochemical characteristics at the inlet and outlet of the cerebral blood-flow system in healthy people and SAH patients. We revealed significant differences in the composition and quality of the sets of correlation relationships, including a lost relationship with end products of cerebral gas metabolism (O2, CO2, etc.) and a lost relationship between the cerebral blood flow and rheological characteristics of the cerebral blood outflow (general and plasma viscosity – F-n, P-n). SAH patients demonstrated inversion or loss of the entire set of Sin P hemodynamic relationships, and loss of relationships with the oncotic pressure, gas pressure, and blood viscosity parameters.

The results of the previous studies[3-8,1] allow us to review and analyze cardiac hemodynamics as a "single entity" consisting of functional units: 1) "atrial unit" - RA and LA; 2) "aorto-pulmonary unit" - bulb of the aorta and PA; 3) "three-chamber unit" of ventricles, consisting of a) " the left chamber" (LV) and b) "the richt chamber" (RV) with blood outflow from the chambers into "aorto-pulmonary unit", and c) "spongy" venous chamber with blood outflow from the myocardium (during the united systole) through CS and Thebesian veins into the "atrial unit." During "united systole"

of "three-chamber unit" of ventricles, the following blood volumes are moved: 1) LV and RV stroke volume into the "aorto-pulmonary unit"; 2) stroke volume from the "spongy" venous chamber into the "atrial unit" (these volumes constitute a united stroke volume of the "three-chamber unit"; and 3) blood inflow from SVC/IVC and pulmonary veins into the "atrial unit" during systolic/membrane blood suction and during the retraction of the tricuspid and mitral valves in the ventricular cavity during the blood expulsion from them. Thus, a united systole of "three-chamber unit" of ventricles is the basis for regulation: 1) the inflow (mobilization) of blood into the "atrial unit"; 2) systolic synchronization of hemodynamics in the "aorto-pulmonary" and "atrial" units; 3) intracardiac conditions of blood outflow from all chambers of the "three-chamber unit" of the ventricles through the fixed rings of CS, truncus pulmonalis, and bulb of the aorta. The volume of the blood moved by ventricular myocardium during this period is more than a united stroke volume by the amount of venous blood mobilized to the "atrial unit."

X-collapses in RA create a stable phase gradient of the venous drainage from the liver into RA.[1] It remains positive (i.e., provides a constant outflow from the right HV into RA) throughout the united systole of the "ventricular unit," when the other phase gradients are zero or negative. It was shown that there is a synchronization of this flow with the same phases of flow outflow from the brain, lungs, and kidneys. As manifestations of a single process (myocardial contractility), all the indicators of a period of united systole change conjugate. We believe that this is the basis of the systolic regulation of venous outflow from the organs (including the liver) into the "atrial unit." Atrial contraction generates the "A" wave and not only pushes blood into the ventricles, but also disperses the shock wave reversely (against the blood flow). While we see the activity of ventricles as a quantum process, the retrograde propagation of the "A" wave is seen as a pre-quantization process. In other words, the activity of angions of functional elements (including metabolic processes) can be regulated by the central organ, the heart, through control wave signals, both down and up the blood flow.

A phase of the diastolic synchronization of intraorgan pressures, which takes place on the background of end-diastolic pressures in the ventricles, aorta, and pulmonary artery, occurs at open atrio-ventricular valves and the created united chambers: 1) "RV, RA, the veins of the great circle"; 2) "LV, LA, pulmonary veins." In this phase, there is a minimization of the differences between the levels and gradients of the pressures inside of the united chambers and between them. Our findings suggest [1] that RA is a zone of unstable hydrodynamic balance within from 0 to 10 mmHg, having a number of fixed values (equal to pressure levels in pressure-bearing veins) synchronized with the phases of evolution of the cardiac cycle, which are the derived values from the dynamics of the cardiac cycle, as well as the threshold values for wave-control signals spreading to the exchange zone of organs, including the human heart. Our data allow to consider CS not only as a vessel for venous blood flow from the myocardium, but also as a channel of intracardiac regulation of LV function by the level of booster pressure in RA, and by the level of pressure in the united chamber (RV-RA) in phase of diastolic synchronization.

The wave impulse of the coronary sinus moves into the relaxing right atrium that has not reached its full diastolic volume and dissipates along the venous collectors. The topographic proximity, coincidence of the axes of the mouths of RHV and CS, and lack of influence of the venous damping of IVC lead to the situation where the right hepatic vein is the first and the closest recipient of a wave impulse from the coronary sinus of the heart.

The capacitance hepatic vessels characterized by negative relationships (see HCM) with the hemodynamic processes that we described earlier [1,3,10-16] – the integrated chamber of RA-RV, RV-PT, vessels of inflow to RA, and CS – are outflow pathways, taking part in generation of blood flows in the venous heart. In norm, the anatomical structure of inflow and outflow pathways of the liver is built on the principle of minimizing the pressure fluctuations inside the liver parenchyma and constancy of liver functions. This structure aims to prevent the impact of the stress effect of the regurgitation from RA. This is achieved by hemodynamic and biochemical damping of arterial blood flow by the organs of the splanchnic system. Hemodynamic damping is provided by the evolutionarily determined pattern of origin and branching of trunks from the aorta. Each organ is characterized by differences in angles where trunks stem from the aorta. As the laminar flow has an elongated telescopic configuration where elements move at different speed, the structured layers, between which friction causes energy dissipation, are characterized by their own parameters.[17]

Thus, the angular differences in the ramifications of the vessels lead to a difference in the pressure levels (information) withdrawn from the spheroid of the pulse wave Ao, which causes the difference in information obtained by various organs. Structural changes in the conductivity of these pathways (for example, in SAH patients) cause malfunction of the compensatory mechanisms responsible for normal functioning.

In norm, the spherical dissipative wave structure of the pulsating flow generated by LV in Ao reaches all points of the microcirculatory bed in the human body before aortic valve closure.[18] The impulse of the pulse wave adapted for each organ with the participation of its own arterial system, outpacing the formation of the bio-informatic flow (BIF) formed by CC, prepares the exchange structures of the organ to receive this BIF.

The stemming pattern typical of trunks makes it possible that each organ receives the respective fixed segment of the pulse wave spheroid. In other words, the biochemical composition of blood is identical for all splanchnic organs, but the impulse of the pulse wave that precedes BIF is individual for each organ. A peripheral vascular resistance with reduced resistance in areas of increased metabolism (increased blood flow) is a mosaic information field (1st feedback) for the source of the laminar arterial generator (heart), with change-informing and control signals in areas of increased blood flow (metabolism). Arterial inflow vessels transporting the laminar flow constitute a damping chamber responsible for stability of the organ's function.

Homeostatic Control Matrix

Variable		Ao				RA					RV		LV				Sin P				HVWP				RHV				CS				PT			
		SP	DP	PP	MP	A	X	V	Y	MP	EDP	MP	SP	PP	CO	SV	SP	DP	PP	MP	SP	DP	PP	MP	SP	DP	PP	MP	SP	DP	PP	MP	SP	DP	PP	MP
Ao	K pl					-	-	-	-	x	x	x	x	x	x	x	+												+	-	+					
	K er									x	x	x	x	x	x	x	-	-		-		-		-			+	-	+		+	+			+	
	Na pl							-	-	x	x	x	x	x	x	x	+	+			+								-	-	-	-				-
	Na er					-	-	-	-	x	x	x	x	x	x	x	-				+	+								-	+		-	-		-
	pH							+	+	x	x	x	x	x	x	x	-	-		-			-		-		-	-	-		-					
	pCO_2		-		-	-			-	x	x	x	x	x	x	x							+	+	+		+			+	-						
	SB		-		-					x	x	x	x	x	x	x	-	-		-	+	+		+			-		-		-	-			-			
	pO_2				+					x	x	x	x	x	x	x	+	+		+																
	HbO_2						+		+	x	x	x	x	x	x	x											+			+	-				+	-
	Ht					+	+	+	+	x	x	x	x	x	x	x	-	-		-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	F-n	+	+			-	+		-	x	x	x	x	x	x	x	-	-		-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	P-n									x	x	x	x	x	x	x	+	+		+	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	H-b							+		x	x	x	x	x	x	x	-	-		-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
SS	K pl					-	-	-	-	x	x	x	x	x	x	x	-	-		-			-	-					+	-	+	-		-		
	K er						-	+	+	x	x	x	x	x	x	x							-	-					+	-	+	-		-	+	
	Na pl		+		+			-	-	x	x	x	x	x	x	x	+	+		+	-	-		-								+		+	
	Na er								-	x	x	x	x	x	x	x					-	-		-	+	+	-	+		-	+	-		-	+	-
	pH					-		+	+	x	x	x	x	x	x	x	-	-		-			+						-	+	-	+		-		-
	pCO_2									x	x	x	x	x	x	x		+			-	-	-				+	-			+	-	+	+		+
	SB				-			+		x	x	x	x	x	x	x	-	-		-			+	+					-	+	-	+				
	pO_2						-	-	-	x	x	x	x	x	x	x																				
	HbO_2					-	-	-	-	x	x	x	x	x	x	x					+	+		+			+		-	+	-	+				-
	Ht						+	+	+	x	x	x	x	x	x	x					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	F-n		+	-		-	-	+	-	x	x	x	x	x	x	x	-	-		-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	P-n									x	x	x	x	x	x	x	+			+	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	H-b						-		-	x	x	x	x	x	x	x	-	-		-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Sin P	SP				+	-	+			+		+	-	-	-		x	x	x	x	-	-	-	-	-	-	+	-	+	-	+	-	+		+	
	DP		+		+		+			+		+	-	-	-		x	x	x	x	-	-	-	-					+	-	+	-	+		+	+
	PP		-				-				+						x	x	x	x	-	-	-	-	-	-		-	-	+	-	+		-	+	-
	MP				+		+		+	+	-	+	-	-	-	-	x	x	x	x	-	-	-	-	-	-	+	-	+	-	+	-	+		+	
HVWP	SP		-	+	-	-	-	-	-	-	-	+	x	x	x	x	x	x	x	x	x	x	x	x	+	+	+	+	-		-	-				-
	DP		-	+	-	-	-	-	-	-	-	+	x	x	x	x	x	x	x	x	x	x	x	x	+	+		+	-		-	-				-
	PP							-	-		+	+	x	x	x	x	x	x	x	x	x	x	x	x		-				+	-	+				+
	MP		-	+	-	-	-	-	-	-	-	+	x	x	x	x	x	x	x	x	x	x	x	x	+	+		+	-		-	-				-
RVH	SP	+		+		+	+		-	+	-	+	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-		-	-			+	
	DP			+		-	+				-		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-		-	-			+	
	PP	+			+	+	+	+					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	+	+		+				
	MP	+		+			+	-	+	_	-		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-		-	-			+	
CS	SP	+	+	-	+	+	+	+	+	+	+	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	+	+	+	+
	DP		+	-	+	+	+	+	+	+	+	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		+	-	
	PP		+	-					+			-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	+		+	+
	MP	+	+	-	+	+	+	+	+	+	+	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	+	+		
PT	SP	+	+		+	+	+	+	+	+	+		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	DP		+		+						+		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	PP	+			+	+				+			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	MP	+	+		+	+	+	+	+	+	+	+	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Biochemical damping of venous blood along the inflow pathways to the liver from splanchnic organs was described earlier. The capacitance venous vessels of the liver are communication channels between the evolutionarily younger (lungs) and older (splanchnic organs) functional systems of the human body. The hepatic venous circulation provides interference of the information and biochemical relationships between the body and the external environment through the venous outflow from GIT (the zone preceding the liver) and arterial flow - CHA (an indicator of aortic hemodynamics and gas exchange in lungs). It results in an integrated BIF of venous outflow from the liver (whose "thesaurus" includes the information volumes of organs preceding the liver), which has uniform hemodynamic and biochemical parameters and interacts with the blood flow of RA and all phases of CC and hemodynamics in IVC/SVC pools and CS (see HCM).

In changeable conditions of the external (gaseous) environment, homeostasis of the open self-regulating system (the liquid medium) can be maintained only by changes in the interaction of such systems as a human being and the external environment through different patterns of adaptive behavior. In the theory of Type 2 functional systems,[19] the integrative BUs that maintain homeostasis through changes in behavior provide functioning, retaining and subsequent elimination of the ideal IG that is developed as a product of the afferent synthesis. BUs constitute structurally identical, regular sequences, which do not depend on any content of IG and final outcome. They have characteristics of bijective reflection, in which they retain the coded structure of the object as an information matrix. Having no dimensions we are aware of, except for a time dimension, IG can include any possible and conceivable type of space and dimension, thus giving grounds for the assumption that they exist (or can be developed) in human mental formations in a compact (potential) form and can be decoded by the operating mental systems (thinking, imagination, etc.). BUs participate in generating, developing and retaining an ideal IG, which is a spatiotemporal entity of activated neural formations of the brain.

We think that the BU afferent stage starts when there is significant imbalance of parameters, misalignment in the system of metabolic, psychological (including social) relationships or, in other words, when there is any deviation from the boundaries of the "normal zone". For metabolism, a deviation from the upper/lower bounds of the normal zone to the level of the suprathreshold sensitivity of the receptors (the first signal system) is involved;[2,20] for structures of the psychological and social spectra, involvement is to the level of "cognized–not-cognized" and "acceptable–not-acceptable" (the second signal system). The distinguishing characteristic of "living beings" is their responsiveness to any disturbance in the homeostatic balance, to outright imbalance, i.e. the selective response to the object of an actual need. The need, being the most significant disposition of the motive, constitutes "an impelling force, a cause of the behavior" [21] and triggers activation of integrative units of behavior (targeted behavior). We intentionally simplify the hierarchy and structure of psychosomatic relationships, and think that the afferent information flow initiated by homeostatic imbalance actualizes an afferent synthesis and generates the image of a future result – the key component of the intelligent regulatory process – a coded IG, which is retained until the goal is reached: the imbalance is rectified, the need is satisfied, the motive loses its effect, and the homeostatic equilibrium (of metabolic and mental nature) is recovered. In our other works we have described our perception of the development stages of adaptive self-regulation, including creation of motivating (reachable and non-reachable) IGs in the behavioral continuum, as well as subjective time and motivation gradient.[20,22]

We see goal-setting caused by a misalignment of the system as a continuous reflection from one space (objective reality) to another (mental structures of reflection), which has different dimensional, temporal, spatial, and other categories. The mental structures of the goal image are further projected into the "external world" through targeted behavior. This results in a correlation between the sets where the near points of reference turn into near points of values. IG has a dualistic nature; it is both an ideal image of the future and a prerequisite to initiating targeted behavior. The synthetized and retained IG includes basic forms of advanced reflection: forecasting and goal-setting. Note that forecasting is advanced modeling of objective sequences, while goal-setting implies involvement of the subject in a targeted activity. Despite these differences, the interference of these forms of advanced reflection is essential for selection of the available parametric information, control signals and, therefore, tactics and strategy of adaptive behavior, the main outcome of which is the achieved homeostatic equilibrium. We define the goal image as an active distributed system capable of spontaneously creating, in response to the information contained in IG, static and spatially heterogeneous structures facilitating adaptive behavior (for example, morphogenesis in a fertilized egg). The result of a specific mental activity (specific human "need"), creating an "artistic image," is that it becomes the "insight," [22] (ie, completion of the unconscious formation (afferent synthesis) of the "artistic image," or IG). The subsequent projection, embodied in visualized forms, cumulatively produces fragments of the cultural environment. As an IG biophysical "marker," we present a hemodynamic, scalar gradient of the fluent venous-liquor pressure (with morphophysiological characteristics) in the exchange fields of actualized neural circuits.[20]

As we do not have our own data, we do not discuss participation of the nervous system, endocrine system, and other organs in homeostatic regulation, assuming that they are included in the system of psychophysiological regulation as it is described in present-day studies.

Conclusion

In sum, the studied organs of the healthy human body have sets of intra-organ regulatory relationships between metabolic and hemodynamic characteristics as well as sets of inter-organ relationships between parameters of systemic and regional hemodynamics. The complex of these relationships, which is given in HCM, makes it possible to maintain the homeostatic equilibrium in the body. The highest hierarchical level of the homeostatic regulation of the human being, where

the permanence of some parameters is maintained through changes in other parameters, is a system of psychophysiological control and regulation of permanently existing parameters of the internal liquid medium in the conditions characterized by changeable parameters of the external (gaseous) environment. The imbalance of metabolic characteristics prior to the level of suprathreshold sensitivity of receptors (for the psychological and social range prior to the level of perceived/not perceived, acceptable/not acceptable) triggers actualization of needs and adaptive patterns of behavior. The psychophysiological control system includes the subsystems we described: 1) the cardiac-hepatic-pulmonary complex having properties of the metabolic and hemodynamic information field providing biological stability of the homeostasis; any significant imbalance of its elements triggers afferent information flows actualizing an afferent synthesis; 2) the mind forming gradient patterns of targeted behavior to eliminate metabolic imbalance, to achieve goals both as coded biological parameters and as the highest forms of behavior, to reach the ultimate goal: parametric, homeostatic equilibrium in the "biosphere" of the human body.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Endocrinology

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Efficiency of Ibandronate in Monotherapy and in Combination with Alfacalcidol in Women with Postmenopausal Osteoporosis

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Abstract

The aim of this study was to carry out a comparative analysis of the efficiency of ibandronate monotherapy and combined therapies with ibandronate and alfacalcidol in women with postmenopausal osteoporosis.

Materials and Methods: A total of 53 women (mean age 60.7 years) with postmenopausal osteoporosis (PMO) were randomized to monotherapy with ibandronate 150 mg/month (Group Ib) (n=25) and therapy with ibandronate 150 mg/month plus alpha-D_3(AD3) 1 μg (alfacalcidol) daily (Group Ib+AD3) (n=28). All women received calcium and vitamin D_3 supplements. Patients were recruited in one center and were followed up for 6 months on a monthly basis. To assess the efficacy of therapy, BMD was measured at LS (L1–L4) and PF at the beginning and end of therapy by DEXA. Biochemical markers of bone turnover were also assessed.

Results: Statistically significant increases in BMD compared with baseline values and the control group were observed in both ibandronate treatment groups. Growth of BMD was significantly higher in Group Ib+AD3 compared to Group Ib. An assessment of CTX dynamics showed a notable decrease in CTX level in patients of both groups compared with levels before treatment. Generally, PTH level decreased insignificantly, but a more pronounced reduction was seen in Group Ib+AD3. TP1NP level significantly increased in Group Ib and was more pronounced in Group Ib+AD3.

Conclusion: Combined therapy with ibandronate sodium and the D-hormone analog alfacalcidol augments the effectiveness of treatment observed in ibandronate sodium monotherapy in PMO women. **(Int J Biomed. 2016;6(3):190-194.)**.

Key Words: menopause • osteoporosis • bone mineral density • ibandronate treatment

Abbreviations

BMD, bone mineral density; **LS,** lumbar spine; **PTH**, parathyroid hormone; **PF,** proximal femur**; BTMs,** bone turnover markers**; CTX**, C-terminal telopeptide; **TP1NP**, type 1 procollagen total N-terminal propeptide.

Introduction

Postmenopausal osteoporosis (PMO) is one of the most important problems of modern health care because of the high prevalence and severity of fractures arising from minor injuries. The medical and social importance of osteoporotic fractures can be seen in the physical disability and increased mortality of patients.[1-4]

One of the main antiosteoporotic drugs used currently in the EU countries and the USA, are the bisphosphonates. In numerous randomized clinical trials, drugs of this group, more than others, prevented occurrence of new and recurrent fractures.[5-11]

Representatives of bisphosphonates improve prognosis of fractures due to their ability to significantly slow down the intensity of resorptive processes in bones, to affect bone remodeling, and to increase bone mineral density. Nitrogen-containing bisphosphonates of third generation ibandronic acid (Bonviva®) possess promising features for clinical practice. Ibandronate has a unique structure, which has allowed combining a high affinity for bone tissue with a

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powerful antiresorptive potential.[12-14]

The clinical efficacy of ibandronate was confirmed by results of multicenter studies. Thus, the results of a multicenter, randomized, double-blind comparative study MOBILE (Monthly Orali Bandronate In Ladi Es) of therapy with ibandronate demonstrate a reduction in the incidence of vertebral fractures (by 4.9% and 6.6%, respectively) and fractures of the thigh bone (by 3,2% and 6,2% respectively) after 1 and 2 years.[15-16] The BONE study (Oral Ibandronate Osteoporosis Vertebral Fracture Trialin North America and Europe) found that women taking ibandronate on a daily or monthly basis have reduced risk of vertebral fractures and non-vertebral fractures in two populations – North America (60 and 54%, respectively) and Europe (50 and 48%, respectively).[17]

The aim of this study was to carry out a comparative analysis of the efficiency of ibandronate monotherapy and combined therapies with ibandronate and alfacalcidol in PMO women.

Materials and Methods

The study included 53 PMO women aged between 51 and 75 years (mean age 60.7±6.13 years, median 61.0 years; interquartile range [IQR] 55.0–64.0 years) with PMO and menopause duration of at least 1 year. Osteoporosis was diagnosed according to WHO criteria (1994) using T score standard deviations (SDs) from the normative values of peak bone mass in healthy women. A value of not more than -1 SD was regarded as normal, a value of -1to-2.5 SD as osteopenia, and ≤-2.5 SD as osteoporosis. Women were randomized to monotherapy with ibandronate 150 mg/month (Group Ib) (n=25) and therapy with ibandronate 150 mg/month plus alpha-D_3(AD3)1μg (alfacalcidol) daily (Group Ib+AD3) (n=28). Both groups were homogeneous, particularly for BMD. All patients were informed about the disease and its complications and gave informed consent to participate in the study. All women received calcium supplements at a dose of 1,000mg and vitamin D_3 800 IU/day. A control group of 16 women received only calcium 1,000 mg and vitamin D3 800 IU/day.

Physical load enhancement in the form of daily 30-min walking was recommended to all patients. Patients were recruited in one center and were followed up for 6 months on a monthly basis. Women with diseases affecting bone metabolism such as hyperparathyroidism, thyrotoxicosis, Cushing's syndrome and disease, hypogonadism, malabsorption syndrome, kidney and liver disease, and malignancies were excluded, as were those taking medications that affect calcium metabolism during 12 months before the study.

BMD Measurements

The primary objective of the study was to assess the evolution of BMD on the background of the therapy, and the second objective was to follow the evolution of BTMs. To assess the efficacy of therapy, BMD was measured at LS (L1–L4) and PF at the beginning and end of therapy by dual energy x-ray absorptiometry (DEXA) using a bone densitometer (Prodigy, CE Lunar Corporation, USA) in private clinic "Doctor Summit" (Summit Trading Company, Ltd., Tashkent, Uzbekistan). In compliance with WHO criteria, BMD was expressed as g/cm² and T-score. Efficacy analyses were conducted according to the intention-to-treat (ITT) principle. The ITT population included all randomized patients who had taken at least one dose of treatment and who had a baseline and one post-baseline evaluation.

Biochemical Measurements

To assess the metabolic activity of bone remodeling, biochemical markers such as CTX (b-CrossLaps test) and TP1NP were measured by electrochemiluminescence assays (Elecsys biochemical analyzer, Elecsys b-CrossLaps) at baseline and after 6 months of therapy. An absorptiometric method was used to determine serum total calcium and nonorganic phosphorous, and a kinetic method was used to determine alkaline phosphatase activity by the amount of liberated 4-nitrophenol. Commercially available kits (CIS Bio International, France) were used to measure levels of parathyroid hormone (PTH).

The study was approved by the local Ethics Committee. Written informed consent was obtained from each patient.

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean ± SD for continuous variables. The data for variation indices with nonparametric distribution are presented with medians and interquartile range [IQR] The Mann-Whitney (U Test) was used to compare the differences between groups. Group comparisons with respect to categorical variables are performed using chi-square test with Yates correction. A probability value of $P<0.05$ was considered statistically significant.

Results

The studied groups were comparable in terms of medical history (Table 1). High BMI was observed in all groups, which is likely associated with a sedentary lifestyle and poor diet (prevalence of pastry, fried food with use of animal fats) among postmenopausal women.

Table 1.

Baseline characteristics of patients

Variable	Treatment		
	Control group (n=16)	Group Ib (n=25)	Group Ib+AD3 (n=28)
Age,y	62.9±6.4	65.7±3.8	61.1±1.35
BMI, kg/m²	31.2±4.8	29.1±4.4	32.7±0.50
Menopause age, y	50.5±3.6	48.9±4.7	46.8±2.00
Menopause duration, y	12.4±4.3	16.8±5.9	14.4±3.08
Fractures in anamnesis, n (%) Hip Wrist Spine Humerus	 1(6.3) 1(6.3)	 1(4.0) 1(4.0)	 1(3.6) 1(3.6) 1(3.6)
Fractures in relatives, n (%)	2(12.5)	2(8.0)	3(10.7)
Menopause before 45 y, n (%)	1(6.3)	3(12.0)	5(17.9)

Patients who had previous fractures were 2 women in Group Ib (fractures of wrist and humerus), 3 women in Group Ib+AD3 (hip, vertebra, and humerus), and 2 women in the control group (hip and wrist). Patients who had a family history of fractures were 2 (8.0% women in Group Ib, 3 (10.7%) women in Group Ib+AD3, and 2 (12.5%) women in the control group. Onset of menopause before the age of 45 years had occurred in 3 (12.0%), 5 (17.9%) and 1 (6.3%) women of Group Ib, Group Ib+AD3 and the control group, respectively.

Basal LS-BMD and PF-BMD equaled to 0.746±0.05 g/cm^2 (median of 0.741 g/cm^2; IQR 0.709-0.781) and 0.747±0.05 g/cm^2 (median of 0.746 g/cm^2; IQR 0.728-0.774), respectively.

Bone Mineral Density

An insignificant increase in BMD was noted in the group of women who used calcium supplements and vitamin D3. In this group, LS-BMD decreased in 8 (50%) women and increased only in 4 (25%) women. The average growth of BMD amounted to 0.24% (Fig.1). In the control group, PF-BMD did not change in 8 (50.0%) patients and an insignificant increase was seen in 7 (43.8%). The average growth amounted to 0.06% (Fig.2).

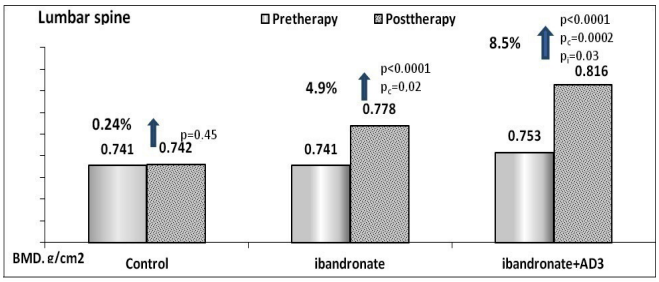

Fig. 1. *BMD-LS at the beginning and end of 6-month therapy (DEXA results).*

p – compared to the parameter before therapy, p_c –compared to the control group, p_i – compared to the ibandronate group after therapy.

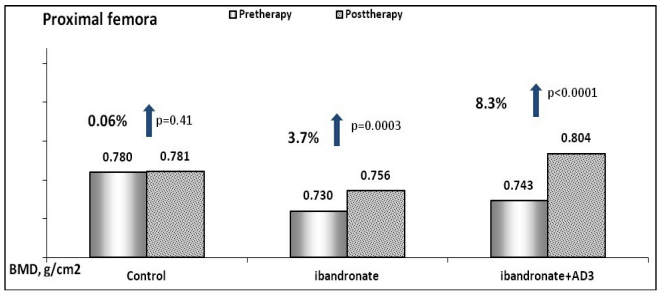

Fig. 2. *BMD-PF at the beginning and end of 6-month therapy (DEXA results).*

p – compared to the parameter before therapy

In Group Ib, a significant increase in LS-BMD and PF-BMD was found. However, we observed a decrease in LS- and PF-BMD in 3 (12.0%) and 4 (16.0%), respectively. Growth of BMD was significantly higher in Group Ib+AD3 compared to Group Ib and the control group; a slight decrease in LS-BMD was seen in 2 patients of Group Ib+AD3.

Biochemical Markers of Bone Turnover

An assessment of CTX dynamics showed a notable decrease in CTX level in patients of both groups compared with levels before treatment (Fig.3). Generally, PTH level decreased insignificantly, but a more pronounced reduction was seen in Group Ib+AD3. TP1NP level significantly increased in Group Ib (30.2%) and was more pronounced in Group Ib+AD3 (52.2%).

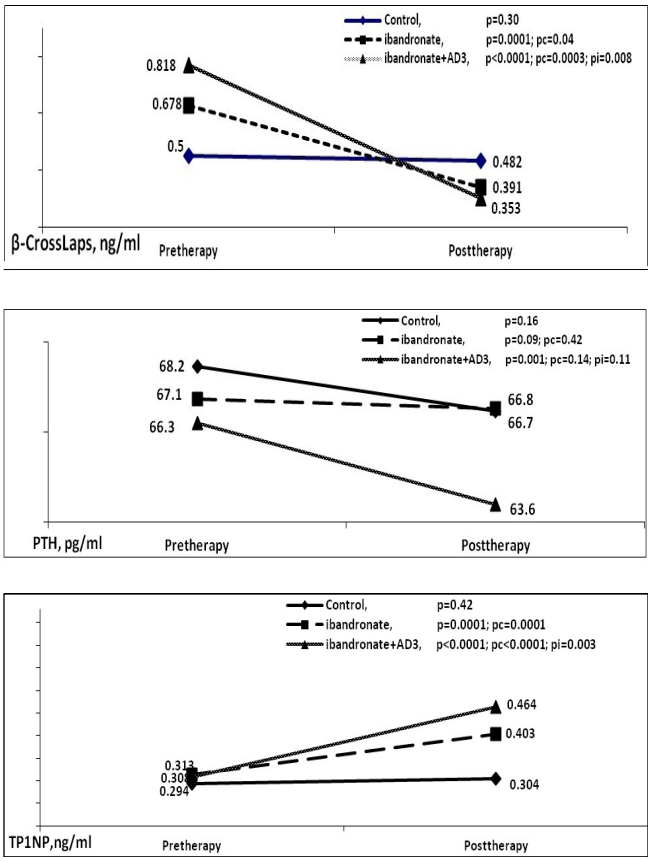

Fig. 3. *Serum markers of bone turnover*

p – compared to the parameter before therapy, p_c – compared to the control, p_i – compared to the ibandronate group after therapy

Laboratory investigations demonstrated normal parameters for calcium–phosphorus metabolism (Ca, P and alkaline phosphatase) in all groups without significant intergroup differences. Levels of calcium and phosphorus as well as alkaline phosphatase activity were in the reference limits, not changing significantly in both groups.

Safety

A flu-like syndrome was observed in 9 women (4 patients in Group Ib and 5 patients in Group Ib+AD3) during study. The intensity of the syndrome was light to moderate, resolved spontaneously or after administration of antipyretics, and did not require discontinuation of therapy.

Discussion

Currently, ibandronate is one of the most in-demand drugs with proven ability to reduce the risk of vertebral

fractures and a dose-dependent activity in relation to peripheral fractures. Ibandronate has been studied in clinical trials involving nearly 9,000 patients. The results of evaluation of ibandronate efficacy in osteoporosis were reported in large randomized studies: BONE, [17] MOPS,[18] MOBILE.[15]

In BONE study, both daily and monthly administration of ibandronate led to a significant increase of vertebral BMD (to 5.4 and 4.4% - North American population, and to 7.1 and 6.3 % - European population) compared to its baseline values. Growth of PF-BMD by 2.6% and 3.7% was seen with daily intake and by 2.5% and 3.1% with monthly intake for North American and European populations of patients.[17] During a 2-year (MOBILE study), LS-BMD of patients receiving ibandronate 150 mg once monthly increased by 7.6% (from baseline), and it increased by 6.4% from baseline in patients receiving 100 mg per month. After 3 years of treatment, patients in both groups showed an increase in PF-BMD as compared with the original mineral density (3.4% - 100 mg and 4.1% - 150 mg).[15]

In our study, within 6 months PMO women received ibandronate 150 mg once monthly, as well as the same dose of ibandronate plus Alpha-D3 1mg/day. Significant improvements of LS-BMD by 4.9% and PF-BMD by 3.7% were observed on the background of ibandronate monotherapy. In combination therapy of ibandronate with Alpha-D3, an increase in LS-BMD and PF-BMD comprised 8.5% and 8.3%, respectively. A decrease in LS-BMD and PF-BMD was seen in 3 and 4 women, respectively, in Group Ib. At the same time, only 2 patients treated with Ib+AD3 demonstrated a slight decrease in LS-BMD.

Two opposed processes, bone formation and bone resorption, characterize bone metabolism. The type 1 collagen degradation product CTX is a parameter characterizing the degree of bone tissue resorption, while TP1NP is a marker of bone matrix formation. Increases in CTX are believed to be the primary and most sensitive parameter of the shift in balance from bone remodeling towards bone resorption.[19]

Study of markers of bone metabolism showed that CTX level decreased significantly in the treatment with ibandronate, and an even greater reduction resulted from the combination therapy. Effects of ibandronate were also associated with a significant increase in TP1NP level, more pronounced in Group Ib+AD3.

Bisphosphonates reduce the number and activity of osteoclasts, induce their apoptosis and inhibit enzymes of the mevalonate pathway, which ultimately results in reduced bone resorption. However, D-hormone deficiency may prevent the bisphosphonates from exerting their antiresorptive effects. D-hormone deficiency may arise from impaired vitamin D conversion into D-hormone and from primary (nutritional) shortage of calcium and vitamin D. As compared with bisphosphonates, active metabolites of vitamin D posses more diverse actions, which have a complementary effect: they reduce osteoclast genesis, stimulate differentiation of preosteoblasts and bone formation (anabolic effect), and also have metabolic effects that appear as reduced levels of PTH and pro-inflammatory cytokines.[20] The addition of alfacalcidol to the bisphosphonates may not only contribute to the effectiveness of therapy, but can also solve such a clinical problem as resistance to bisphosphonates, which sometimes is as high as 83%.[21]

PTH is a key regulator of calcium–phosphorus metabolism. It is synthesized by the parathyroid glands in response to a reduction in extracellular calcium concentrations, and it activates osteoclasts to increase bone resorption resorption (demineralization, bone destruction); as a result, calcium and phosphorus are released into the circulation. Increases in PTH levels, particularly in elderly individuals with vitamin D3 deficiency, can result in osteomalacia, increased bone remodeling, bone mass reduction, and fractures.[22] Reduction of PTH levels in our study was observed in both treatment groups with more significant reduction in combined therapy.

Conclusion

Thus, combined therapy with ibandronate sodium and the D-hormone analog alfacalcidol augments the effectiveness of treatment observed in ibandronate sodium monotherapy in PMO women.

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Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Hepatology

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Hepatocellular Carcinoma in Patients with Chronic Hepatitis C

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Abstract

The purpose of the study was to examine the clinical and epidemiological data in patients with chronic hepatitis C (CHC) and hepatocellular carcinoma (HCC) before they sought specialized medical care.

The study included 92 patients with CHC. All patients were divided into 2 groups: Group 1 consisted of CHC patients with HCC (n=45), and Group 2 (n=47) consisted of CHC patients without HCC.

With the development of HCC in CHC patients, clinical manifestations were absent only in 2.2% of patients. Determining factors in HCC development are male sex, mature age, the maintained HCV replication, moderate and severe fibrosis, disease duration of more than 10 years, and the lack of effect of antiviral treatment. (**Int J Biomed. 2016;6(3):195-198.)**.

Key Words: chronic hepatitis C • hepatocellular carcinoma • risk factors • clinical manifestations.

Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide.[1] In most countries, HCC accounts for 70%–85% of primary liver cancer cases,[2] with the burden of disease expected to increase in coming years.[3] HCC causes ~600,000 deaths worldwide per year.[1]

HCC is a complex disease associated with many risk factors and cofactors.[4,5] Most cases of HCC are secondary to chronic infection with HBV or HCV. About 10% to 25% of HCC cases worldwide are thought to be a result of HCV infection.[6,7]

There is a need for further investigation of the clinical and pharmacological aspects of this disease. Algorithms for HCC treatment depend on the stage of the disease at diagnosis and the availability of complex therapies.[8] However, the disease is incurable in advanced stages, when its management is very expensive and effective only in terms of quality adjusted life years (QALY).[9] According to Bolondi et al.[10] the cost per treatable HCC was $17,934 with a cost per life year saved of $112,993.

The rating and ranking of risk factors (RFs) for the formation of cirrhosis and primary liver cancer in patients with chronic hepatitis C (CHC) is a serious health problem. Its relevance is unquestionable and requires immediate development of organizational models of treatment and prevention of viral hepatitis and primary liver cancer, because this pathology is one of the threats to national security.

The purpose of the study was to examine the clinical and epidemiological data in patients with CHC and HCC before they sought specialized medical care.

Materials and Methods

Diagnosis was established based on the clinical-laboratory data as well as the results of the PCR (RNA-HCV) and IEA (anti-HCV). The expression of the hepatic cytolysis syndrome was determined according to the International Classification (Los-Angeles, 1994). Viral loading and the genotype of the C virus were defined by PCR. A percutaneous liver biopsy was performed to confirm HCC diagnosis and to grade and stage histological disease. Liver biopsies that were at least 1.5 cm in length and had 3-5 portal tracts were considered as informative. Coded histological sections of liver biopsies were scored independently by two different histopathologists using the Knodell Histology Activity Index (HAI)[11] and Metavir system.[12] A consensus score was calculated after discussion on the points of differences for comparison of various classification and statistical calculations. Patients with PCC were distributed in stages of the disease according to AJCC TNM 6th edition.[13]

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The study included 92 patients with CHC. All patients were divided into 2 groups: Group 1 consisted of CHC patients with HCC (n=45), and Group 2 (n=47) consisted of CHC patients without HCC (Table 1).

Table 1.

Baseline characteristics of patients

Variable		Group 1 n=45	Group 2 n=47	*P*
Male, n(%)		29 (64.4)	26 (55.3)	*0.3723*
Female, n(%)		16 (35.6)	21 (44.7)	
Age, y		56.0±2.5	32.1±2.1	*0.0000*
Weight, kg		66.1±3.2	72.8±2.9	*0.1237*
Duration of disease, y		17.5±2.7	7.9±2.1	*0.0059*
HCV genotype, n(%)	1b	33 (73.3)	27 (57.4)	*0.1097*
	3a	7 (15.6)	12 (25.5)	*0.2374*
	2	5 (11.1)	8 (17.0)	*0.4158*
METAVIR stage F3		19 (42.2)	6 (12.8)	*0.0015*

In accordance with the purpose of the study, we designed a specific observation card, which included demographic data, duration of HCV infection, routes of HCV transmission, alcohol consumption, smoking status, and the results of physical examination and laboratory/instrumental investigations.

The study was approved by the local Ethics Committee. Written informed consent was obtained from each patient.

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean ± SEM for continuous variables. The Mann-Whitney (U Test) was used to compare the differences between groups. Group comparisons with respect to categorical variables are performed using chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Among CHC patients with HCC, 29(64.4%) were male and 16(35.6%) female. The average age of patients at the first examination ranged from 40 to 72 years (mean age 56.0±2.5 years), with no significant differences between men and women.

A history of icteric forms of acute viral hepatitis (AVH) was found in 5(11.1%) patients. The presence of HCV infection RFs was found in 39(86.7%) patients. These patients were those with the following conditions: earlier acute surgical diseases, which were a reason for blood transfusion and surgical interventions (30.8%); a combination of RFs for infection (professional risk and blood transfusion) (12.8%); elective surgical operation and parenteral manipulations in hospital (28.2%); occupational exposure to blood (12.8%); drug use by injection (10.3%); and tattoos (5.1%).

The duration of HCV infection was determined in 42 patients of Group 1: up to 10 years in 14.3% of patients, from 10 to 15 years in 33.3% of patients, and more than 15 years in 52.4% of patients.

In Group 1, the characteristic clinical signs of the disease (by the frequency of their appearance) were asthenovegetative syndrome(fatigue, irritability, sleep disturbances, a decreased performance, and general weakness, malaise, mood instability, headache), dyspeptic syndrome(loss of appetite, abdominal discomfort, nausea, bloating, belching and vomiting in some patients), and dull aching pain in the epigastric zone or right upper quadrant. Almost all patients of Group 1 had complaints (Table 2); the difference was only in the degree of the severity and various combinations of the complaints.

Table 2.

Clinical syndromes and symptoms in patients of both groups

Clinical manifestations	Group 1 n (%)	Group 2 n (%)	*P*
Asthenovegetative syndrome	44 (97.8)	28 (59.6)	*0.000*
Dyspeptic syndrome	31 (68.9)	27 (57.4)	*0.2557*
Hepatomegaly	37 (82.2)	29 (61.7)	*0.0289*
Splenomegaly	23 (51.1)	10 (21.3)	*0.0028*
Hemorrhagic syndrome	16 (35.6)	4 (8.5)	*0.0017*
Right hypochondrium syndrome	43 (95.6)	30 (63.8)	*0.0002*
Icteric skin/sclera	12 (26.7)	2 (4.3)	*0.0028*
Weight loss	27 (60.0)	2 (4.3)	*0.0000*
Arthralgia	28 (62.2)	12 (25.5)	*0.0004*
Myalgia	26 (57.8)	15 (31.9)	*0.0126*
Depression	19 (42.2)	6 (12.8)	*0.0015*
Skin rashes	5 (11.1)	2 (4.3)	*0.397**

**- Yates' p-value*

In Group 1, the first clinical manifestations of the disease in 36(80%) patients were the hepatic manifestations (hepatomegaly, jaundice, an enlarged spleen), whereas in Group 2, these manifestations were found only in 10(21.3%) patients. In early disease, extrahepatic manifestations were detected in 27(60%) patients in Group 1 and 12(25.5%) patients in Group 2. The combination of the hepatic and extrahepatic manifestations occurred in 29 (64.4%) patients in Group 1 and 9 (19.1%) patients in Group 2.The absence of the hepatic and extrahepatic manifestations was noted only in 3(6.7%) patients of Group 1 and 19(40.4%) patients of Group 2. Disease debut with hepatic manifestations was found in 21(74./2%) men and 7(43.7%) women, whereas disease debut with extrahepatic manifestations was in 8(50%) women and 6(20.6%) men ($p<0.001$).

In Group 1, the nonspecific complaints were also frequent: fatigue (weakness and decreased performance) (97.8%), headache (61.1%), heaviness (88.2%) and pain

in the right upper quadrant (47.2%), poor appetite(57.6%), epigastric pain (45.1%), nausea (42.4%), flatulence (61.1%), and abdominal discomfort (63.2%). In addition, itchy skin was found in 4 patients. Among the objective data from the physical examination, we found enlargement of the liver and spleen in 82.2% and 51.4% of patients, respectively; hemorrhagic syndrome in 35.6% of patients; and extrahepatic signs of liver disease as a palmar erythema and vascular "sprockets" in 68% of patients.

In general, the combination of CHC and HCC has no strictly specific clinical symptoms that can help to distinguish this concomitant pathology from other liver diseases. However, the comparative analysis shows the predominance of the asthenovegetative and hemorrhagic syndromes, hepatosplenomegaly, arthralgia, myalgia, depression, and weight loss in patients of Group 1. Clinical manifestations of the disease often did not meet the severity of liver injury; to evaluate the activity and prognosis, we used laboratory, instrumental, and morphological methods of diagnosis.

In Group 1, the degree of inflammatory activity by HAI was as follows: minimal (score 1-3) - 5(11.1%), mild (score 4-7) - 10(22.2%), moderate (score 8-12) - 14(31.1%), and marked (score 13-18) - 16(35.6%). Distribution of patients by METAVIR stage of liver fibrosis is shown in Fig. 1.

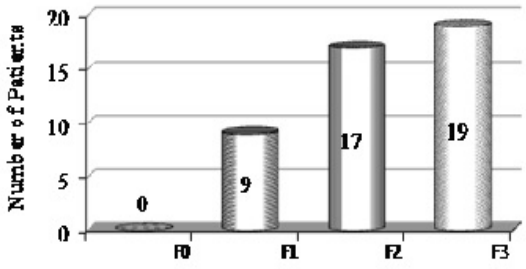

Fig. 1. *Distribution of patients by METAVIR stage of liver fibrosis*

The distribution of Group 1 patients according to the HCV genotypes was as follows: genotype 1b - 33(73.3%), genotype 3a - 7(15.6%), and genotype 2 - 5(11.1%). HCV RNA was detected in 41(91.1%) patients. The average viral load was $2.3x10^6$ IU/ml.

In Group 1, 18(40%) patients received the combined antiviral treatment: peginterferon-alpha2a plus ribavirin. However, antiviral treatment did not lead to a sustained virologic response. On the background of antiviral treatment, virological relapse and virological breakthrough were noted in 6 and 12 patients, respectively; 22 people refused to initiate antiviral treatment, and one patient did not receive antiviral treatment due to the presence of contraindications.

Indicators of alpha-fetoprotein (AFP) remained within the normal range (from 0.5 to 2.5 MoM) in 50% of patients. In 24% of patients, AFP increased from 5 to 30 MoM, and 26% of patients had AFP from 170 to 12000 MoM. According to MRI results, the left lobe of the liver was moderately enlarged in 40% of patient, the right lobe in 10% of patients, and both lobes in 10% of patients. The liver size within the age norm was found in 40% of patients. We found the following localization of the pathological process: S7-60%, S8-40%, and the combination of 3 or more segments in 30% of patients.

The presence of concomitant diseases was noted in 38(84.4%) patients in Group 1: gastrointestinal disease in 71% of cases, including 15(33.3%) patients with two or more comorbidities.

Conclusion

The clinical picture of CHC without HCC was low symptomatic, and clinical signs were absent in 36% of patients. With the development of HCC in CHC patients, clinical manifestations were absent only in 2.2% of patients. In some patients, the disease was diagnosed in connection with the "accidental" discovery of elevated levels of serum transaminases and/or detection of anti-HCV. Often, especially in women, the first clinical signs of the disease were extrahepatic signs. Determining factors in HCC development are male sex, mature age, the maintained HCV replication, moderate and severe fibrosis, disease duration of more than 10 years, and the lack of effect of AVT.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Obstetrics and Gynecology

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Influence of Perinatal Risk Factors on Premature Labor Outcome

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Abstract

In this article, for the first time, the problem of premature labor (PL) is considered from the standpoint of the concept of perinatal obstetric risk. The obtained results show that the optimal choice of the mode of delivery must be based on gestational age and perinatal risk factors with calculation of their intrapartum gain (IG). **(Int J Biomed. 2016;6(3):199-201.)**.

Key Words: premature labor • perinatal risk factors • perinatal mortality • Cesarean section.

Introduction

Each year, an estimated 15 million infants are born prematurely,[1] and this number is rising. Complications from preterm birth (PB) are the leading global cause of perinatal mortality (PNM) in developed countries. Prematurity takes the first place in the incidence of PNM: up to 60-70% of early neonatal mortality and 70-75% of infant mortality. Stillbirth at premature labor (PL) is 8 to 13 times higher than with labor at term.[2] According to WHO and UNICEF, the number of children under 5 years of age dying each year declined from more than 12.7 million in 1990 to 5.9 million in 2015,[3] but among children under 5 years of age, PB complications are the leading cause of death, responsible for nearly 1 million deaths in 2013.[4] Reducing the burden of PB requires effective maternal care including comprehensive obstetric care (with caesarean section, if needed). Questions about the delivery method in PL are relevant and debated.

In the Russian Federation, there are not clear guidelines and indications for a surgical delivery by Cesarean section (C-section) in PL. National guidelines in obstetrics published with the participation of leading specialists of the Russian Federation recommend individually determining the indications for abdominal delivery in PL.[5] There is no guidance on accounting for the degree of PR in choosing a method for early delivery in PL.[6]

The aim of this study was to improve perinatal outcomes of PL based on the assessment of PR factors and a differentiated selection of mode of delivery.

Materials and Methods

The study was performed in Municipal clinical hospital № 29 named after N.E. Bauman. We carried out a prospective analysis of 236 medical records of pregnant women with PL at 28 to 33 weeks (plus 6 days) of gestation (code ICD X O60). The study was conducted in accordance with ethical principles of the Declaration of Helsinki.

According to PL classification, all pregnant women were divided into 2 cohorts: Cohort I (gestational age from 28 to 30 weeks plus 6 days) and Cohort II (gestational age from 31 to 33 weeks plus 6 days). Depending on the amount of PR factors, each cohort was divided into 3 groups: a low PR, a moderate PR, and a high PR.

We identified the degree of PR based on the scale developed by O.G. Frolova and E.I. Nikolaeva (1981) and modified in 2003 by V.E. Radzinsky et al.[7] The scale includes a number of parameters divided into blocks: socio-biological block, data of obstetric and gynecological history, extragenital diseases (EGD) of mother, complications of pregnancy, and fetal assessment.

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A specific group for PR was defined in accordance with the number of points derived from S. Knyazev's scale (2003): low risk (<15 points), moderate risk (from 15 to 20 points), and high risk (≥ 25 points). Calculation of PR factors was performed twice: at admission and during labor. The ratio of these indexes determines the so-called "intrapartum gain" (IG) of PR factors.[6]

Inclusion criteria were singleton pregnancy, PB (gestational age from 28 to 33 weeks plus 6 days), and no treatment for cervical insufficiency.

Exclusion criteria were polycyesis, congenital malformations of the fetus revealed during currently pregnancy and after childbirth, induced PB, the use of assisted reproductive technologies, and the scar on the uterus.

In accordance with the purpose of the study, we designed a specific questionnaire for the clinical assessment of the state of health of the pregnant women.

Statistical analysis was performed using the statistical software «Primer of Biostat 4.0» and «STATISTICA 7». Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Comparative clinical and statistical analysis of the age and anthropometric indicators, social and marital status, and status of somatic and reproductive health of surveyed pregnant women showed that the two cohorts were comparable, according to analyzed parameters.

The average age of patients was 29.1±0.4 years. Overall, 126 (53.4%) of the women were aged between 21 and 30 years, and 72 (30.5%) between 31 and 35 years.

Analyzing the complications of the first trimester of gestation, we revealed the following features. It is well known that smoking increases the risk of PB.[8,9] Our study showed that 103 (43.6%) women were smokers. Moreover, the premature term was significantly higher in smokers ($P<0.05$). In both cohorts, we revealed moderate correlations between smoking and PB (r=0.32, $P<0.05$ and r=0.37, $P<0.05$). Pregnant women with a high PR were smokers more often than were pregnant women with low/moderate PR ($P<0.05$). We found a moderate direct correlation between the degree of PR and smoking (r=0.62, $P<0.05$).

The overall rate of extragenital diseases (EGD) was 100%. In Cohort II, the incidence of EGD was the highest in women with a high PR (8[33.3%]) compared to women with a moderate PR (6 [11.5%]) (*Yates' P=0.04998*) and a low PR (3 [9.4%]) (*Yates' P=0.05830*). Diseases of the genitourinary system (chronic cystitis, pyelonephritis, urolithiasis) were diagnosed in 48 (20.3%) women without significant differences between groups. Diseases of the genitourinary system were diagnosed in every third pregnant woman with moderate to high PR in Cohort II. There was a moderate correlation between the PR degree and diseases of the genitourinary system in Cohort II (r=0.43, $P<0.05$) and a less pronounced correlation for Cohort I (r=0.31, $P<0.05$).

Among 236 pregnant women, every ninth woman had obesity (25[10.6%]), and every third woman (76[32.2%]) was underweight. Underweight women were identified significantly more often in groups with a high PR.

The study of reproductive health showed that an inflammatory disease of the body of the uterus and the uterine appendages occurred in 43 (18.2%) pregnant women. In Cohort II, these diseases were diagnosed significantly more often in women with a high PR compared to women with moderate to low PR (7 [29/2%] versus 8 [9.5%]) (*Yates' P=0.03405*). Recurrent inflammatory diseases of the external genitalia and vagina occurred in 87 (36.9%) women. Around 44.3% and 58.3% of women (Cohort I and Cohort II, respectively) with a high PR had history of vulvovaginitis. In Cohort II, sexually transmitted infections (STIs) were diagnosed significantly more often in women with a high PR compared to women with low to moderate PR (8 [33.3%] vs. 11[13.1%], $P=0.04632$).

Evaluation of the parity status showed that the number of primigravida women was significantly higher in the low PR groups. Three out of four pregnant women with a high PR were multigravida and gave birth for the first time. In women of both cohorts with a low PR, the number of abortions was lower than in women with moderate to high PR (1.6±0.4 and 1.9±0.3, respectively). PL history in 15 (9.1%) pregnant women showed that almost half of them were in the high PR groups (46.7%).

The frequency of a complicated course of pregnancy was 100%. The first trimester and the beginning of the second trimester of gestation is an essential period for the further course of pregnancy and, therefore, affects the prognosis of the perinatal outcomes. This period was characterized by the threat of spontaneous abortion in the majority of patients (60.6%). Continuous threat of pregnancy termination, requiring repeated hospital treatment (the average rate of hospitalizations was 2.1±0.9), was diagnosed in 59 (25%) pregnant women; most of these women (43[72.9%]) had a high PR. Almost every second woman had one abortion in anamnesis; 78% and 47.6% of women had two or more abortions. In Cohort II, cervical insufficiency was found in 6 (25.0%) women with a high PR and 7/8.3% women with low to moderate PR (*Yates' P=0.06325*).

In the structure of the pathology of the amniotic fluid, oligohydramnios (45.3%) was predominant. Oligohydramnios was diagnosed 2 to 3 times more often in women with high to moderate PR compared to women with a low PR. The incidence of oligohydramnios significantly increased with gestational age (r=0.547, $P<0.01$). Polyhydramnios was found in 30(12.7%) women.

Preeclampsia was diagnosed in 43 (18.2%) women and was a frequent complication in pregnant women with a high PR in Cohort II: 13 (54.2%) vs. 2 (6.2%) pregnant women with a low PR ($P=0.0001$).

Anemia of pregnancy was diagnosed in 108 (48%) women without significant differences between cohorts and was more common with increasing gestational age.

PL was often associated with a premature rupture of membranes (63%) without an association with gestational age. Tocolytic therapy was administered to all pregnant women,

including an intrapartum tocolysis. The duration of tocolysis in Cohort I was significantly longer than in Cohort II (*P<0.05*), apparently motivated by the desire to prolong the pregnancy as much as possible.

Vaginal birth occurred in 154 (65.3%) women. C-section was performed in 82 (34.7%) patients: 16 (19.5%) women with a low PR, 32 (39.0%) – a moderate PR and 34 (41.5%) – a high PR.

Fetometry showed that intrauterine growth retardation (IUGR) was diagnosed in 71(30.1%) patients. The main share of women with IUGR was presented by those with a high PR (38.0%) in Cohort I and those with a moderate PR (23.9%) in Cohort II. Diagnosis of IUGR was confirmed in only 16(22.5%) newborns (Table 1), and every second newborn was born to a mother of Cohort II with a high PR.

Table 1.

Analysis of IUGR frequency

Cohort	Degree of PR	Women diagnosed with IUGR		IUGR infants	
		n(%)	Statistics	n(%)	Statistics
I	Low (n=20)	3(15.0)	χ^2=0.397 *P=0.81996*	1(33.3)	Yates' χ^2=0.28 *P=0.86936*
	Moderate (n=38)	8(21.1)		2(25.0)	
	High (n=70)	12(17.1)		4(33.3)	
	n=128	23(18.0)*		7(30.4)	
II	Low (n=32)	17(53.1)	χ^2=2.178 *P=0.33655*	2(11.8)	Yates' χ^2=3.65 *P= 0.16098*
	Moderate (n=52)	23(44.2)		3(13.0)	
	High (n=24)	8(33.3)		4(50.0)	
	n=108	48(44.4)*		9(18.8)	
Total	236	71(30.1)		16(22.5)	

**- chi-square=19.521 and P=0.0000 between two cohorts*

Disorders in the uteroplacental and fetal-placental circulation were detected in 79 (33.5%) of pregnant women (2 and 3 degree in 16[20.2%] women). These disorders were most common in groups of women with a moderate PR.

Analysis of PNM showed that 8 (3.39%) newborns died, including 6 (75.0%) newborns born to mothers at 28 to 30 weeks (plus 6 days) of gestation. In Cohort 1, the vast majority of the deceased newborns (66.7%) were born to mothers with a moderate PR that was significantly more than in other groups (*P<0.05*).

In Cohort 2, two newborns died; they were born to mothers with moderate to high PR. These data confirm a known fact: PNM decreases with increasing gestational age.

A retrospective analysis of PNM according to the revised risk factors, taking into account their IG, showed another distribution of the analyzed indicators. After recalculation of PR factors with regard to IG, we found that the vast majority of deceased newborns (87.5%) were born to mothers with a high PR. In this regard, we have analyzed the critical threshold of IG for PR factors that affected the perinatal outcomes.

It was found that 6 of 8 deceased newborns were born vaginally and belonged to groups of low and moderate PRs. The decision for vaginal delivery was based only on PR factors, despite the fact that the women were patients with moderate and high PRs, according to IG. In general, if the situation with the choice of delivery method in pregnant women with a high PR is very clear, namely, a planned *en caul* (within intact membranes), C-section is the method of choice for delivery in PL at 28-33 weeks (plus 6 days) of gestation, the choice of mode of delivery in women with moderate to low PR is ambiguous.

After recalculation of PR factors with regard to their IG, all pregnant women (100%) with a low PR was transferred into moderate PR groups; every second pregnant woman (47[52.2%]) with a moderate PR was transferred into high PR groups. The remaining women (43[47.8%]) were still in moderate PR groups. Analysis of perinatal mortality and morbidity has demonstrated the presence of statistically significant differences (*P<0.05*) in the selection of the priority mode of delivery in women with PL at all stages of gestation, depending on PR. Thus, the perinatal mortality and morbidity rate was lower among pregnant women with a high PR who gave birth to premature babies delivered by C-section.

The optimal choice of method of delivery based on PR factors and IG is a resource for reducing perinatal mortality and morbidity. Risk strategy must involve a dynamic recalculation of PR factors, IG, in childbirth.

Competing interests

The authors declare that they have no competing interests.

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Neonatal Outcomes in the Postpartum Period depending on Perinatal Risk Factors, Terms and Mode of Delivery

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Abstract

The aim of this study was to evaluate neonatal outcomes of preterm birth in the postpartum period, depending on perinatal risk factors, terms, and mode of delivery. Regardless of the mode of delivery, more than 60% of the infants died in the early neonatal period. The main diseases in the early neonatal period were asphyxia, respiratory distress syndrome and intraventricular hemorrhage. **(Int J Biomed. 2016;6(3):202-206.)**.

Key Words: preterm birth • perinatal mortality • perinatal morbidity • Cesarean delivery.

Introduction

Preterm birth (PB) is a significant cause of infant and child morbidity and mortality. Human viability, defined as gestational age at which the chance of survival is 50%, is now approximately 23–24 weeks in developed countries.[1] Despite technological advances, the extremely premature infant born at 22 to 25 weeks of gestation and with extremely low birth weight (<1000 g) remains at high risk for death and disability. Infants born less than 34 weeks comprise almost 60% of infant deaths.[2] Recent studies have demonstrated little progress in reducing the mortality and morbidities associated with extremely PB.[3-5] Nearly one-half of newborn deaths occur during the first 24 hours after birth.[1] Approximately 80% of low-birth-weight infants require resuscitation and stabilization at delivery.[6,7]

Over the last generation, a dramatic decline in infant mortality has been associated with medical innovations in the management of neonates, particularly those born preterm. Initiation of rational intravenous fluid therapies, development of artificial airways and breathing circuits, application of mechanical ventilation and airway distending pressure, and development of a resuscitation scoring system, the Apgar score (AS), have become important factors in the effective resuscitation of the newborn.

The aim of this study was to evaluate neonatal outcomes of PB in the postpartum period, depending on perinatal risk (PR) factors, terms, and mode of delivery.

Materials and Methods

The study was performed in Municipal clinical hospital № 29 named after N.E. Bauman. We carried out a prospective analysis of 236 medical records of pregnant women with premature labor (PL) at 28 to 33 weeks(plus 6 days) of gestation (code ICD X O60) and 236 infants born alive. The study was conducted in accordance with ethical principles of the Declaration of Helsinki.

According to PL classification, all pregnant women were divided into 2 cohorts: Cohort I (gestational age from 28 to 30 weeks plus 6 days) and Cohort II (gestational age from 31 to 33 weeks plus 6 days). Depending on the amount of PR factors, each cohort was divided into 3 groups: a low PR, a moderate PR, and a high PR.

We identified the degree of PR based on the scale developed by O.G. Frolova and E.I. Nikolaeva (1981) and

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modified in 2003 by V.E. Radzinsky et al.[8] A specific group for PR was defined in accordance with the number of points derived from S. Knyazev's scale (2003): low risk (< 15points), moderate risk (from 15 to 20 points), and high risk (≥ 25 points). Calculation of PR factors was performed twice: at admission and during labor. The ratio of these indexes determines the so-called "intrapartum gain" (IG) of PR factors.[9]

Inclusion criteria were singleton pregnancy, PB (gestational age from 28 to 33 weeks plus 6 days), and no treatment for cervical insufficiency (CI).

Exclusion criteria were polycyesis, congenital malformations of the fetus revealed during currently pregnancy and after childbirth, induced PB, the use of assisted reproductive technologies, and the scar on the uterus.

Statistical analysis was performed using the statistical software «STATISTICA 7». Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction. Multiple comparisons were performed with one-way ANOVA and post-hoc Tukey HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Neonatal anthropometric parameters of newborns are shown in Table 1. Evaluation of AS in the first and fifth minutes of life has revealed statistically significant differences between groups ($P<0.05$). In both cohorts of women with a low PR, and with a moderate PR in Cohort I, first- and fifth-minute ASs was significantly lower compared to newborns born to women with a high PR ($P<0.05$) (Table 2). Further analysis of the reasons for this paradoxical situation has shown that a low AS in newborns born to mothers with low to moderate PR was due to the fact that infants born to mothers of these groups died in the early neonatal period.

Table 1.

Anthropometric parameters of newborns

Cohort	Degree of PR	n	Birth weight (g)	Body length (cm)	HC (cm)	ChC (cm)
I	L (1)	20	1398.2±311.9	37.6±3.1	29.3±1.9	26.8±2.0
	M (2)	38	1462±363.1	38.2±3.3	28.0±1.3	24.0±1.9
	H (3)	70	1315.4±239.5	36.9±1.8	27.3±1.2	24.0±1.1
	Statistics	ANOVA Tukey HSD test	*P=0.0146* P_{1-2}=0.6260 P_{1-3}=0.3940 P_{2-3}=0.0118	*P=0.0404* P_{1-2}=0.6704 P_{1-3}=0.5250 P_{2-3}=0.0331	*P=0.0000* P_{1-2}=0.0021 P_{1-3}=0.0000 P_{2-3}=0.0312	*P=0.0000* P_{1-2}=0.0000 P_{1-3}=0.0000 P_{2-3}=0.9948
II	L (1)	32	1881.7±350.4	43.7±3.9	30.2±2.2	27.2±2.6
	M (2)	52	1907.3±337.5	42.3±2.3	31.9±1.8	29.4±1.7
	H (3)	24	1850.9±543.0	41.1±2.9	31.2±1.8	28.6±2.2
	Statistics	ANOVA Tukey HSD test	*P=0.8425* P_{1-2}=0.9552 P_{1-3}=0.9551 P_{2-3}=0.8320	*P=0.0062* P_{1-2}=0.0971 P_{1-3}=0.0047 P_{2-3}=0.2377	*P=0.0007* P_{1-2}=0.0004 P_{1-3}=0.1375 P_{2-3}=0.3084	*P=0.0001* P_{1-2}=0.0000 P_{1-3}=0.0413 P_{2-3}=0.2788

L- low, M- moderate, H- high, HC- head circumference, ChC- chest circumference.

Table 2.

Evaluation of AS in the first and fifth minutes of life

Cohort	Degree of PR	n	AS	
			the first minute of life	the fifth minute of life
I	Low (1)	20	4.6±1.8	6.1±1.4
	Moderate (2)	38	4.6±1.7	6.1±1.4
	High (3)	70	6.7±1.0	7.2±0.8
	Statistics	ANOVA Tukey HSD test	*P=0.0000* P_{1-2}=0.9948 P_{1-3}=0.0000 P_{2-3}=0.0000	*P=0.0000* P_{1-2}=0. 9948 P_{1-3}=0.0004 P_{2-3}=0.0000
II	Low (1)	32	5.6±1.7	6.7±1.1
	Moderate (2)	52	7.1±0.7	7.7±0.5
	High (3)	24	6.7±0.9	7.4±0.7
	Statistics	ANOVA Tukey HSD test	*P=0.0000* P_{1-2}=0.0000 P_{1-3}=0.0013 P_{2-3}=0.3247	*P=0.0000* P_{1-2}=0.0000 P_{1-3}=0.0028 P_{2-3}=0.2553

In each group of mothers, preterm infants had two to three neonatal diseases. Despite the prevention of respiratory distress syndrome (RDS), the frequency of apnea and respiratory failure was high (92.0% - 95.0%) in infants with a gestational age of 28 to 30 weeks (plus 6 days) without statistically significant differences, depending on the degree of PR (Figure 1).

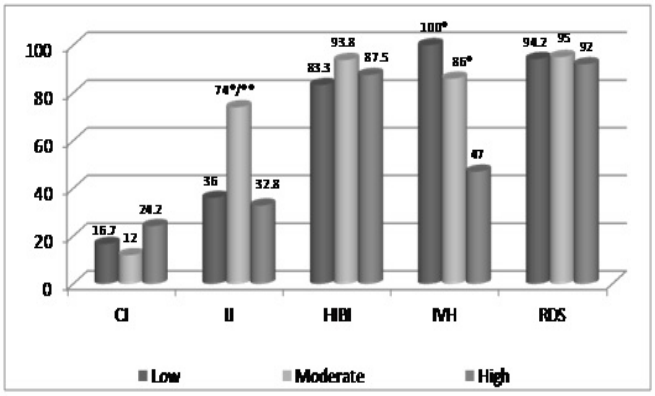

Fig. 1. *Perinatal morbidity (%) in Cohort 1 (vaginal delivery)*

**, **- P<0.05 compared with a high PR and a low PR, respectively*

A different situation was observed in newborns with intraventricular hemorrhage (IVH). IVH was diagnosed significantly more often in infants born to mothers with low and moderate PR (100% and 86%, respectively), whereas only 4.2% of infants born to mothers with a high PR had IVH. Hypoxic-ischemic brain injury (HIBI) was found with equal frequency in all groups of Cohort I. Intrauterine pneumonia (IP) was diagnosed significantly more often (75%) in infants born to mothers of Cohort I with a moderate PR compared

to infants born to mothers with low and high PR (every third newborn) ($p<0.05$). In Cohort II, IVH, HIBI and RDS were dominant in the structure of neonatal morbidity. IVH was diagnosed significantly more often (*P<0.05*) in infants born to mothers with low and moderate PR (78.0% and 58.5%) (Figure 2).

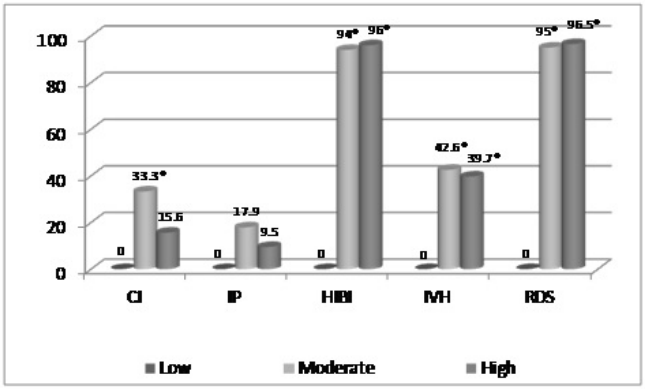

Fig. 2. *Perinatal morbidity (%) in Cohort 1 (*Cesarean delivery)

** - P<0.05 compared with a low PR*

A comparison between the two cohorts showed that a statistically significant reduction in the RDS frequency occurred only in newborns of Cohort II to mothers with a high PR (*P<0.05*). In other groups, despite a longer period of gestation, we did not find a significant reduction in the RDS frequency.

There were no significant differences in IVH rate depending on the term of delivery. With regard to the term of delivery, statistically significant differences were found for IP. In Cohort II, the incidence of IP was significantly lower in all analyzed groups compared with Cohort I (*P<0.05*).

We evaluated the frequency of perinatal morbidity depending on the mode of delivery. The frequency of IVH with a Cesarean delivery decreased 2.3 and 2.5 times in the groups with moderate and high PRs, respectively. We found no significant differences (Figure 3, 4) between groups in such diseases as HIBI, IP and conjugated jaundice.

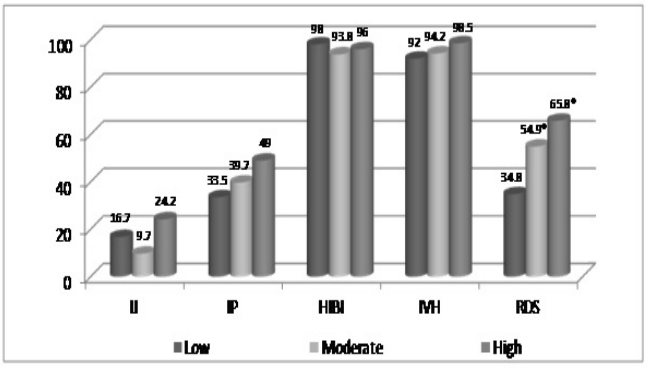

Fig. 3. *Perinatal morbidity (%) in Cohort 2 (vaginal* delivery)

** - P<0.05 compared with a low PR*

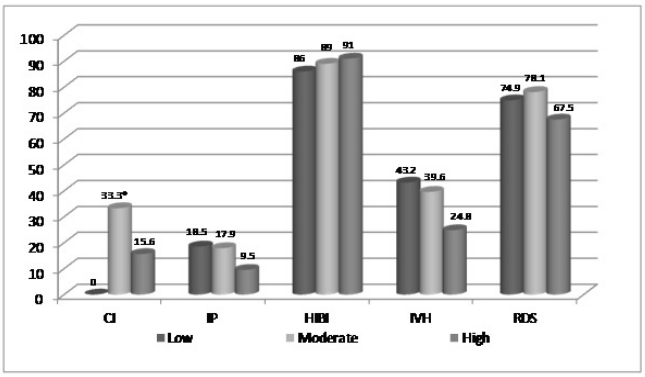

Fig. 4. *Perinatal morbidity (%) in Cohort 2 (*Cesarean delivery)

** - P<0.05 compared with a low PR*

Evaluation of the duration of mechanical ventilation (MV) showed (Table 3) that the most long-term MVs were in infants born to mothers of Cohort I with low to moderate PR that was associated with a significantly higher incidence of intrauterine growth retardation. The MV duration was in 3-4 times lower in infants born by Cesarean delivery. In both cohorts, the infants born to mothers with moderate to low PR were transferred to the second stage of nursing and treated significantly later in the postnatal period (Table 4) because they were significantly longer on MV.

Table 3.

Duration of MV and CPAP

Cohort	Degree of PR	n	MV, min	CPAP, min
I	Low (1)	20	260.7±462.1	103.1±112.0
	Moderate (2)	38	212.1±480.1	68.0±63.4
	High (3)	70	72.2±84.6	79.9±76.7
	Statistics	ANOVA Tukey HSD test	*P=0.0235* P_{1-2}*=0.8498* P_{1-3}*=0.0597* P_{2-3}*=0.0848*	*P=0.2837* P_{1-2}*=0.2515* P_{1-3}*=0.4860* P_{2-3}*=0.7393*
II	Low (1)	32	194.8±240.4	47.8±27.6
	Moderate (2)	52	164.2±29.8	38.5±18.6
	High (3)	24	41.5±33.4	67±73.9
	Statistics	ANOVA Tukey HSD test	*P=0.0001* P_{1-2}*=0.5643* P_{1-3}*=0.0001* P_{2-3}*=0.0009*	*P=0.0176* P_{1-2}*=0.5545* P_{1-3}*=0.1801* P_{2-3}*=0.0126*

Table 4.

Transfer time for second stage of nursing and treatment (age in days)

Cohort	Degree of PR	n	Transfer to specialized care for second stage of nursing (days)	ANOVA	Tukey HSD test
I	Low (1)	20	4.0±1.9	*P=0.0002*	P_{1-2}*=0.5855* P_{1-3}*=0.0012* P_{2-3}*=0.0049*
	Moderate (2)	38	5.1±3.9		
	High (3)	70	7.7±4.5		
II	Low (1)	32	3.2±2.7	*P=0.0006*	P_{1-2}*=0.0088* P_{1-3}*=0.0008* P_{2-3}*=0.3576*
	Moderate (2)	52	4.8±2.5		
	High (3)	24	5.6±1.3		

Analysis of PNM showed that 8 (3.39%) newborns died, including 6 (75.0%) newborns born to mothers at 28 to 30 weeks (plus 6 days) of gestation. In Cohort I, the vast majority of the deceased newborns (66.7%) were born to mothers with a moderate PR that was significantly more than in other groups ($P<0.05$). In Cohort II, two newborns died; they were born to mothers with moderate to high PR (Table 5). These data confirm a known fact: PNM decreases with increasing gestational age.

Table 5.

PNM and degree of PR

Cohort		I (n=128)						II (n=108)					
Degree of PR		Low (n=20)		Moderate (n=38)		High (n=70)		Low (n=32)		Moderate (n=52)		High (n=24)	
Number of infant (<30 days) deaths	n	abs	%	abs	%	abs	%	abs	%	abs	%	abs	%
	8	1	12.5	4	50	1	12.5	0	0	1	12.5	1	12.5

A retrospective analysis of PNM according to the revised risk factors, taking into account their IG, showed another distribution of the analyzed indicators (Table 6). After recalculation of PR factors with regard to IG, we found that the vast majority of deceased newborns (87.5%) were born to mothers with a high PR. In this regard, we have analyzed the critical threshold of IG for PR factors that affected the perinatal outcomes. It was found that 6 of 8 deceased newborns were born vaginally and belonged to groups of low and moderate PRs. The decision for vaginal delivery was based only on PR factors, despite the fact that the women were patients with moderate and high PRs, according to IG. In general, if the situation with the choice of delivery method in pregnant women with a high PR is very clear, namely, a planned en caul (within intact membranes), C-section is the method of choice for delivery in PL at 28-33 weeks (plus 6 days) of gestation, the choice of mode of delivery in women with moderate to low PR is ambiguous.

Table 6.

PNM according to the revised PR factors, taking into account IG

Cohort		I				II			
Degree of PR		Moderate (n=58)		High (n=70)		Moderate (n=84)		High (n=24)	
Number of infant (< 30 days) deaths	n	abs	%	abs	%	abs	%	abs	%
	8	1	12.5	5	62.5	0	0	2	25

Clinical diagnoses of the preterm infants who died

Vaginal delivery

1. Severe RDS, respiratory failure III. First- and fifth-minute ASs of 3-6. Neonatal resuscitation. Rapid labor (3hrs and 5 min). Newborn died at the age of one day from severe asphyxia and multiple organ failure. Histologically: placental lesions with inflammation (foci of necrosis, diffuse amnionitis, accumulation of inflammatory cells).

2. RDS, moderate asphyxia. First- and fifth-minute ASs of 5-6. Neonatal resuscitation. Waterless interval of 10 minutes, rapid labor (5hrs and 55 min) on the background of intrapartum tocolysis with Gynipral. Newborn died at the age of one day from severe asphyxia and multiple organ failure. Histologically: the expressed pathological villous immaturity

3. Hypoxic-ischemic brain injury (depressed fracture of the parietal bone, subdural hematoma, IVH III), bilateral intrauterine pneumonia (antibiotic-resistant Staphylococcus epidermidis), aplasia of thymus and adrenal glands. Skin hemorrhage, hemorrhagic anemia, multiorgan failure. First- and fifth-minute ASs of 5-6. Neonatal resuscitation. Waterless interval of 10 minutes, rapid labor (4hrs and 40 min). Newborn died at the age of 11 days. Histologically: the expressed pathological villous immaturity on the background of inflammation.

4. Severe RDS, respiratory failure III, fetal malnutrition III, IVH. First- and fifth-minute ASs of 3-6. Waterless interval of 18 hours. Neonatal resuscitation. Newborn died at the age of 2 days. Histologically: the expressed pathological villous immaturity on the background of inflammation.

5. Neonatal CNS depression, hemorrhagic disease, antibiotic-resistant E. coli infection, RDS. First- and fifth-minute ASAs of 6-7. Neonatal resuscitation. Rapid labor (3hrs and 35 min) with birth injuries. Newborn died at the age of 30 days. Histologically: the expressed pathological villous immaturity and inflammation.

6. IVH, RDS. intrauterine pneumonia. First- and fifth-minute ASs of 4-5. Neonatal resuscitation. Newborn died at the age of 4 days. Histologically: the expressed pathological immaturity of placental tissues on the background of inflammation.

Abdominal delivery

7. Acute hypoxia, IVH grade 2, RDS, respiratory failure II, HIDI, intrauterine infection. First- and fifth-minute ASs of 2-5. Neonatal resuscitation. Abdominal delivery due to the increasing severity of preeclampsia. Newborn died at the age of 15 days from IVC and bilateral intrauterine pneumonia complicated by DIC. Histologically: the expressed pathological immaturity of placental tissues on the background of inflammation.

8. Severe asphyxia, IVH, multiple organ failure, DIC, segmental pneumonia of the left lung. Emergency C-section due to bleeding placenta previa. First- and fifth-minute ASs of 3-5. Waterless interval of 35 minutes. Neonatal resuscitation. Newborn died at the age of 8 days. Histologically: the expressed pathological villous immaturity, placental insufficiency.

Thus, regardless of the mode of delivery, more than 60% of the infants died in the early neonatal period. The main diseases in the early neonatal period were asphyxia, RDS and IVH. Newborns with severe asphyxia and comorbidity (perinatal brain damage and intrauterine infection/ inflammation) died in the late neonatal period.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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New Trends in Management of Epilepsy in Patients with Cerebral Venous Malformations: Our Experience

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Abstract

Background: Venous vascular malformations, also known as venous angiomas or, more exactly, developmental venous anomalies (DVAs), represent congenital, anatomically variant pathways in the normal venous drainage of the brain area. In general neurological practice, DVAs are not considered epileptogenic, therefore, in conducting neuroimaging as a rule, not taken into account. A positive correlation, however, between the location of DVAs and the electroencephalographic seizure focus is debated.

Materials and Methods: The present study provides a complete analysis of clinical and MRI characteristics of symptomatic epilepsies associated with cerebral venous malformations in children and adults. Patients were selected after a retrospective search through the database of the University Clinic into which, since 2016, patients were prospectively entered. To date (February 2016), there is a total of 5,856 patients with epilepsy of which there are 105 patients with congenital malformations of the brain, and 32 of them were found to have principal diagnosis of DVA.

Results: Cavernous angiomas prevailed among venous anomalies (53.1%); DVAs were registered in 46.9% of cases. The associated analysis of DVA localization and the epileptic seizure types showed a direct relationship in 60.0% cases.

Conclusion: DVAs as a cause of seizures are important to consider when examining patients with epileptic seizures. **(Int J Biomed. 2016;6(3):207-212.)**.

Key Words: brain • developmental venous anomalies • cavernous malformation • epilepsy • management

Abbreviations

CM, cavernous malformation; **CT**, computed tomography; **CVM**, cerebral venous malformations; **DVA**s, developmental venous anomalies; **GRE**, gradient echo; **ILAE**, International League Against Epilepsy; **MRI**, magnetic resonance imaging; **SGTCS**, secondary generalized tonic-clonic seizures; **SWI**, susceptibility weighted imaging; **VEM**, video EEG monitoring.

Introduction

Cerebral venous malformations (CVMs), also known as venous angiomas or, more exactly, developmental venous anomalies (DVAs), represent congenital, anatomically variant pathways in the normal venous drainage of the brain area. They consist of converging dilated medullary veins that drain centripetally and radially into a transcerebral collector that opens either into the superficial subcortical or deep pial veins.[1]

DVAs have no proliferative potential, no direct arteriovenous shunts, and normal brain parenchyma between the dilated veins.[2] Once thought to be rare, they are now considered to be the most common vascular malformations in the central nervous system (CNS).[3,4] Although for many years DVAs were commonly called venous angiomas, the

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newer term DVA has been recommended as more appropriate because the involved vessels are not abnormally formed, but apparently merely dilated. The majority of DVAs are found occasionally and never cause symptoms, although there are isolated reports of patients with syndromes attributed to DVAs. For example, DVAs have been reported to cause epilepsy, progressive neurologic deficits, and haemorrhage.[5,6] Chronic, often undetected, microhemorrhages of these lesions result in iron deposition in adjacent brain tissue in the form of hemosiderin, and the iron in this perilesional hemosiderin is thought to play a major role in their epileptogenicity.[7,8] Frequently, convulsions have been associated with venous malformations.[9] A positive correlation, however, between the location of DVAs and the electroencephalographic seizure focus is unusual.

Contrast-enhanced CT, which is no doubt responsible for the recent increase in the number of reported cases of DVAs, is yielding to the far superior imaging ability of magnetic resonance as it becomes routinely available. MRI is thus becoming the primary study medium of choice and the means by which diagnosis of DVAs is verified.[10] Although the standard contrast-enhanced MRI is excellent in depicting DVAs, adjacent hemosiderin from associated cavernomas may not be assessed without the use of gradient-echo or echo-planar imaging, especially with fast spin-echo techniques. On a contrast-enhanced MRI, the cluster of veins in developmental venous anomalies has a spoke-wheel appearance; the veins are small at the periphery and gradually enlarge as they approach a central draining vein.[11] GRE or T2* sequences are able to delineate these lesions better than T1 or T2 weighted images. In patients with familial or multiple cavernous angiomas, GRE T2* sequences are very important in identifying the number of lesions missed by conventional spin echo sequences. SWI may have sensitivity equal to that of GRE in detecting these capillary telangiectasias in the brain. SWI is also highly sensitive in detecting calcification as compared to T1 and T2 images.[12]

Epilepsy associated with cavernous angioma of the brain is widely recognized. However, in general neurological practice, DVAs are not considered epileptogenic: therefore, in conducting neuroimaging, as a rule they are not taken into account.

Materials and Methods

We analyzed clinical and MRI characteristics of symptomatic epilepsies associated with CVMs in children and adults. Patients were selected after a retrospective search through the database (October 2008 – February 2016) of the Neurological Center of Epileptology, Neurogenetics and Brain Research of the University Clinic into which, since 2016, patients were prospectively entered. To date (February 2016), there is a total of 5,856 patients with epilepsy of which there are 105 patients with congenital malformation of the brain, and 32 of them were found to have principal diagnosis of DVAs. We analyzed epidemiological variables such as age, gender, associated risk factors, clinical presentation, radiological data, treatment options, and follow-up. It was performed as a part of complex research No 210-16 «Epidemiological, genetic and neurophysiological aspects of nervous system disorders (central, peripheral, autonomic) and preventive medicine» (state registration No 0120.0807480).[13,14] The present study was approved by the Ethics Committee of Krasnoyarsk State Medical University. Written informed consent was obtained from each patient.

From 2008 to 2016, we included 32 patients with CVMs and symptomatic epilepsy in our study. All patients underwent preliminary anamnestic and clinical selection using stratified randomization. All of the participants were residents of the Siberian Federal District, and had certain diagnosis of symptomatic epilepsy.

Symptomatic epilepsy diagnosis in all patients enrolled in this study was verified using VEM along with carrying out stress tests. All patients underwent brain MRI (1.5 Tesla or higher), including GRE T2* and SWI sequences. Detailed analysis of case history for each patient included debut age, the type of epileptic seizures at debut, and the dynamics of the disease progression.

All statistical analyses were carried out using licensed software package SPSS, version 20.0 (USA). Categorical variables are presented using frequencies and percentages The data for variation indices with nonparametric distribution are presented with medians and quartiles (Me [P25; P75]).

Results

Symptomatic epilepsies associated with CVMs were registered in 17 (53.1%) male patients and in 15 (46.9%) female patients. The age of patients at the time of the survey varied between 4 and 71 years with median of 27.5 (17.5:41.5) years; there were 8 (25.0%) children and 24 (75.0%) adults. The epilepsy onset age varied between 0.4 and 67 years with a median of 9.5 (5:26.5) years. Peak onset of the epileptic seizures varied between 0 and 10 years (17 [53.1%]).The period from epilepsy onset to the brain MRI and DVA identification varied between 0 and 52 years with median of 5.5 (1:11) years.

Distribution of epilepsy seizure types was as follows: simple focal (partial) seizures in 18.8% of cases, complex focal (partial) seizures in 3.1% of cases, combined simple and complex focal seizures in 18.8%, SGTCS in 3.1%, combined simple focal seizures and SGTCS in 15.6%, both complex focal seizures and SGTCS in 21.3%, and simple and complex focal seizures with SGTCS in 25% of cases.

Analysis of pedigrees identified 13 (40.6%) patients with family members or close relatives presenting with congenital malformations and 5 (15.6%) with epileptic seizures.

According to VEM-results, the location of the epileptic discharges was principally presented by frontal lobe seizures (13 [40.6%]) and temporal lobe seizures (13 [40.6%]).

The associated analysis of all CVM localization (by MRI) and location of the epileptic activity (by VEM) showed a direct relationship in 12 (37.5%) cases, a partial relationship with one DVA in 6 (18.8%) cases, and no associations in 14 (43.8%) cases. The associated analysis of DVA localization and the epileptic seizure types showed a direct relationship in 9(60.0%) (Fig.1) cases and no association in 6(40.0%) patients. In the latter group of patients, focal cortical dysplasia could serve as an area of beginning epileptic seizures.

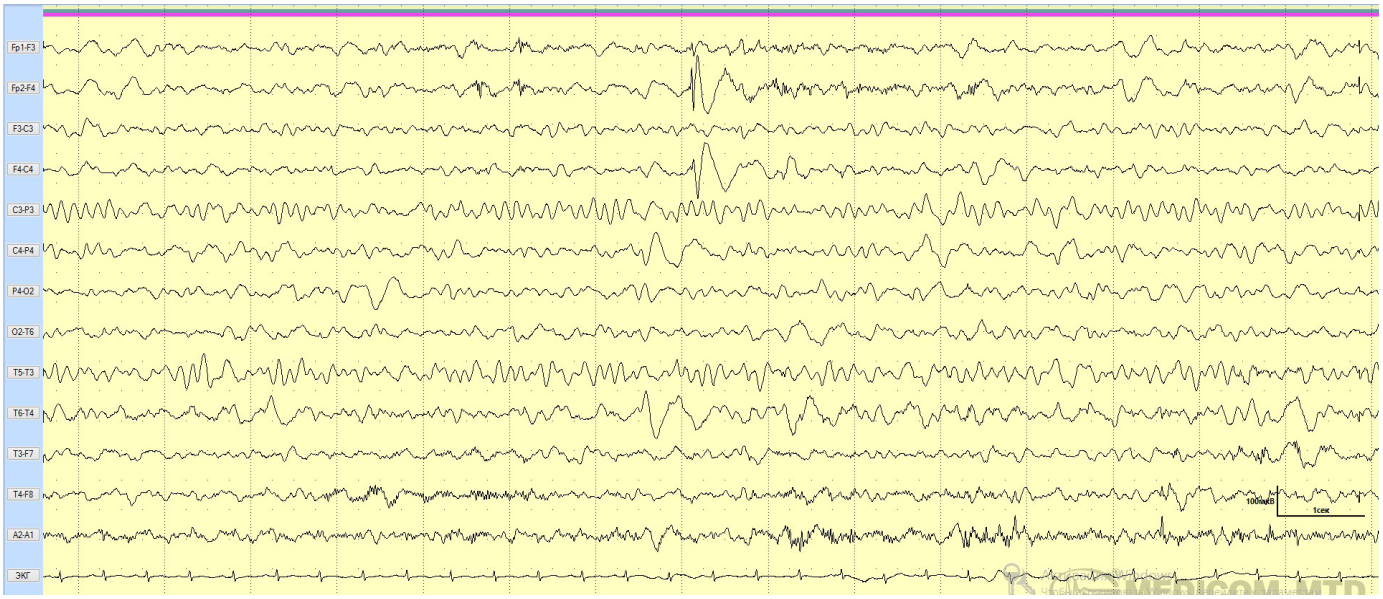


Fig. 1a. *EEG of a 7-year-old girl: regional epileptiform activity in the right frontal lobe.*

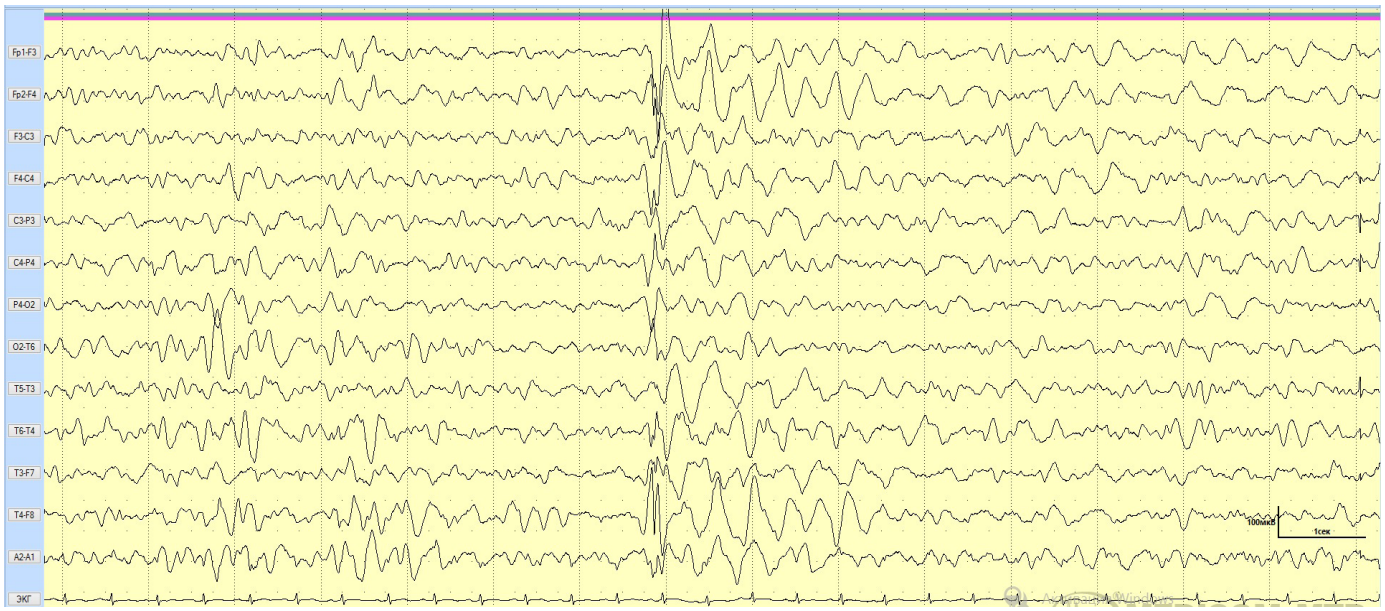
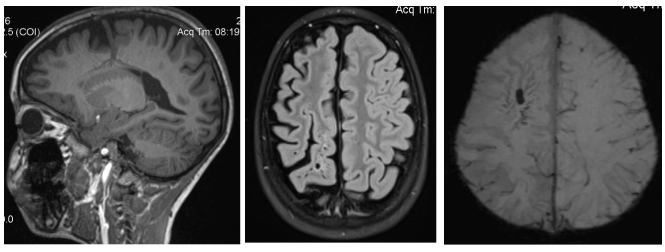


Fig. 1b. *EEG of a 7-year-old girl: regional epileptiform activity in the right frontal lobe with secondary bilateral synchrony.*



A B C

Fig. 2. *Brain MRI of a 7-year-old girl with symptomatic focal epilepsy associated with the isolated DVA.*

(A). T1 sagittal image: venous angioma of the right frontal lobe involving massive superficial vein.
(B). T2 FLAIR image: atrophy of the right frontal lobe.
(C). SWI: venous angioma of the right frontal lobe.

Venous anomalies were detected in hereditary diseases such as Von Hippel–Lindau disease in 13 (40.6%) patients, Sturge–Weber syndrome (encephalotrigeminal angiomatosis) in 2 (6.2%) cases, Gorlin-Goltz syndrome in 3.1% cases, and tuberous sclerosis complex in 3.1% cases. Cerebral venous anomalies were isolated in 17 (53%) cases (Fig. 2). Surgical treatment was recommended for focal epilepsy. Cavernous angiomas prevailed among venous anomalies (53.1%); DVAs were registered in 46.9% of cases.

Monotherapy was administered in 78.1% cases; prevailing medications were carbamazepine group drugs (13 [52.0%]). A drug-resistant epilepsy was observed only in 3(9.4%) cases of CVMs, including two cases of CM and one case of DVAs.

Discussion

DVAs are extreme variations of normal transmedullary veins that are necessary for the drainage of the white and gray matter.[15] Currently, due to progress in brain investigation, they can be diagnosed with a higher frequency.[16-20]

DVAs are the most commonly encountered vascular malformation in the CNS, accounting for up to 60% of them. Their prevalence is about 2.5% to 9%,[21] and they are usually solitary. DVA is a variation of normal venous drainage. It is considered to be formed during Padget's fourth to seventh stage of development.[22]

A typical venous angioma is composed of a large parent vein that receives an array of radially-oriented tributary veins in a spoke-wheel configuration which looks like «the caput medusa».[23]

It is generally accepted that DVAs are formed during intrauterine life,[24,25] but no consensus exists regarding the mechanism leading to their formation. Their etiology and mechanism of development are unknown, but it is currently accepted that they act like a compensatory system of cerebral parenchyma venous drainage due to early failure, abnormal development, or an intrauterine occlusion of normal capillaries or small transcerebral veins and thrombosis of normal parenchymal veins.[22] These drainage pathways may have developed as a method for maintaining the hemodynamic equilibrium of the transcortical venous drainage.

During embryogenesis, occlusion or maldevelopment occur during the formation of the medullary veins or their tributaries, and as a result, compensation DVA is formed. Thus, the main suggested etiology for DVA formation is an embryologic event that results in either arrested formation or thrombosis of the developing venous drainage of the specific region.[26-28] That is followed by a secondary compensatory mechanism in which embryologic medullary venules persist and cluster locally in a large draining vein.[27,29-31] These might occasionally develop as a result of dominant inheritance of a gene mutation in the short arm of chromosome 9.[32]

DVAs can either drain into deep subependymal veins and the galenic system or drain into superficial cortical veins. The superficial pattern is present in about 70%, while the deep drainage pattern is present in 20% of the population.[22,29,33] The remaining 10% have a combination of the superficial and deep drainage.[22,29,33] DVAs are mostly supratentorial and are found most frequently in the frontal lobe (36% to 56%) followed by the parietal (12% to 24%), occipital (4%) and the temporal lobes (2% to 19%); in the cerebellum (14% to 29%); in the basal ganglia (6%); in the thalamus and ventricles (11%), and in the brainstem (less than 5%).[22,29,33] DVAs may also be present adjacent to brain tumors, infarctions, demyelinating areas, and moyamoya malformations; also associated congenital anomalies of the cerebral arterial system, such as the primitive trigeminal artery, fetal origin of the posterior cerebral arterial system, as well as fetal venous anomalies, such as retention of the primitive facial, occipital, and marginal tentorial sinuses.[34]

The initial diagnosis is typically made in the third decade.[35,36] There is an equal prevalence in men and women. Cerebral venous angioma is usually asymptomatic and may be found occasionally at autopsy or by angiography.[21,37]

DVAs may present with headache, seizure, dizziness, and ataxia.[35,38-40] Prospective studies on venous angiomas have demonstrated a very low rate of both symptomatic hemorrhage (0.34% per year) and neurologic symptoms; bleeding, when it rarely occurs, has been hypothetically blamed on putative neighboring cavernous malformations.[41] Blood flow through venous angiomas is low, and they are thought to drain normally from the brain.

Symptoms can be produced either by venous congestion related to flow obstruction or mechanical compression (hydrocephalus or nerve compression). The clinical sequelae of DVA are likely related to the regional changes that occur near it.[42] They reported histopathologic evidence of vascular remodeling related to altered hemodynamics in the region of DVA, including microvascular wall hyalinization and calcification, which are consistent with chronic regional blood flow alternation and venous hypertension.[43]

Very few cases have been reported in which DVAs were located in the same area as the EEG focus of the seizure.[39] The incidence of seizures associated with symptomatic DVAs ranges from 8% to 29%.[35,44,45] In most of the cases, DVAs were located in a different region with respect to the focus of the seizure [9,45] or there was another lesion found that could be the cause of epilepsy.[38] DVAs have been reported to be associated usually with generalized seizures,[44,46] but some patients have experienced partial seizures,[44,47] complex partial seizures [46] or even Jacksonian march of motor seizures.[1]

Although cases of existence of DVAs and seizures have been reported, the correlation between them has not been firmly determined. [15,22,38,41,45] The study of Striano et al.[15] revealed that DVAs are rarely found in epileptic patients, as distinct from other vascular malformations, cavernomas in particular. Topographic and/or etiological relationships between DVA and epilepsy are still undefined. Similarly, seizures have been localized to areas not associated with DVA in several studies,[9,35] or to associated cortical dysplasias.[15]

DVAs may be associated with abnormal neuronal migration and possible susceptibility to epileptogenesis.[39] However, recent literature suggests that DVAs may be the cause of focal epilepsies in cases where no epileptogenic lesions can be detected.[48] Several mechanisms are postulated based on the following: (1) subclinical hemorrhage, more likely when a DVA is associated with a cavernous malformation [9] and (2) increased inflow or restricted outflow, resulting in intermittent cortical hyperemia and dysfunction creating an epileptic focus.[22]

Patients with DVAs associated with epilepsy require a precise analysis of the seizure pattern and EEG findings, because another epileptogenic lesion may be present which is surgically curable.[45]

MRI is the diagnostic method of choice, showing a starburst pattern of white matter veins converging on a large draining vein in the case of a venous malformation.[49] When cerebral DVAs present symptoms such as cerebral hemorrhage, epilepsy, headache, cranial nerve paresis, and/or cerebral ataxia, surgical intervention has been carried out.[37,50-53] Clinicians should be aware that, though generally benign, DVAs and their associated lesions may represent a complex entity

with potential for clinical complication requiring, in certain cases, additional imaging investigations and specific medical management.[54,55]

Conclusion

A cavernous angioma was found more frequently in our patients with SE. However, DVAs were diagnosed in 46.9 % of patients. Association localization of DVAs with localization-related epileptic seizures was observed in 60.0 % of cases.

Thus, DVAs as a cause of seizures are important to consider when examining patients with epileptic seizures. The inclusion of SWI in the protocol of neuroradiological studies has allowed us to improve the quality of diagnostic care for patients with symptomatic focal epilepsy in our clinic, and it was useful in the selection of surgical treatment for drug-resistant forms of epilepsy.

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Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Traumatology/Orthopedics

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Soluble Thrombomodulin and Major Orthopedic Surgery

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Abstract

Background: A high level of soluble thrombomodulin (sTM) is associated with a lower risk of thrombosis but can cause severe bleeding after operations. Deep vein thrombosis (DVT) and blood loss are serious threats after orthopedic surgery. The aim of our pilot study was to evaluate the effect of the preoperative level of sTM on coagulation and inflammation as well as the blood loss and the development of symptomatic DVT after total large joint replacement.

Methods and Results: In all patients (n=50) who underwent total hip or knee replacement, sTM, PrC, D-dimer, vWF, CRP, and platelets were determined before and after the operation. According to the preoperative sTM level, patients were divided into 2 groups: the thrombomodulin low (TML) group (n=25) and thrombomodulin high (TMH) group (n=25). The concentration of sTM was 4.4 [3.4, 4.7] ng/ml in the TML-group and 8.7[7.3, 10.6] ng/ml in the TMH-group. After surgery, D-dimer, vWF, platelet count and CRP were higher and total blood loss was lower in the TML group**.** In the TML-group, a symptomatic DVT was detected in 3(12%) patients; in the TMH-group, a symptomatic DVT was identified only in 1(4%) case.

Conclusion: These findings support the important role of sTM in coagulation, inflammation, bleeding, and presumably in venous thrombosis after major orthopedic surgery. **(Int J Biomed. 2016;6(3):213-217.)**.

Key Words: thrombomodulin • coagulation • bleeding • total hip and knee replacement.

Abbreviations

CRP, C-reactive protein; **DVT**, deep vein thrombosis; **IQR**, interquartile range; **PrC**, protein C; **TM**, thrombomodulin; **sTM**, soluble TM; **vWF**, von Willebrand factor.

Introduction

Human TM is a single-chain, type 1 transmembrane glycoprotein mainly expressed on the luminal surface of the vascular endothelium.[1] TM binds thrombin and modifies its conformation, which allows activation of PrC.[2] In addition, TM plays an important role in mediating anti-inflammatory activity.[3] Plasma sTM is formed by proteolysis of membrane-bound TM[4] with anticoagulant and anti-inflammatory properties.[5] Experimental and clinical studies have shown that a low level of sTM is associated with a prethrombotic state and venous thrombosis.[6-9] Application of recombinant human sTM is effective in prevention of the venous thrombosis in a rat model and after total hip replacement [10,11] for the treatment of disseminated intravenous coagulation.[12] On the other

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hand, the increased level of sTM causes severe bleeding after injuries and operations. [13]

Thromboembolic complications and increased blood loss are serious threats after major orthopedic surgery.[14] The incidence of venography-detected DVT ranges from 42% to 57% in total hip replacement and 41% to 85% in total knee replacement, in the absence of prophylaxis. [15]

The aim of our pilot study was to evaluate the effect of the preoperative level of sTM on coagulation and inflammation as well as the blood loss and the development of symptomatic DVT after total large joint replacement.

Materials and Methods

The study included 50 patients (22 men, aged from 30 to 74 years) admitted for total hip or knee replacement. Exclusion criteria were coagulopathy and anticoagulant or antiplatelet therapy before surgery. The study was conducted in accordance with ethical principles of the Declaration of Helsinki and approved by the local Ethics Committees. Written informed consent was obtained from each patient.

All patients received antithrombotic prophylaxis with enoxaparin (40 mg daily), first administered in the evening before the operation, and then administered daily for two weeks. In order to limit blood loss, all patients included in the study received tranexamic acid (15-20 mg/kg intravenously). The first dose was administered 20-30 minutes before the surgical incision and the second dose was administered 6 hours after the first infusion. Intraoperative and postoperative bleeding was monitored. Clinical parameters indicative of thrombosis were recorded, and patients with suspected DVT were admitted for Doppler ultrasound verification.

All patients were divided into 2 groups according to the preoperative sTM level (Fig.1). The median value for sTM was 5.6 ng/mL. The data obtained from patients with sTM level below the median was combined into a thrombomodulin low (TML) group (n=25); the data obtained from patients with sTM levels equal to or above the median made up the thrombomodulin high (TMH) group (n=25). The concentration of sTM was 4.4 [3.4, 4.7] ng/ml in the TML-group and 8.7[7.3, 10.6] ng/ml in the TMH-group. The normal reference range was 5.1[2.9, 7.6] ng/ml, according to the manufacturer of the reagents. Characteristics of patients and surgical procedures in selected groups are presented in Table 1. The age of patients in the TMH-group was higher than in the TML-group, and this fact was taken into account when carrying out a statistical analysis of research results.

Blood sampling was performed at set time (T) points during the perioperative period: preoperatively 1 to 2 days (T1), 30 minutes after completing the replacement (T2), postoperative Day 1 (T3), Day 3 (T4), Day 7 (T5), and Day 14 (T6). Venous blood samples were collected in the morning into a test tube containing a 3.2% sodium citrate solution in a ratio of 1:9 sodium citrate/blood. Platelet count was determined in EDTA-stabilized blood. Blood serum was used for quantitative determination of C-reactive protein.

The following analyses were carried out for all patients: TM («Human sCD141 ELISA Kit», Gen Probe Diaclone, France); PrC («Technozym Protein C ELISA», Technoclone, Austria); D-dimer («Technozym D-dimer ELISA», Technoclone, Austria); von Willebrand factor («Technozym vWF: Ag ELISA», Technoclone, Austria), platelet count. C-reactive protein («High Sensitivity C-Reactive Protein Enzyme Immunoassay Kit», Biomerica, Germany) was determined for 38 patients. All analyses were performed in accordance with the manufacturer›s instructions.

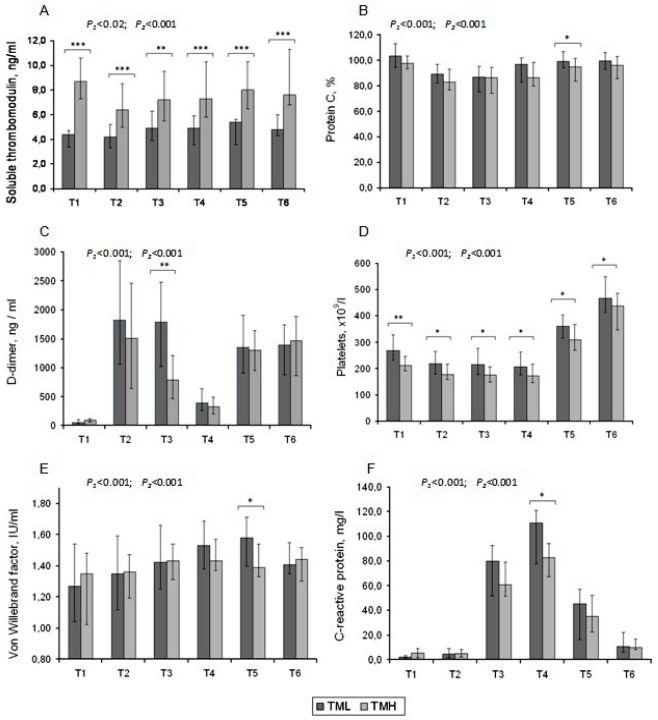

Fig 1. *Coagulation and inflammation parameters before and after total large joints replacement.*

Differences in concentrations and activity of sTM (A), PrC (B), D dimer (C), platelet count (D), vWF (E), CRP (F) for TML and TMH groups are shown in histogram (-P<0.05; **-P<0.01; ***-P<0.001). P1 - statistical significance of postoperative changes for the TML-group; P2 - statistical significance of postoperative changes for the TMH-group.*

Table 1.

Baseline characteristics of patients and surgical aspects

Variable	TML group	TMH group	*P*
Gender (male/female), No (%)	10/15 (40/60)	12/13 (48/52)	*0.573*
Age, years, median [IQR]	51 [47, 63]	62 [57, 65]	*0.032*
Type of surgery (THR/TKR), No. (%)	21/4 (84/16)	20/5 (80/20)	*0.818*
Prostheses (cementless/cemented), No.(%)	19/6 (76/24)	15/10 (60/40)	*0.335*
Duration of surgery, min, median [IQR]	100 [85,110]	100 [90,120]	*0.397*
Type of anesthesia (total/regional), No.(%)	10/15 (40/60)	6/19 (24/76)	*0.335*

THR - total hip replacement, TKR – total knee replacement. The results are expressed as a Number (percentage) and Median [IQR].

Statistical differences between two groups were analyzed by nonparametric Mann Whitney U test; the differences between time points T1 – T6 - by Friedman's ANOVA by ranks. A probability value of $P<0.05$ was considered statistically significant. The effect of age was analyzed by regression analysis and analysis of covariance. The results are expressed as Median [interquartile range (IQR)].

Results and Discussion

We observed that the patients with higher levels of sTM were older. A similar association between sTM and age was found earlier.[16] Blood sTM level is determined by hereditary factors [9,13] and by pathological changes that lead to increased proteolytic cleavage of endothelial TM.[17] TM expression is depressed in the aged.[18] On the other hand, aging is often accompanied by increasing endothelial dysfunction;[19] it can enhance the shedding of the soluble fragments of TM into circulating blood. Regression analysis showed that in the TMH-group, the patients' age affects only the preoperative level of PrC and CRP, as well as the platelet count at day 7 after the operation. After analysis of covariance, results for the dependent parameters were adjusted.

sTM level in the TML-group increased by Day 1 after surgery and stayed above the initial level until Day 14. On the contrary, in the TMH-group, TM level decreased after the operation and then was gradually restored ($P<0.02$ and $P<0.001$ respectively in Friedman's ANOVA, Fig. 1A). Despite the decline in the level of sTM in the TMH-group, significant differences in sTM levels between the groups were observed throughout the study period, and we can conclude that the postoperative level of sTM depends on its preoperative level.

PrC levels in both groups significantly decreased after the operation, and then gradually recovered ($P<0.001$ in Friedman's ANOVA for both; Fig. 1B). We observed a tendency to a lower level of PrC in the TMH-group compared with the TML-group throughout the study period. The observed differences were statistically significant by Day 7 ($P=0.034$). We cannot exclude that when there is a decrease in the liver production of PrC, an increase of the TM cleavage may have a compensatory effect for the anticoagulant pathways.

The concentration of D-dimer in TML-group was greatly increased immediately after surgery and Day 1 after the joint replacement. We observed a significant shift towards normalization in the concentration of D-dimer by postoperative Day 3, which was followed by another increase in the D-dimer level ($P<0.001$ in Friedman's ANOVA for both; Fig. 1C). The TMH-group had a more rapid decrease in the activity of coagulation after the operation. The concentration of D-dimer was substantially lower in this group than in the TML-group by Day 1 after surgery ($P=0.002$). Soluble TM fragments have full cofactor activity and the ability to bind thrombin and activate PrC.[1] A more rapid decrease of the D-dimer level is apparently due to more effective inactivation of the thrombin in patients with high levels of TM. We can assume that higher levels of sTM provide a more effective inhibition of coagulation induced by total large joint replacement.

The platelet count performed before surgery in the TMH-group was significantly lower than in the TML-group. After a total large joint replacement in both groups, the platelet count decreased for 3 days followed by a gradual increase exceeding the preoperative level ($P<0.001$ in Friedman's ANOVA for both; Fig. 1D). Significant differences between the groups remained in the postoperative period. A negative relationship between platelet count and sTM level was found in both healthy persons and those with pathological conditions.[16,20] We observed a similar relationship in our study before and after major orthopedic surgery.

vWF is considered to be one of the known endothelial markers, and changes in its concentration reflect the degree of the inflammatory response after large joint replacement.[21] We observed a gradual increase in vWF over time in both groups ($P<0.001$ in Friedman's ANOVA for both; Fig. 1E). However, the increase in vWF was less prominent in the TMH-group than in the TML-group, and these differences reached statistical significance by Day 7 ($P=0.032$).

The serum level of CRP is an indicator of the acute phase inflammatory response and can be used as a monitoring tool.[22] The concentration of CRP was significantly increased after surgery, reaching maximum values by Day 3. After that, the CRP level had a tendency to normalize but was never able to reach the baseline during the study period ($P<0.001$ in Friedman's ANOVA for both; Fig. 1F). CRP dynamics showed a similar character in both groups; however, the maximum CRP level was significantly lower in the TMH-group than in the TML-group ($P=0.019$). TM has been shown to mediate anti-inflammatory activities using activated protein C-dependent and activated protein C-independent mechanisms.[3] In our study, a higher level of sTM was associated with less increase of CRP and vWF after surgery. We concluded that sTM reduces the intensity of the inflammatory response after a total large joint replacement.

Intraoperative and postoperative bleeding monitoring has shown that the total blood loss (Intraoperative and postoperative within 24 hours) was significantly higher in the TMH-group than in the TML-group (Fig.2).

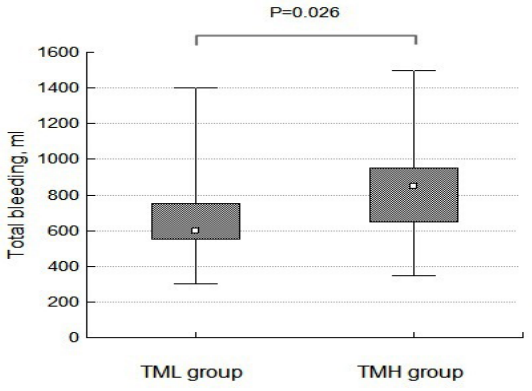

Fig. 2. *Total bleeding (ml) after a total large joint replacement.*

The box represents the median and IQR, the whiskers represents the minimum and maximum, P=0.026.

There were no significant differences in the localization of the operated segment, the duration of surgery, and the type of anesthesia between groups with high and low levels of sTM (Table 1). However, the blood loss was significantly greater in patients with higher sTM levels. Apparently, a high sTM level promotes rapid activation of PrC and early interruption of thrombin generation. This worsens the propagation phase of coagulation and increases blood loss. Our results are in line with the observation of Y. Dargaud et al. (2015); they have shown that increased levels of sTM cause severe bleeding after injuries and operations.[13]

Platelets play a critical role in hemostasis during vessel damage. In our study, the high sTM level was associated with a relatively low platelet count in the perioperative period, which suggests that a synergy of these factors contributes to the blood loss.

Previous experimental studies suggest that TM deficiency is associated with the prethrombotic state.[6,7] Significantly lower levels of plasma sTM were detected in patients with thrombosis in comparison with healthy individuals.[8] We have detected less venous thrombosis after total large joint replacement in patients who have a higher sTM level before and after surgery. In the TML-group, a symptomatic DVT was detected in 3(12%) patients; in the TMH-group, a symptomatic DVT was identified only in 1(4%) case. Although the differences did not reach statistical significance, our results do not contradict previously published data.

Our study has some limitations: a relatively small number of patients; participants were divided into groups retrospectively according to the median of the preoperative sTM level; connection of sTM with age; Doppler ultrasound was used only for confirming a symptomatic DVT, so we could not detect a "silent" thrombosis. Despite these limitations, we assume the obtained results support a link between the variation in preoperative sTM level and the activity of coagulation and inflammation after total large joint replacement. Further research may help in preoperative preparation and post-operative treatment of patients who require major orthopedic surgery.

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Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Epidemiology/Population

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Abortion in the Republic of Sakha (Yakutia): Incidence and Trends

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Abstract

Background: The abortion incidence is influenced by many medical and socioeconomic factors. In some way, it indicates the wellbeing of the population, and the abortion statistics can show the ways to improve medical services and to raise the living standards of people. The objective of the study was to estimate the abortion incidence and to describe its current trends in the Republic of Sakha (Yakutia) (the RS(Y)).

Materials and Methods: The study was designed as a population-based descriptive study, based on the results of a longitudinal analysis of national and regional reports of the Yakut healthcare services and an analysis of medical records describing 34,220 abortions among women living in all regions of Yakutia, which occurred between 2010 and 2014 and in the first 9 months of 2015.

Results: The absolute number of abortions performed each year, the rate of abortions per 1,000 women of fertile age, and the rate of abortions per 100 deliveries declined ($P<0.01$) by about 37%, 32% and 37%, respectively, between 2006 and 2014. The rate of abortions per 1000 women of fertile age in the first 9 months of 2015 decreased by 1.3%, compared to the same period of 2014 ($P=0.05$). Though the number of abortions in primigravida women decreases every year, the percentage of them is still rather high, especially at the age of 20 to 24 and 15 to 19, accounting for approximately 2.4% of all abortions. The relative number of miscarriages before 12 weeks of pregnancy increased. Changes in the relative number of abortions performed between 12 to 21 weeks of pregnancy characterized by a decrease in the percentage of miscarriages from 2012 to 2015 and a dramatic increase in the percentage of therapeutic abortions.

Conclusion: The revealed trends of the absolute number of abortions and the rates of occurrence in the RS(Y) can be considered in total as favorable, but compared to the data obtained in Russia in total, the dynamics of these trends cannot be regarded as satisfactory. Simplicity of medication abortions can lead to an increase in the absolute number of abortions, especially in adolescents and young women. Analysis of spatial and temporal distribution of the incidence of abortions did not reveal any association with the ethnicity of women.**(Int J Biomed. 2016;6(3):218-221.)**.

Key Words: abortion incidence • adolescents • primigravida women • reproductive health care.

Introduction

Abortion care is known to be a critical component of comprehensive reproductive health care. According to WHO, in 2008, an estimated 43.8 million induced abortions were performed throughout almost all major regions of the world, a slight decline from 45.6 million in 1995. Developing countries accounted for 86% of all induced abortions.[1,2] The United Nations defines the policy on fertility level in Russia in total, and particularly in Yakutia, as "no intervention" and qualifies the grounds on which abortion is permitted as "least restrictive" (to save a woman's life, in case of rape or incest, and because of fetal impairment).[3]

In the Republic of Sakha (Yakutia) (the RS(Y)), women have the fundamental right to abortion as determined by the law. The decision to continue or terminate a pregnancy

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belongs to the pregnant woman, and the decision makers are not restricted by regulations that limit or delay access to care. The Ministry of Health Care of the RS(Y) tracks yearly the news on demography indices and abortion in order to perform management duties and to implement the federal policies, procedures and regulations in a timely manner.

According to the World Factbook, in 2015 Russia ranked 179th among 224 countries in the latest global survey with its 1.61 estimated total fertility rate.[4,] Though the trends of demographic indices in the RS(Y) are rather favorable (Fig.1), fertility can still be increased.

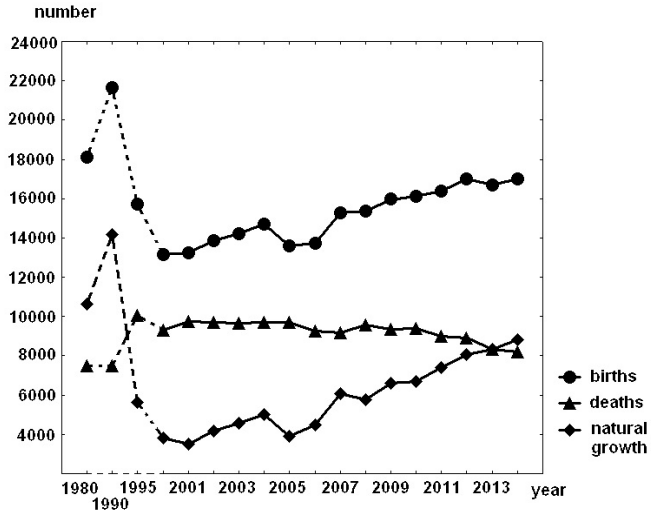

Fig. 1. *Number of births, deaths and natural population growth in the RS(Y) since 1980 [5]*

The abortion incidence is influenced by many medical and socioeconomic factors. In some way, it indicates the wellbeing of the population, and the abortion statistics can show the ways to improve medical services and to raise the living standards of people.

The objective of the study was to estimate the abortion incidence and to describe its current trends in the RS(Y).

Materials and Methods

The study was designed as a population-based descriptive study, based on the results of a longitudinal analysis of national and regional reports of the Yakut healthcare services and an analysis of medical records describing 34,220 abortions among women living in all regions of Yakutia, which occurred between 2010 and 2014 and in the first 9 months of 2015.

Gestational age was determined according to the recommendations of the Committee on Obstetric Practice American Institute of Ultrasound in Medicine Society for Maternal-Fetal Medicine.[6] Types of abortions were defined according to WHO recommendations.[7]

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). Group comparisons with respect to categorical variables are performed using chi-square tests. A probability value of $P<0.05$ was considered statistically significant.

Results

The female population of the RS(Y) by age groups as of 2014 is presented in Table 1. Around 50% of all women of Yakutia are of child-bearing age, a percentage which is higher than, for example, 43% in the USA as of July 2014,[8] and 46% in Russia in total as of January 2014.[9] Moreover, the relative number of girls aged between 0 to 14 years in the female population of Yakutia is also greater: about 22% compared to 15% in Russia in total.[9]

Table 1.

The structure of female population of the RS(Y) in 2014

Female population	absolute number
Total population	954 896
Females, total	491 349
women of child-bearing age (15 – 49 years old)	248 115
adolescent girls (15 – 17 years old)	18 793
girls (0 – 14 years old)	106 232

Trends in the number of abortions in Yakutia and in Russia in total by year are presented in Table 2. The absolute number of abortions performed each year, the rate of abortions per 1,000 women of fertile age, and the rate of abortions per 100 deliveries declined ($P<0.01$) by about 37%, 32% and 37%, respectively, between 2006 and 2014. The rate of abortions per 1000 women of fertile age in the first 9 months of 2015 decreased by 1.3%, compared to the same period of 2014 ($P=0.05$).

Table 2.

Number of abortions in the RS(Y) and in the Russian Federation in total [10-12]

Year	Absolute number of abortions	Rate of abortions per 1,000 women in fertile age		Rate of abortions per 100 deliveries	
		Yakutia	Russia	Yakutia	Russia
2006	14 164	53.0	41.0	107	107
2007	14 090	53.0	38.0	95	92
2008	13 120	50.0	36.0	89	81
2009	12 059	44,6	-	78	74
2010	10 848	41.0	31.7	80	67
2011	9 900	38.1	30.5	71.9	63
2012	9754	37.6	29.3	65.9	56.2
2013	9105	35.8	28.3	71.3	53.7
2014	8976	35.9	25.9	67.6	48.1
2015*	6385	28.4	-	67.6	-

**-the first 9 months*

In 2015, the absolute number of abortions did not decrease in 16(47%) regions of Yakutia and in the city of Yakutsk. The absolute number of abortions increased in

Srednekolymsky (+32%), Churapchinsky (+24%), Niurbinsky (+20%), Tattinsky (+19%), Amginsky (+18%), Bulunsky (+17%), Verkhoyansky (+16%), and Namsky (+15%) regions. In the remaining 18 regions, the absolute number of abortions decreased, in 6 of them by more than 20%.

Though the number of abortions in primigravida women decreases every year, the percentage of them is still rather high, especially at the age of 20 to 24 and 15 to 19, accounting for approximately 2.4% of all abortions. It must be mentioned that 82 teen abortions were performed during the first 9 months of 2015.

Changes in relative numbers of abortions before and after 12 weeks of pregnancy are shown in the Figures 2 and 3. The relative number of miscarriages before 12 weeks of pregnancy increased during the last 3 years (17.3% in 2012 vs 20.6% in 2014, *P<0.000*), and this trend continued in 2015. The percentage of therapeutic and social abortions was the same, and the visible but slight decrease can hardly be interpreted even as a tendency (71.7% in 2012 vs 70.7% in 2014, *P>0.05*). The percentage of unspecified abortions decreased from 2012 to 2014 (8.4% in 2012 vs 6.4% in 2014, p<0.000), but then increased in 2015 (6.4% in 2014 vs 7.2% in 2015, *P<0.05*).

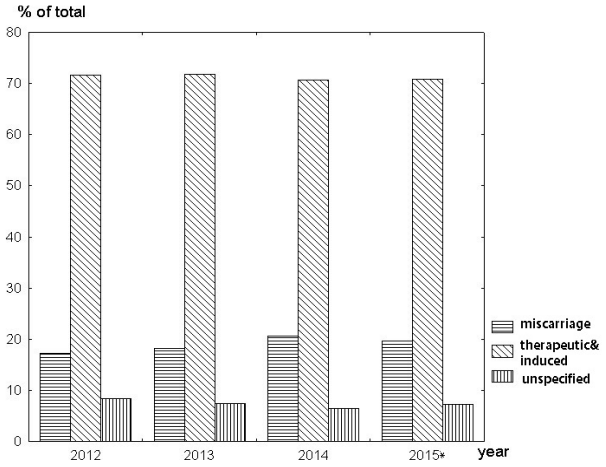

Fig. 2. *Relative number of abortions before 12 weeks of pregnancy (% of all abortions)*
** - the first 9 months*

Changes in the relative number of abortions performed between 12 and 21 weeks of pregnancy (Figure 3) were characterized by a decrease in the percentage of miscarriages from 2012 to 2014 (47.4% in 2012 vs 40.3 in 2014, p<0.000) and a further slump in 2015, a dramatic increase in the percentage of therapeutic abortions (34% in 2012 vs 45.5% in 2014 and 43.2% in 2015, *P<0.000*) and a decrease in the percentage of unspecified abortions from 2012 to 2014 (17.4% vs 13.4%, *P<0.000*), with an unexplained increase by 10.7% (*P<0.000*) in 2015. In the first 3 quarters of 2015, the percentage of therapeutic abortions increased in 17 regions and in the city of Yakutsk. In Nizhnekolymsky, Momsky, Bulunsky, Churapchinsky and Ust'-Yansky regions, the increase was more than by 15% (*P<0.01*). The percentage of miscarriages decreased in 20 regions and increased in 11 regions and in the city of Yakutsk. A significant (*P<0.05*) increase was revealed in Anabarsky (+24%), Zhigansky (+17%), Tattinsky (+14%), Verkhnevilyuisky (+7.1%) and Oymiakonsky (+6.3%) regions.

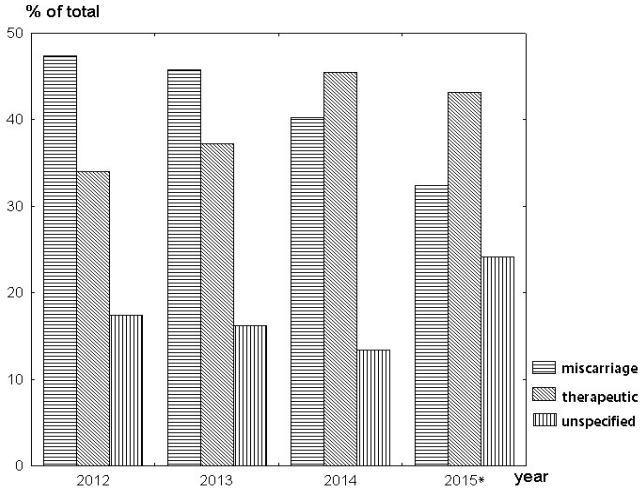

Fig. 3. *Relative number of abortions between 12 and 21 weeks of pregnancy (% of all abortions)*
** - the first 9 months*

The percentage of different types of medical abortions is presented in Figure 4. From 2012 to 2014, the percentage of surgical abortions (D&E) decreased (68.1% in 2012 vs 63.6% in 2014, *P<0.000*) with a further 6% drop in 2015; the percentage of vacuum aspirations also decreased (29.4% in 2012 vs 15.4% in 2014 and 13.8 in 2015, *P<0.000*), and the percentage of medication abortions changed dramatically (2.5% in 2012 vs 20.8% in 2014, *P<0.000*) with a further increase of almost 8% in 2015.

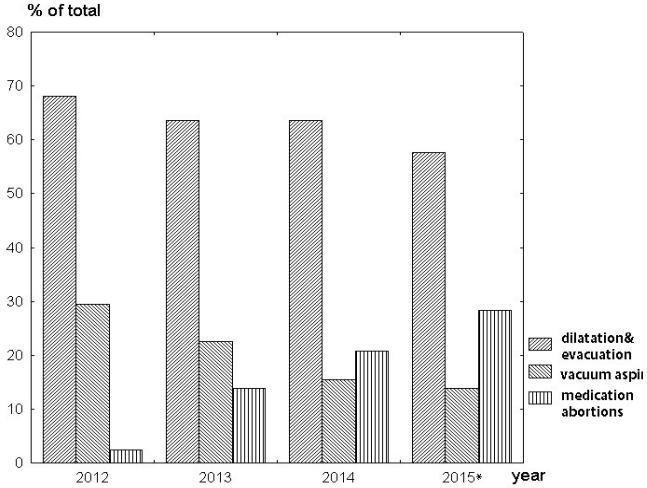

Fig. 4. *Relative number of different types of therapeutic abortions (% of all therapeutic and social abortions); *- the first 9 months*

An increase in the percentage of medication abortions was due to successful implementation of the Regional Program of State Assurance, which since 2012 has included

financial coverage for this kind of abortion. The result was that during only the first 9 months of 2015 the absolute number of medication abortions reached 1,214, which is almost twice as many as during the first 9 months of 2014. Of those 1,214 abortions, 75% were performed in the regions and only 25% in Yakutsk.

Conclusion

The revealed trends of the absolute number of abortions and the rates of occurrence in the RS(Y) can be considered in total as favorable, but compared to the data obtained in Russia in total, the dynamics of these trends cannot be regarded as satisfactory.

One of the most concerning issues is the multi-directional pattern of changes in different regions, which most probably depends on the quality of regional medical services, education, and socioeconomic status of the individual region. Differences in the incidence of abortions between regions were revealed, but it is unknown whether these differences can be assigned to lower standards of living in the inhabited locality with poor medical services rather than to ethnicity and the associated style of living. Analysis of spatial and temporal distribution of the incidence of abortions did not reveal any association with the ethnicity of women, though this analysis was based only on registered places of their habitation because ethnicity is not registered in the standard medical documentation.

Another matter of concern is the rather high incidence of abortions in adolescents and in primigravida women, which issue can be resolved only after a long period of teamwork with social services. The increase in the percentage of medication abortions within last 4 years is a positive trend, but the simplicity of performing them and the fact this procedure is free of charge can lead to an increase in the absolute number of abortions, especially in adolescents and young women.

An unsettling finding is a very high number of the so-called “unspecified” abortions. The absence of individual statistics on criminal abortions makes us suppose they are included in the “unspecified” group, though most probably this is just a fault of registration.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Epidemiology/Population

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Dynamics of Incidence of Chronic Hepatitis B and C in the Population of Almaty city for 2001-2014

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Abstract

The results of a retrospective epidemiological analysis revealed a sharp decline in the incidence of acute hepatitis B among the entire population of Almaty and the absence of acute hepatitis B, acute hepatitis C and chronic hepatitis C among children under 14 years of age. We found an increased incidence of chronic hepatitis B and chronic hepatitis C among the population of Almaty. Assessment of the hepatitis C incidence by the cumulative indices more objectively reflects the epidemiological situation for this disease. **Int J Biomed. 2016;6(3):222-224.)**.

Key Words: hepatitis B virus • hepatitis C virus • morbidity • cumulative indices.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) remain an urgent health problem in Kazakhstan. An encouraging trend is that the incidence of HBV in Almaty city decreased 9.3 times and has not registered among children under 14 years since 1998, attributable to effective vaccination programs; the prevalence of HBV carriers also dropped to 2.3%-2.6%.[1,2] At the same time, it has been shown that 80% of HBsAg carriers are people between the ages of 20 and 40 years.[2]

Official registration of HCV infections in Kazakhstan and Almaty city started in 1998. In the beginning, only acute hepatitis C (AHC) was registered; new cases of chronic hepatitis C (CHC) began to register in 2008. The first observations have shown that the AHC incidence is characterized by low levels(0.4–2.1 in the general population and up to 0.75 in children).[2-4] However, the CHC incidence was characterized by higher prevalence and tended to increase, largely covering adults as chronic hepatitis B (CHB).[2-4] These features of the incidence of CHB and CHC require improving the disease surveillance.

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Materials and Methods

For the retrospective epidemiological analysis, we used the data of the official registration of the Department of Sanitary and Epidemiological Surveillance of Almaty for HBV and HCV in the intensive indicators from 2001 to 2014. The behavior of the epidemic process was assessed by the annual dynamics of the cumulative incidence of hepatitis B and C.[5]

Results and Discussion

Table 1 shows the average incidence rate for acute hepatitis B (AHB) in the general population and in children under 14 years of age at 4-5-year intervals for 2001-2014. As can be seen, the AHB morbidity dynamics among the general population tend to decrease.

Table 1.

Dynamics of AHB incidence in Almaty for 2001-2014

Nosology	population category	The average annual incidence rate ($^0/_{0000}$) for			Multiplicity reduction
		2001-2005	2006-2010	2011-2014	
AHB	General population	20.0	5.5	1.5	13.3
	children under 14 years of age	1.01	0.0	0.0	Absence of AHB

The average rate for 2001-2005 was 20.0 per 100,000 and dropped to 1.5 in 2011-2014. Multiplicity reduction amounted to 13.3 times. More striking dynamics were observed for AHB among children under 14 years of age. The AHB incidence was recorded only during 2001-2004. The average rate was 1.01 per 100,000 children. Since 2005 in Almaty, AHB has not been registered among children. These data conclusively demonstrate the epidemiological effectiveness of HBV vaccination in children.

The results of a similar analysis for AHC incidence are shown in Table 2. During the analyzed period, the isolated cases of AHC were recorded annually: figures ranged from 0.6 to 1.14$^{0}/_{0000}$. AHC cases were not registered among children. Generally, AHC is uncommon in childhood,[6,7] and most chronically infected children are asymptomatic with normal growth and development.

Table 2.

Dynamics of AHC incidence in Almaty for 2001-2014

Nosology	population category	The average annual incidence rate ($^{0}/_{0000}$) for			Multiplicity reduction
		2001-2005	2006-2010	2011-2014	
AHC	General population	1.14	0.24	0.6	1.9
	children under 14 years of age	0.00	0.00	0.00	-

The standard case definition for AHCV was adapted from World Health Organization recommendations.[8] It is necessary to diagnose AHCV based on epidemiological and clinicobiochemical findings, such as the presence of newly identified markers of HCV – antibodies to HCV (anti-HCV) and HCV RNA. Unlike with AHB—in which the IgM antibody to the hepatitis B core antigen is diagnostic of acute infection and precedes the appearance of IgG—with HCV infection the IgM antibody responses are variably detected in both acute and chronic phases.[9] Anti-HCV IgM cannot therefore serve as a diagnostic marker of acute HCV infection. In this context, the continuing practice of determining IgM for the diagnosis of acute hepatitis C in Kazakhstan is incorrect.

In recent years, the incidence of CHB and CHC has increased.[11,12] Table 3 illustrates the dynamics of incidence of CHB and CHC in Almaty for 2003-2014 years.

Table 3.
Dynamics of incidence of CHB and CHC in Almaty for 2003-2014

Nosology	The average annual incidence rate ($^{0}/_{0000}$) for			Multiplicity increase
	2003-2006	2007-2010	2011-2014	
CHB	6.1	5.1	11.6	1.9
CHC	4.7	4.1	14.8	3.1

In particular, from the time of registration in 2003 the incidence of CHB and CHC has tended to increase, and has increased 1.9 and 1.3 times, respectively. Taking into account the incorrectness of the separate account of AHC cases and treating them as the manifestation of CHC cases, we calculated the cumulative incidence rates of HCV, the average indices of which were compared with the average annual rates of CHB (Table 4).

Table 4.

Dynamics of the cumulative incidence rates of CHC and CHB in Almaty for 2003-2014

Nosology	The average annual incidence rate ($^{0}/_{0000}$) for			Multiplicity increase
	2003-2006	2007-2010	2011-2014	
CHC	5.5	4.3	15.0	2.7
CHB	6.1	5.1	11.6	1.9

CHC incidence, according to cumulative indices, was slightly higher than the CHB incidence, especially in 2011-2014. These results indicate an epidemiological potential of CHC and require attention to the public health problem. Analysis of the distribution of patients with CHC and CHC by age groups showed that 80% to 90% of patients are between 20 and 49 years of age.

Thus, the results of a retrospective epidemiological analysis revealed a sharp decline in the AHB incidence among the entire population of Almaty and the absence of AHC and AHC among children under 14 years of age. Registered isolated cases of AHC, apparently, are a manifestation of CHC cases.

We found an increased incidence of CHB and CHC among the population of Almaty. Assessment of the hepatitis C incidence by the cumulative indices more objectively reflects the epidemiological situation for this disease. These circumstances have to be understood by epidemiologists for effective and quality monitoring of hepatitis C. CHB and CHC, being a risk factor for hepatocellular carcinoma and liver cirrhosis, should be a focus of the healthcare system in Kazakhstan.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Epidemiology/Population

INTERNATIONAL JOURNAL OF BIOMEDICINE

Epidemiological Characteristics of Hepatitis A in Some Regions of Kazakhstan with Different Degrees of the Severity of Ecological Disaster

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Abstract

The results of a retrospective epidemiological analysis of the hepatitis A incidence among children in the studied territories showed no connection between the incidence rate and the ecological status of the territory. the strategic vaccination of children in areas of high endemicity is the most effective way to control HAV, or possibly to eliminate it. (**Int J Biomed. 2016;6(3):225-227.)**.

Key Words: hepatitis A virus • incidence rate • cumulative indices • ecological disaster • vaccination.

Introduction

Hepatitis A (HA) is the most common form of acute viral hepatitis worldwide. The incidence rate is strongly related to socioeconomic indicators and access to safe drinking water. The hepatitis A virus (HAV) endemicity level for a population is defined by the results of age-seroprevalence surveys; a systematic review on the global prevalence of HAV infection was recently published by WHO.[1] Areas of the world can be characterized as having high, intermediate, low, and very low endemicity for HA. Areas of high endemicity include most of Africa, Asia and Central and South America. In areas of high endemicity, the prevalence of anti-HAV IgG reaches 90% in adults, and most children have been infected by 10 years of age.[2] In most middle-income regions in Asia, Latin America, Eastern Europe, and the Middle East, surveys of anti-HAV antibody in the population show a mix of intermediate (≥50% are immune by age 15 years) and low (≥50% are immune by age 30 years) prevalence.[3] Safe water supply, food safety, improved sanitation, hand washing and the hepatitis A vaccine are the most effective ways to combat the disease.[3-5]

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HA is still an important problem for Kazakhstan, despite the marked decline in the HA incidence of 12.2 times among children. It should be noted that the decrease in morbidity in different areas ranges from 5.3 times to its complete absence. HA is more frequently registered in the Kyzylorda region and South-Kazakhstan region, where the annual incidence among children under 14 years is 3 and 2.5 times higher than in the whole country.[6,7]

Kyzylorda region is a larger zone of the Kazakhstan part of the Aral Sea region, which was declared by government decree [8] in 1990 as a zone of ecological disaster due to a sharp reduction in the water area of the sea. This area covers mainly the territory of Kyzylorda oblast and extends over part of the territory of neighboring South Kazakhstan oblast.

According to the severity of this ecologically unfavorable situation, the area of ecological disaster in the Aral Sea area is divided into three zones: 1) ecological catastrophe, 2) ecological crisis, and 3) ecological pre-crisis state.

Within the zone of ecological catastrophe are the Aral and Kazaly regions of Kyzylorda oblast and Shalharsky region of Aktobe oblast. The zone of ecological crisis encompasses Karmakchinsky, Zhalagash, Shieli, Syr Darya and Zhanakorgan regions, Kyzylorda city, and Baikonur, including the surrounding villages. Within a zone in an ecological pre-crisis state are Irgiz region in Aktobe oblast,

Aris city and Turkestan in South Kazakhstan oblast, and Ulytau region in Karaganda oblast.[8]

Among epidemiologists and practitioners, it is believed that the relatively high incidence in South Kazakhstan and Kyzylorda oblasts is associated with the current environmental situation. This study is devoted to analysis of the HA incidence in the areas located in the zones with different degrees of severity of the environmental disaster.

Materials and Methods

For the retrospective epidemiological analysis, we used the data of the official registration of the Departments of Sanitation and Epidemiological Surveillance of the republic, regions and oblasts for the HA incidence in children for 2005-2014.

Results and Discussion

The generalized average annual epidemiological parameters for HA in the study areas, belonging to three different zones of ecological disaster, are given in Table 1. As can be seen, in Aralsk, Kazalinsk and Arys, relating to the zone of ecological catastrophe, the average annual incidence rates for children under 14 years of age amounted to 185.9, 86.3 and 334.7, respectively. In Karmakchi, Zhalagash and Shieli, relating to zone of ecological crisis, these indices were 101.8, 418.1 and 151.6, respectively.

In areas, relating to the area of an ecological pre-crisis state (villages Irgiz and Ulytau), these indices amounted to 11.3 and 77.9 respectively. In the control and environmentally safe village of Atasu, this index was $36.3^{0}/_{0000}$. These data suggest that in the studied territories, there is no direct connection between the HA incidence in children and the severity of the environmental disaster.

In all studied regions of Kyzylorda oblast and Aris city of South Kazakhstan oblast, the highest incidence rates were observed among children between 3 and 6 years of age(from 158.8 to 2642.2); the relatively high rates were observed among children of 7 to 10 years of age. The proportion of children among HA patients in these territories ranged from 78.3% to 87%. The identified epidemiological features of HA are most common for the naturally flowing HA epidemic process.[9]

We have previously shown that the effective vaccination program for HA prevention among children leads to noticeable changes in the epidemiological characteristics of HA. In particular, there is a decrease in the proportion of children in the structure of the annual incidence in population, a reduction of morbidity among young children with transference into the teenage group and young people group (15-19 years of age), the elimination or significant decrease in amplitude of cyclic recurrence of epidemic process.[10,11]

In the villages of Shalhar and Irgiz, as well as in Aktobe oblast as a whole, the incidence rate among children reached 11.3-14.2, and the proportion of children, 28.1% to 30.3%.

Table 1.

The epidemiological parameters of hepatitis A in areas with different degrees of the severity of ecological disaster

Oblast	Zones of ecological disaster	Region	The average annual incidence rate for 10 years ($^{0}/_{0000}$)								
			Children under 14 years	Proportion of children (%)	Age groups, y				Socio-professional groups		
					1-2	3-6	7-10	11-14	organized	unorganized	schoolkids
Kyzylorda oblast	ecological crisis	Oblast	247.1	73.2	193.3	343.4	250.0	120.2	191.8	154.6	137.9
	ecological catastrophe	Aralsk	185.9	84.1	167.2	251.5	226.5	99.7	283.4	165.8	149.8
		Kazalinsk	86.3	87.0	72.5	189.5	174.7	81.3	149.9	130.1	119.3
	ecological crisis	Karmakchy	101.8	78.3	54.9	158.8	151.0	78.9	139.6	50.1	106.1
		Zhalagash	418.1	82.1	182.1	254.3	192.5	117.9	154.9	247.4	371.9
		Shieli	151.6	84.5	133.7	181.4	152.1	84.2	71.5	257.2	191.7
Aktobe oblast	–	Oblast	13.3	29.1	6.7	10.4	16.6	16.8	8.3	8.7	19.9
	ecological catastrophe	Shalhar	14.2	30.3	8.5	9.2	12.1	14.5	9.1	11.7	15.2
	ecological pre-crisis state		11.3	28.1	0.0	7.4	13.0	13.4	10.2	9.8	12.5
South Kazakhstan oblast	–	Oblast	336.9	88.9	218.3	466.5	368.5	98.5	282.2	362.1	311.1
	ecological pre-crisis state	Aris	334.7	85.7	420.0	2642.2	914.0	245.6	4.3	75.0	114.1
Karaganda oblast	–	Oblast	54.2	52.3	43.1	211.3	205.1	186.9	28.3	49.5	85.7
	ecological pre-crisis state	Ulytau	77.9	60.8	22.3	45.3	145.6	161.0	61.1	21.3	114.3
	outside the disaster zone (control)	Atasu	36.7	25.8	0.0	9.9	7.4	29.2	2.4	5.1	39.4
The Republic of Kazakhstan	–	–	108.1	75.3	115.3	257.9	210.1	105.6	48.5	109.9	104.7

We found a shift in morbidity from zero among children between 1 and 2 years of age to 7.4, 13.0 and 13.43$^{0}/_{0000}$ in the following age groups (3-6, 7-10, 11-14). The same trend was observed in the villages of Ulytau and Atasu, the latter of which is located outside the disaster zone and is defined as a reference control village. It was noted earlier that the vaccination against HA was effective in these areas.[6]

Thus, the analysis of HA incidence among children in the studied territories showed no connection between the incidence rate and the ecological status of the territory. At the same time, if we consider the relationship between HA incidence and the environmental contamination with HAV-Ag in different regions within same province, a certain connection is revealed. The region rank positions for the environmental HAV-Ag contamination and the HA incidence in the different territories of Kazakhstan are shown in Table 2. As can be seen, in regions of the same area (oblast), there is an identity or similarity in the rank positions according to the environmental HAV-Ag contamination and the HA incidence. This fact is seen most clearly in the regions of Kyzylorda oblast. So, in the Aral region, the rank positions were close (1 and 2). Similar proximity of these ranks was noted in Kazalinsk region (4 and 5), and these positions were the same (3 and 3) in Shiel region. An evident discrepancy was noted in the Zhalagash region (1 and 5). In Aktobe and Karaganda oblasts, the rank positions for the environmental HAV-Ag contamination and the HA incidence fully coincided. These data suggest the presence of a causal link between the environmental HAV-Ag contamination and the HA incidence.[12] This relationship is leveled by vaccination, making it more difficult to analyze in Kazakhstan, where vaccination of children against HA has been conducted from 2004 to 2005.[11,13,14]

Table 2.

The region rank positions for the environmental HAV-Ag contamination and the HA incidence

Oblast	Ecological status	Region	Contamination & a rank position %; (№)	Incidence rate & a rank position $^{0}/_{0000}$; (№)	Ranks
Kyzylorda oblast	ecological catastrophe	Aralsk	1.3; (1)	185.3; (2)	1-2
		Kazalinsk	0.52; (4)	86.3; (5)	4-5
	ecological crisis	Karmakchy	0.9; (2)	101.8; (4)	2-4
		Zhalagash	0.5; (5)	418.3; (1)	5-1
		Shieli	0.6; (3)	151.6; (3)	3-3
Aktobe oblast	ecological catastrophe	Shalhar	0.3; (1)	14.2; (1)	1-1
	ecological crisis	Irgiz	0.2; (2)	11.3; (2)	2-2
South Kazakhstan oblast	ecological crisis	Aris	1.2	334.7	
Karaganda oblast	ecological crisis	Ulytau	0.4; (1)	77.9; (1)	1-1
	control zone	Atasu	0.3; (2)	36.7; (2)	2-2

Thus, in the studied regions of Kazakhstan, the average annual incidence rates for HA vary from 11.3$^{0}/_{0000}$ in Irgiz region of Aktobe oblast to 418.3$^{0}/_{0000}$ in the Zhalagash region of Kyzylorda oblast. The degree of the environmental HAV-Ag contamination varies from 0.2% to 1.3%. Almost full similarity or the proximity of rank positions among regions for HAV-Ag contamination and morbidity shows a link between these parameters. However, we did not find an association between the degree of HAV-Ag contamination/HA and the severity of the environmental disaster. Thus, the strategic vaccination of children in areas of high endemicity is the most effective way to control HAV, or possibly to eliminate it.

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ORIGINAL ARTICLE

Medical Biochemistry

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7-(1-Methyl-3-Pyrrolyl-)-4,6-Dinitrobenzofuroxan Reduces the Frequency of Antibiotic Resistance Mutations Induced by Ciprofloxacin in Bacteria

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Abstract

The aim of the present study was to investigate biological properties of the novel nitrobenzoxadiazole derivative 7-(1-methyl-3-pyrrolyl-)-4,6-dinitrobenzofuroxan.

Materials and Methods: We used a bioluminescent test based on a set of lux-biosensors, which are genetically modified *E.coli* strains able to react on different types of factors that can induce an SOS-response with light emission. The spontaneous and induced mutation frequencies of antibiotic resistance in *E. coli* were determined by methods of classical genetics of microorganisms.

Results: 7-(1-methyl-3-pyrrolyl-)-4,6-dinitrobenzofuroxan demonstrated inhibition of SOS-response in a biosensor model system and significantly reduced the frequency of spontaneous mutations and mutations induced by ciprofloxacin of antibiotic resistance.

Conclusion: Based on our data, we can recommend using compound **1** as a starting point for the development of drugs that block mutagenesis associated with the emergence of antibiotic-resistant bacteria. (**Int J Biomed. 2016;6(3):228-232.)**.

Key Words: antibioitic resistance • fluoroquinolones • SOS-response • SOS-inhibitors • nitrobenzoxadiazole derivatives

Introduction

Resistance of microorganisms to fluoroquinolones is a big problem for international medicine, especially as regards nosocomial infections. The fastest resistance is formed by *Pseudomonas aeruginosa*. There are data an increase of resistance of pneumococci.[1] Currently, the natural and acquired mechanisms of antimicrobial resistance are well known.[2] In general, the mechanism of resistance is realized through modifying the target of action, inactivation of the antibiotic, actively removing it from the microbial cell, violation of the permeability of outer structures of the microbial cells (efflux), violation of the permeability of outer structures of the microbial cells, or the formation of a "metabolic shunt."

In the case of fluoroquinolones, the mechanism of target modification is implemented by modifying two bacterial enzymes: DNA gyrase and topoisomerase IV, which mediate conformational changes in the molecule of bacterial DNA, necessary for its normal replication.

Currently used anti-microbial agents often have the ability to induce mutations in microorganisms, thus causing the appearance of forms which are resistant to a range of antibiotics. A paper by Cirz et al.[3] describes the mechanisms of mutations of resistance to rifampicin and ciprofloxacin caused by the use of these drugs. It was shown that the main generator of resistance mutations is the SOS-or error-prone repair system.[4]

In order to compensate for the negative effects identified in the works of Cirz and Wigle et al.,[3,5] it was proposed to synthesize new compounds, which would be able to switch off the launch of SOS-response and thus increase the sensitivity of the bacterial population to antibiotics. Currently, several

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laboratories are conducting screening for SOS-response inhibitors that would prevent the rapid development of bacterial resistance to antibiotics. In the course of these studies, ATP-competitive nucleotide analogs,[6,7] polysulfated naphthyl compounds,[8] alpha-helical peptides,[9] and the variable valence metal cations [10] were investigated. Those compounds proved effective as inhibitors of RecA in vitro. However, none of these classes of compounds showed biological activity *in vivo*, being unable to penetrate the membrane barrier.[11]

In the study on the biological properties of the novel nitrobenzoxadiazole derivatives,[12] we described the synthesis of previously known 7-(1-methyl-3-pyrrolyl -)-4,6-dinitrobenzofuroxan (**1**), which possesses a high DNA-protective effect, and negates damage caused by the oxidating mutagen Dioxidine. Compound **1** includes both electron-excess (N-methylpyrrole) and electron-deficient (4,6-dinitrobenzofuroxan) fragments, resulting in significant intramolecular charge transfer and a high polarity of the structure **1** as a whole. In this study, we investigated the ability of this compound to inhibit the launch of SOS-response induced by ciprofloxacin of the fluorquinolone antibiotic group. This class of antibiotics is distinguished by the ability to start the SOS-repair (and, consequently, mutagenesis), without chemical modification of the bases, only by inhibiting the topoisomerase.[3,13,14] Our experimental approach implies using *E. coli*-based Lux-biosensors, responding to the activation of the SOS pathway with an increase in luminescence.[15] Through this approach, it became possible to identify the inhibitors, effectively, working not only in model cell-free systems, but also within bacterial cells. Shifts in spontaneous and induced mutation frequency of antibiotic resistance in *E. coli,* induced with substance-**1,** were determined by methods of classical genetics of microorganisms.

Materials and Methods

We thoroughly described the parameters of 7- (1-methyl-1H-pyrrol-3-yl)-4,6-dinitro-2,1,3-benzoxadiazolyl1-oxide (substance **1)** in our early papers.[12,16]

To analyze the expression of stress-inducible operons and mutagenesis we used recombinant strain of *Escherichia coli* MG1655 (pRecA-lux), with a plasmid containing luxCDABE operon from photobacteria *Photorhabdus luminescens*, put under control of PrecA promoter of *E. coli*. Biosensors with pRecA plasmids react to factors causing induction of the SOS-response in the cell.

A test compound was dissolved in DMSO (dimethylsulfoxide, analytical grade, Ugreaktiv, Russia) to a concentration of 10mg/ml. From this solution serial dilutions were then made in DMSO to 10^2mg/ml. Thus, the following concentrations were tested: 1; 0.1; 0.01 mg/ml. DMSO was used as a control for each dilution in respective concentrations.

Bioluminescent test

We used a slightly modified bioluminescent test protocol described in detail in our early papers.[12,15]

The culture of *E. coli* MG 1655 pRecA-lux was grown in LB broth. Bacteria were grown in liquid culture at 37 °C. The overnight culture was diluted with fresh medium to the density of 0.01 - 0.1 McFarland units (concentration $3 \cdot 10^7$- $3 \cdot 10^6$ cells/mL).

For growth on solid medium, LB-agar (LB + 20 g / liter of microbiological grade agar) was used. Both liquid and solid medium were supplemented with ampicillin (100 μg/ml).

The measurements were made using a DEN-1B («Biosan») densitometer. Then the suspension was grown for 2 hours to early logarithmic phase. Aliquots of the culture (90 μl) were transferred into the wells and they were added with 10 μl of the test preparation and 10 μl of the control inductor (except of control wells). The control wells were added with 10 μl of DMSO.

As an inducer to activate the recA promoter, we used ciprofloxacin solution (pharmaceutical grade, KRKA, Slovenia) in deionized water at a concentration of 4.10 mg /ml.

After treatment, the plate with the sample was placed into the luminometer and incubated at 30^0C. The intensity of the bioluminescence was measured every 10 - 15 minutes.

For luminescence measurements, microwell plate luminometer LM-01T (Immunotech Co.) was used.

SOS induction response factor (I^s) was calculated according to the formula:

$$I^s = {}^{L_e}/_{L_k} - 1$$

where: L_k - the intensity of the luminescence in the control sample (in arbitrary units);

L_e- luminescence intensity of the test sample (in arbitrary units).

We considered statistically significant excess of L_e over L_k as an indication of the SOS-inducing effect. The excess was measured with t-test, calculated in the Microsoft Excel program. Anti-SOS activity indicator (A,%) was calculated by the formula:

$$A = (1 - {}^{I_a}/_{I_p}) \times 100$$

where: I_a - factor SOS-response induction caused by the influence of the inhibitor under investigation, I_p- factor of ciprofloxacin SOS-response induction.

All experiments were performed in three independent replicates. Confidence intervals for protective activity value were calculated by the described method.[15, 16]

Determination of spontaneous and induced mutagenesis parameters

Cells of E.coli MG1655 (pRecA-lux) strain were inoculated into 4 ml of the LB medium and stirred evenly. The induced culture was divided into four equal aliquots. One aliquot served as a control. To the remaining three aliquots were added 100 μg of the substance 1 to the concentration of 1 mg/ml; 100 μg of ciprofloxacin was added to the concentration of 0.1 μg/ml; 100 μl of the mixture of ciprofloxacin and the substance 1 were added to reach the above concentrations.

Cultures were incubated at 37° C for 18-20 hours.

The resulting culture were diluted with fresh LB to the density of 1 McFarland unit ($3 \cdot 10^8$ CFU), and a number of serial 1:10 dilutions was prepared in saline. The optical density of the solution was measured using a DEN-1B densitometer («Biosan», Latvia)

About 100 ul were taken from the 1/10 dilution and plated onto the surface of plates with LB agar with 70 µg/ml of rifampicin to count the frequency of rifampicin-resistant mutants appearance. From the dilutions, 10^{-6} and 10^{-5} 100 µl of the culture were plated to LB agar plates with no addition of rifampicin to count the baseline number of cells.[17] Luminescence measurements were performed in quadruplicates. Colony counting was carried out in 48 hours.

Survival rate was measured by the formula:

Survival rate, % = (n_e / n_c) 100%,

where: n_e - the number of colonies after incubation with the inducer, n_k - number of colonies after incubation without inducer.

The frequency of mutants resistant to rifampicin was calculated by the formula:

$F_m = n_a/a\ n_b$,

where: n - the number of colonies on the plate containing an antibiotic –containing medium; n_b - number of colonies on the medium without antibiotics; a - the dilution factor.

Statistical significance of the mutagenic effect was evaluated by statistically significant difference (t-test, $P < 0.05$) in the number of colonies between the experiment and control, considering dilution factor.

Results

Figures 1, 2 and 3 show the luminescence of biosensor cultures in the control, in the presence of ciprofloxacin and/or substance-**1** in concentrations of 0.01, 0.1 and 1mg/ml.

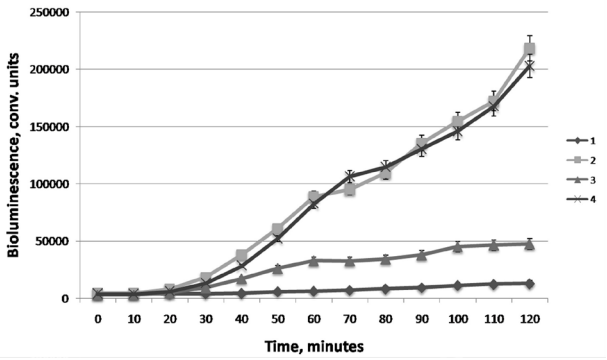

Fig. 1. *Luminescence of biosensor E. coli MG1655 (pRecA-lux) in the presence of Ciprofloxacinum with and without substance* **1** *(0.01 mg/ml).*

1 –control; 2 – 0.0001 mg/ml of Ciprofloxacinum; 3 – 0.01 mg/ml of substance **1;** *4 - Ciprofloxacinum with 0.01 mg/ml of substance* **1.**

As can be seen in the figures, the introduction of ciprofloxacin causes an increase in luminescence of cultures used in our study biosensor strain. That fact illustrates the activation of SOS-response. The maximum value of I_f obtained for the ciprofloxacin concentrations used in our experimental system was 16.06. Substance **1** in concentrations of 0.01, 0.1 and 1mg/ml also causes a weak SOS-induction. Maximum I_s values were 2.84, 1.95, 0.5, respectively. This effect could be explained by the interaction between substance **1** and RecA protein and/or other components of the SOS-reparation system. A lower concentration of substance **1** reduces the intensity of SOS-induction caused by ciprofloxacin. However, a statistically significant effect was observed in the first 60 minutes of incubation. Its absence in further processing seems to be due to bacteria metabolizing active ingredients.

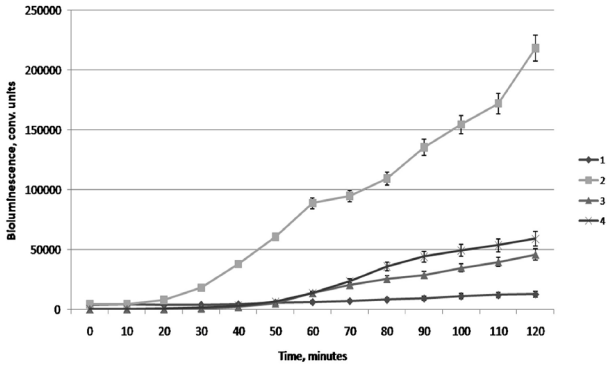

Fig. 2. *Luminescence of biosensor E. coli MG1655 (pRecA-lux) in the presence of Ciprofloxacinum with and without substance* **1** *(0.1 mg/ml).*

1 -control, 2 – 0.0001 mg/ml of Ciprofloxacinum; 3 – 0.1 mg/ml of substance **1;** *4 - Ciprofloxacinum with 0.1 mg/ml of substance* **1**

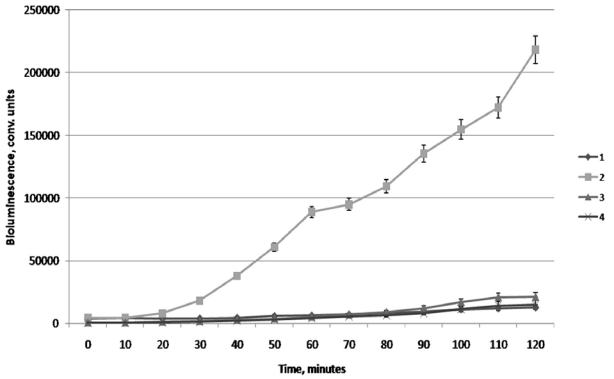

Fig. 3. *Luminescence of biosensor E. coli MG1655 (pRecA-lux) in the presence of Ciprofloxacinum with and without substance* **1** *(1 mg/ml).*

1 -control, 2 – 0.0001 mg/ml of Ciprofloxacinum; 3 – 1 mg/ml of substance **1;** *4 - Ciprofloxacinum with 1 mg/ml of substance* **1**

Concentrations of 0.1 and 1mg/ml gave more significant suppression of SOS-induction that was observed during the whole experiment. Thus, the anti-SOS effect of substance-**1** is generally dose-dependent, although the dependence is not linear (Fig.4).

Results of experiments on mutagenesis are given in Table 1. In our experimental system, substance 1 slightly (12%) but statistically significantly reduced the frequency of spontaneous mutagenesis. Ciprofloxacin more than doubled the frequency of mutants resistant to rifampicin. When ciprofloxacin and substance **1** were added together, a statistically significant mutagenic effect was also observed. However, in this case the increase in the frequency of mutations was about five times lower than in the case of a single action of ciprofloxacin.

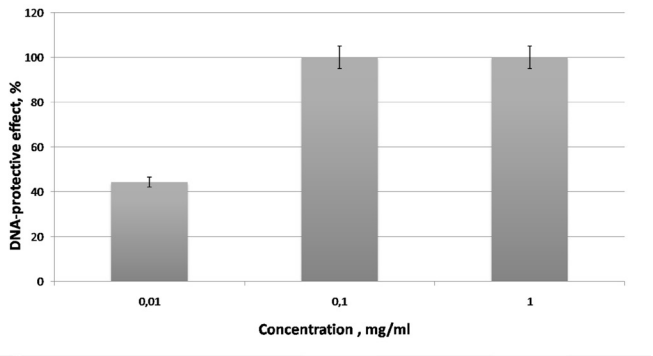

Fig. 4. *The maximum DNA-protective effect of substance* **1**

It should be noted that substance **1** slightly decreased the cell viability when compared to control. However, on the background of stress caused by ciprofloxacin, substance **1**, on the contrary, increased the survival rate of the bacteria. Therefore, the observed antimutagenic effect cannot be the result of an artifact associated with survival.

Discussion

Of considerable interest is the question of this phenomenon's mechanism. Besides DNA-protective activity, it was found earlier that substance-**1** has an ability to generate nitric oxide (II) *in vivo*.[12,16]

Another feature of this compound is its ability to exist in two isomeric forms, which are in **1** ⇌ **1'** equilibrium (Fig.5). This process of dynamic restructuring of the furoxan cycle is called 1,3-N-oxide tautomerism and may potentially facilitate its conformational adjustment to binding sites of biopolymers.

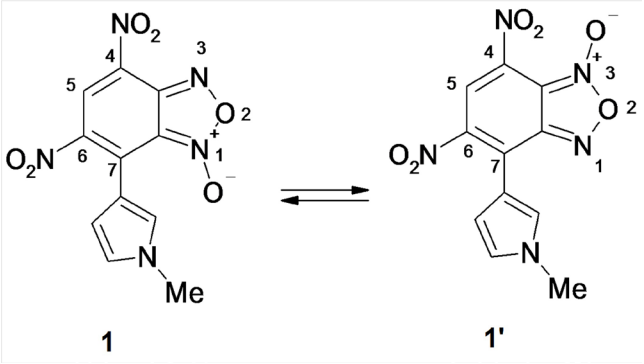

Fig. 5. *Two isomeric forms of substance* **1**

Figuring out how this activity relates to the ability to inhibit a RecA-mediated SOS-response will be the subject of our further research.

Extensive use of ciprofloxacin in medical and veterinary practice has led to the development of resistance up to 25% of species such as *Escherichia coli, Salmonella typhimurium, Streptococcus pneumoniae*, *Pasteurella multocida*, and *Staphylococcus aureus*.[18, 19] However, most of the standards of care for septic and infectious diseases include fluoroquinolones in general and ciprofloxacin in particular as the first-line drugs. That fact determines the utility of search for methods to reduce resistance.

Comparing our data on the biosensor test and the test for mutagenesis, it should be noted that the concentration of the substance **1** that causes the complete suppression of the Rec-induction did not block ciprofloxacin-induced mutagenesis completely. Apparently, the SOS-path is the main, but not the only, mechanism for the induction of mutations of antibiotic resistance by ciprofloxacin. However, based on our data, we can recommend using compound **1** as a starting point for the development of drugs that block mutagenesis associated with the emergence of antibiotic-resistant bacteria.

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Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Medicinal Plants

INTERNATIONAL JOURNAL OF BIOMEDICINE

Antidiarrheal Activity of Three Medicinal Plants in Swiss Albino Mice

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Abstract

Background: Different parts of *Allamanda neriifolia (AN)*, *Crinum latifolium (CL),* and *Bruguiera cylindrica (BC)* are used in folk medicine to treat diarrhea. Therefore, the aim of this study was to investigate and compare possible antidiarrheal activity of the crude extracts from barks, stems, and roots of AL, CL, and BC in Swiss albino mice.

Methods: Antidiarrheal activities of extracts were evaluated at three doses (100 mg/kg, 200 mg/kg and 400 mg/kg) and compared with Loperamide in a castor oil-induced diarrhea and charcoal meal test model in the Swiss albino mice.

Results: The aqueous extract of CL and BC administered at doses of 100, 200 and 400 mg/kg showed 0%, 24.5%, 62.26% and 5.66%, 37.11%, and 62.26% diarrhea inhibition, respectively. This reduction in diarrheal episodes is significant, and maximum effect was observed at the dose of 400 mg/kg similarly in the alcohol extracts of both CL and BC. AN administered at the dose of 100, 200 and 400 mg/kg showed 55.97%, 74.84% and 74.84% diarrhea inhibition, respectively. The aqueous extracts of AN, CL and BC were able to increase the percentage inhibition of the charcoal meal movement.

Conclusion: The antidiarrheal effect of the AN extract, in contrast to CL and BC, against the castor oil-induced diarrhea model prove its efficacy in an extensive range of diarrheal conditions.**(Int J Biomed. 2016;6(3):233-236.)**.

Key Words: Allamanda neriifolia • Crinum latifolium • Bruguiera cylindrica • antidiarrheal activity.

Introduction

Diarrhea is one of the leading death-causing diseases, especially in developing countries, so this is the most concerning issue for these countries. In view of this, WHO has initiated a Diarrheal Disease Control Program to study traditional medical practices and other related aspects.[1]

Children are more susceptible to this disease, which is the second leading cause of death of children under five years old. [2] The major causative agents of diarrhea in humans include *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli, Salmonella typhi,* and *Candida albicans*.[3,4] Diarrhea is a gastrointestinal tract (GIT) dysfunction, which is considered as a common symptom of infection and one of the causes of intestinal motility disorder.[5] It causes loss of water and important nutrients from GIT in addition to increasing intestinal motility.[6] The rate of material movement through the intestinal lumen is directly associated with its motility. As diarrhea causes high intestinal motility, the increased motility also heightens diarrheal effects through increasing the rate of movement of intestinal content.[7,8]

Castor oil is known to induce GIT enteropooling similar to that observed in diarrhea.[9-11] Its effect is mediated by ricinolic acid, which can induce a hypersecretory response from the gut wall, leading to diarrhea. [12-14] For this present study, three medicinal plants were selected and tested as antidiarrheal plants. The general information about these plants are shown in Table 1.

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Table 1.

General information about Allamanda neriifolia, Crinum latifolium and Bruguiera cylindrical

Plants	Local Name	Family	Traditional Use
Allamonda neriifolia	Gonta	Apocynaceae	Antifungal and antibacterial agent, cathartic.
Crinum latifolium	Sukhdarshan	Amaryllidaceae	Rheumatism, fistula, tumors, earaches, rubefacient, tubercle and whitlow agent.
Bruguiera cylindrica	Bakau Putih (Oriya)	Rhizophoraceae	Bleeding stopper and blood pressure controller.

Materials and Methods

Plant material

The bark, stem and leaves of these plants were collected in Bangladesh. *Allamanda neriifolia* and *Crinum latifolium* were collected from the rural area of the Noakhali region and *Bruguiera cylindrica* from Sonadia Island, off the coast of Cox's Bazar. The plant material was originally identified and authenticated by the Bangladesh National Herbarium, Dhaka.

Preparation of the plant extracts

The bark, stem and leaves were washed under a running tap to remove adhered dirt and then dried in shade at a temperature between 21 and 30°C for 15 days. After complete drying, these were ground into fine powders. The 400 g of each powdered crude plant was subjected to a cold extraction process by maceration with 2000 ml of 98% methanol at room temperature for 7 days. At the end, each macerate was filtered with Whatman No.1 filter paper, and the filtrate evaporated to dryness using a rotary evaporator (Buchi 011, USA). Residue left at the bottom of the beaker after evaporation was crude methanol extract of *Allamanda neriifolia*, *Crinum latifolium*, and *Bruguiera cylindrica*. The filtrate was air dried and stored in a refrigerator at 4°C for further testing.

Experimental Animal

Swiss albino mice of both sexes, aged 4-5 weeks, collected from Jahangirnagar University Animal House were used for the experiment. The animals were housed in polypropylene cages (30 cm x 20 cm x 13 cm) in standard conditions (room temperature 21±1.0 ºC and a 12-hr light/dark cycle) for 7 days before the experiment. The animals were fed with a standard diet and water *ad libitum*. The experiment was done in the Physiology Laboratory of University of Dhaka.

Preparation of Test Materials

Antidiarrheal activities of extracts were evaluated at three doses (100 mg/kg, 200 mg/kg and 400 mg/kg). Administering the crude methanolic extracts of *Allamanda neriifolia*, *Crinum latifolium* and *Bruguiera cylindrica* at noted doses required the amount of each extract to be measured and triturated in a unidirectional way by the addition of a small amount of suspending agent Tween-80. After proper mixing of extracts and the suspending agent, normal saline was slowly added. The final volume of the suspensions made was 2.5 ml. To stabilize the suspension, it was stirred well by a vortex mixer.

Antidiarrhoeal activity

Castor oil-induced diarrhea

This experiment was carried out by the slightly modified procedure previously described by Uddin et al.[15] and Awouters et al.[16] In this method, castor oil is used to induce diarrhea in all the experimental groups. Defecation is the primary way to measure the antidiarrheal effect. Each animal was constantly observed for consistency of fecal matter and frequency of defecation. The feces were collected with an absorbent sheet of paper placed beneath the transparent cages.[16] The wet feces were read at the end of the experiment by lifting up the upper part of the cage containing the sheet of paper and animals. The percentage inhibition of defection was measured using the following formula:

% Inhibition of defecation = (1 - B/A) x 100, were

A - Mean number of defecation by castor oil

B - Mean number of defecation by drug or extract

Experimental Design

Fifty-five mice were randomly divided into groups consisting of 5 mice in each group and fasted overnight before the experiment. Each group received a particular treatment. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. As it was difficult to observe the biological response of 5 mice at a time receiving the same treatment, it was necessary to identify individual animals of a group during the treatment. Each animal's tail was numbered with a marker to distinguish it from the others and marked as M1, M2, M3, M4, and M5. (Fig.1)

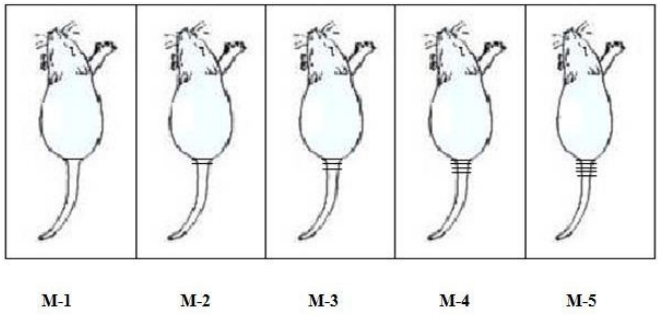

Fig. 1. *Identification of test animals*

Procedure

On the day of the experiment, the animals were divided into groups of 5 mice each. Control group (I) received 1% v/v Tween-80 in normal saline, 0.5 ml/mice); Positive control group (II) received Loperamide (Square Pharmaceuticals Ltd., Bangladesh, 5 mg/kg body weight). Other groups received crude extracts of selected plants at different doses.

Thirty minutes after the administration of the controls and test samples, 0.5 ml of castor oil was administered to each animal orally. The animals were placed in transparent cages

to observe for consistency of fecal matter and frequency of defection for four hours. After 4 hours, the total number of defecations for each mouse was taken, and then the data were evaluated statistically to find their significance. Each mouse of all groups was observed for consistency of fecal matter and frequency of defecation. The percentage inhibition of defection was calculated.

Intestinal motility test

A gastrointestinal (GI) motility test was done according to standard methods[7,17] with slight modifications. Control group I received 0.2 ml PSS. Group II (positive control) received atropine sulfate (5 mg/kg). Effect of the extracts on intestinal motility was evaluated at three doses (100 mg/kg, 200 mg/kg and 400 mg/kg). All administrations were made orally by gavage. Mice were given 1 ml of charcoal meal (5 g of activated charcoal suspended in 50 ml PSS) 30 min later through the same route. After another 30 minutes of charcoal treatment, animals were sacrificed in compliance with the NIH Guide for the Care and Use of Laboratory Animals. The distance traveled by the charcoal meal from pylorus to cecum was measured, and the percentage of inhibition of movement was calculated.[18]

%Inhibition=(MTLI-MDCC)/MTLI x100, were MTLI - mean total length of the intestine and MDCC - mean distance covered by the charcoal

Statistical analysis was performed using StatSoft Statistica v6.0. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Multiple comparisons were performed with one-way ANOVA and post-hoc Tukey HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results

The acute toxicity study showed that oral administration of alcohol and aqueous extracts of *Crinum latifolium* and *Bruguiera cylindrica* up to 200 mg/kg dose showed neither mortality nor any visible clinical signs of general weakness in the animals. The aqueous extract of *Crinum latifolium* and *Bruguiera cylindrica* administered at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg showed 0%, 24.5%, 62.26% and 5.66%, 37.11%, and 62.26% diarrhea inhibition, respectively (Table 2). This reduction in diarrheal episodes is significant, and maximum effect was observed at the dose of 400 mg/kg similarly in the alcohol extracts of both *Crinum latifolium* and *Bruguiera cylindrica. Allamanda neriifolia* administered at the dose of 100, 200 and 400 mg/kg showed 55.97%, 74.84% and 74.84% diarrhea inhibition, respectively. This shows significant reduction in diarrheal episodes with maximum effect at 200 mg/kg and 400 mg/kg dose level. In Group II, Loperamide showed a significant reduction in diarrheal episodes by 54.72%. The study reveals that the aqueous and alcohol extracts exhibited significant diarrheal activity. The antidiarrheal effect of the Allamanda neriifolia extract, in contrast to *Crinum latifolium* and *Bruguiera cylindrica*, against the castor oil-induced diarrhea model prove its efficacy in an extensive range of diarrheal conditions.

Table 2.

Effect of methanol extract of Allamanda neriifolia, Crinum latifolium, Bruguiera cylindrica on castor oil- induced diarrhea in mice

Group/Treatment	Dose (p.o.)	Number of stools	Inhibition (%)	*Statistics*
Control group I	0.5 ml/ mice	5.3 ±1.18		
Control group II (loperamide)	5 mg/kg	2.4 ±0.26	54.72	$P_{I\text{-}II}=0.000$
Allamanda neriifolia (1a)	100 mg/kg	2.3±0.33	55.97	$P_{I\text{-}1a}=0.000$ $P^{1a\text{-}2a}=0.000$ 3-9 0.000
Allamanda neriifolia (1b)	200 mg/kg	1.3±0.66	74.84	$P_{I\text{-}1b}=0.000$ $P^{1b\text{-}2a}=0.000$ $P^{1b\text{-}2b}=0.000$ $P^{1b\text{-}3a}=0.000$ $P_{1b\text{-}3b}=0.020$
Allamanda neriifolia (1c)	400 mg/kg	1.3±0.33	74.84	$P_{I\text{-}1c}=0.000$ $P^{1c\text{-}2a}=0.000$ $P^{1c\text{-}2b}=0.000$ $P^{1c\text{-}3a}=0.000$ $P_{1c\text{-}3b}=0.020$
Crinum latifolium (2a)	100 mg/kg	6.3±0.88	0	$PI_{I\text{-}2a}=0.000$ $P_{2a\text{-}2b}=0.004$ $P^{2a\text{-}2c}=0.000$ $P_{2a\text{-}3b}=0.000$
Crinum latifolium (2b)	200 mg/kg	4.0±1.00	24.53	$P_{2b\text{-}2c}=0.020$
Crinum latifolium (2c)	400 mg/kg	2.0±0.00	62.26	$P_{I\text{-}2c}=0.000$ $P_{2c\text{-}3a}=0.000$
Bruguiera cylindrica (3a)	100 mg/kg	5.0±1.73	5.66	$P_{II\text{-}3a}=0.000$
Bruguiera cylindrica (3b)	200 mg/kg	3.3±0.66	37.11	$P_{I\text{-}3b}=0.020$
Bruguiera cylindrica (3c)	400 mg/kg	2.0±0.00	62.26	$P_{I\text{-}3c}=0.000$ $P_{3a\text{-}3c}=0.000$

Table 3.

Effect of methanol extracts of Allamanda neriifolia, Crinum latifolium, Bruguiera cylindrica on inhibition of GI motility

Group	Dose (mg/kg)	MTLI (cm)	MDCC (cm)	Inhibition (%)
Group I	0.2 ml PSS	42	30	
Group II	5 mg/kg	37.1	32.5	12.4
Allamanda neriifolia	100 mg/kg	39	24	38.5
	200 mg/kg	40	26	35
	400 mg/kg	40	27	32.5
Crinum latifolium	100 mg/kg	43	21	51.2
	200 mg/kg	42	22	47.6
	400 mg/kg	39	26	33.3
Bruguiera cylindrica	100 mg/kg	40	25	37.5
	200 mg/kg	41	27	34.1
	400 mg/kg	40	29	27.5

Inhibition of GI motility by crude extracts of *Allamanda neriifolia, Crinum latifolium* and *Bruguiera cylindrica* is presented in Table 3. The studied extracts were able to increase the percentage inhibition of the charcoal meal movement.

Discussion

For present investigations, the castor oil-induced diarrhea model is used for the evaluation of the antidiarrheal property of drugs. Ricinoleic acid, the active component of castor oil, is responsible for its diarrhea-inducing property.[19] It stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestine mucosa. Its action also stimulates the release of endogenous prostaglandins, which in turn stimulate motility and secretion.[20] Medicinal plants are a promising source of antidiarrheal drugs.[21] The present study showed that the methanol extracts of *Allamanda neriifolia, Crinum latifolium*, and *Bruguiera cylindrica* at a dose of 400 mg/kg exhibited a significant inhibition of castor oil-induced diarrhea in experimental mice, although *AN* exhibited significant inhibition in all cases (100 mg, 200 mg and 400 mg). Tanin, alkaloids, saponins, sterols and terpenoids present in plants are responsible for antidiarrheal activity.[21] The above constituents may be present in the studied extracts. Further studies are needed to isolate the active substances from *Allamanda neriifolia*, *Crinum latifolium* and *Bruguiera cylindrica* for a clear understanding of the mechanisms of their actions.

Competing interests

The authors declare that they have no competing interests.

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Point of View

The Epistemology of Wrinkles: From Geology and Anatomy to Physiology

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Abstract

The author studies the geometry and physics of wrinkling – drawing from geology and animal biology. The general principles behind tension and compression wrinkles are the same in geology or dermatology. But what is the structure in the subcutaneous tissue that marks every overlying wrinkle? Given we now know wrinkles have both anatomical and pathological correlations considering wrinkles as pure anatomic lines is fallacious and the article offers a unique perspective of skin creases and also avenues for further research. **(Int J Biomed. 2016;6(3):237-239.)**.

Key Words: wrinkles • surgery • geology • anatomy • physiology • ageing • skin

Discussion

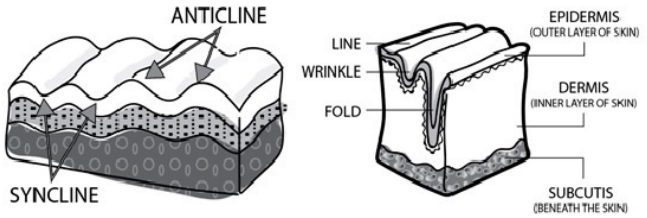
In 2003, Cerda and Mahadevan[1] studied the physics and geometry of membrane wrinkling – they took a food-grade plastic sheet, cut ribbons from the middle and stretched the sheet. They noted that wrinkles appeared parallel to the ribbon, and the wavelength (λ) was proportional to the square root of the sample size. They then extrapolated their research to human skin. They noted that human wrinkling is generally more notable where there is excess skin (such as back of hands) or where skin is close to bone (such as forehead or crow's feet).

If human skin is viewed as a mere physical membrane, wrinkles fundamentally occur because a keratinocyte-stiffened epidermis drapes a softer and thicker dermis. Of course, anatomical sites like knees and elbows have wrinkles that can be considered 'tension' wrinkles (two-dimensional, due to geometry, pre-tension and joint action) and in other areas like forehead, muscle action causes 'compression' wrinkles (one-dimensional due to muscle action only).

The author has been fascinated by the concept of wrinkles and relaxed skin tension lines being used as surgical guidelines, given these have become widely adopted with no conclusive scientific basis – and both types of lines differ in direction, yet are considered acceptable for surgical incisions. In animals such as pigs, for example, with age and fat deposition skin tension lines begin to run transversely, whereas in thinner animals, skin tension lines run more obliquely.[2] To get a better understanding of any condition, it is better to study its loss or excess. In 2010,[3] there was a report of a Japanese baby with Michelin Tire Baby Syndrome, a condition that causes folding of excess skin. However, as was noted in this case report, in some babies with this syndrome spontaneous recovery occurs i.e. wrinkles simply disappear. Most notably, skin fold biopsies from these babies showed no epidermal, dermal or subcutaneous tissue abnormalities. Surely, there must be an underlying anatomical reason why wrinkles occur?

Geologically speaking, folds occur in the Earth's 'skin' are conceptually no different to wrinkles on any multi-layered surface. These geological folds occur when flat and stacked planar surfaces, such as sedimentary strata, are bent or curved as a result of permanent deformations. To the author, geological folds seem epistemologically related to cutaneous folds, as illustrated in the figure. Berg [4] proposed a model for anticline formation -- showing that ranges became raised along a partially listric, sub-horizontal thrust fault. However, this model showed the down-dip portion of the fault plunging at depth, which was only possible if basement rocks were plastic -- and therefore this theory was later discredited.[5] Later, geologists adopted the model of 'balanceable cross sections'.[5] On observing the anticline formations in old rocks, it appears that it is the resistance to forward movement of the layers in front causes the rocks to bend upward in asymmetric fashion, causing wrinkling. Anticlines occur

in old rocks just as wrinkling occurs in aging skin. These geological 'compression' wrinkles gives rise to shortening of the beds across the structure as the area becomes horizontally compressed, not dissimilar conceptually to the formation of cutaneous wrinkles with age. However, there was one matter unresolved – geologically there are differences notable in the underlying strata, -- but histologically, as we discussed in the Michelin Tire Baby case above, there was no underlying differences noted on anatomic pathology examination.



Pessa[6] and others set out to answer this question – is there an underlying anatomical basis for wrinkles – can biology follow geology? Pessa studied both creases such as crow's feet, and forehead wrinkles. They found that each and every wrinkle they studied occurred within ±1 mm of a major lymphatic vessel and its surrounding tube of adipose tissue. They concluded that an anatomical basis for wrinkles does exist and these are lymphatic vessels, along with the surrounding distinct peri-lymphatic fat, which are present directly beneath wrinkles and creases. This may have implications for surgery. Until recently, incisions were planned in wrinkle or crease lines with the theory being as collagen runs parallel to wrinkle lines, collagen will be laid down in the same direction as is normal within wrinkle lines and the septa between skin and muscle -- leaving to the best possible scars. However, the author's group recently noted that incisional and excisional lines are different i.e. when skin is excised and tension created, as is done after skin cancer surgery, different dynamics apply and wrinkle lines no longer have the least wound tension.[7]

More recently, it has come to light that wrinkles can be caused due to aging and oxidative stress – and indeed increased wrinkling may be a sign of impending heart disease, metabolic disorders, osteoporosis or degenerative disorders.[8] And, while photo-damage from sunlight is one of the most common causes in Caucasian skin, recent studies have shown that UV light also damages lymphatic channels.[9] Some of the genetic mechanisms behind wrinkling are disturbed lipid metabolism, altered insulin and STAT3 signaling, up regulation of apoptotic genes partly due to the deregulation of FOXO1, down regulation of members of the jun and fos family, differential expression of cytoskeletal proteins (e.g., keratin 2A, 6A, and 16A), extracellular matrix components (e.g., PI3, S100A2, A7, A9, SPRR2B), and proteins involved in cell-cycle control (e.g., CDKs, GOS2).[10]

The direct finding of an anatomical relationship between lymphatic channels and wrinkles is a good direction for new research. This has implications for not only surgical lines, but also in understanding why chronic illness or cancer often accelerates the aging process. Tybjærg-Hansen and others studied over 10,000 Danes and found that ear lobe creases indicated the body's biological age, and was an indicator for heart disease.[11] Studies on Shar Pei dogs suggests that these animals have mucinosis – high hyaluronic acid levels are found in both cutaneous tissues and the blood stream -- and these high amounts are due to an excess in the activity (overexpression) of the HAS2 enzyme.[12] Such research avenues are promising as they can shed more light on processes such as cell recognition and ageing.

Conclusion

In surgery and medicine, thus far, wrinkles have been considered purely as a surface anatomical phenomenon -- with the main purpose in surgery being the concealment of skin incision scars. However, given we now can see that below each such crease, there exists an anatomical correlation to a lymphatic vessel, the implications are still poorly understood. For example closing a wound in a fashion that creates compression of the lymphatic vessels may be less than desirable. Further, we now know that wrinkles in certain locations indicate both the body's biologic age, as well as a risk for heart disease. We also know that high hyaluronic acid levels due to enzymatic dysfunction can cause deep wrinkling in animals – and therefore when it comes to wrinkles beauty may indeed be more than skin-deep. The author's perspective is that until now we have been viewing wrinkles purely as fault lines of skin i.e. using a geological or anatomical approach -- when what is needed is more research behind the physiology of wrinkles.

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Author contributions

S.P was the sole author and conceptualized and conducted this study.

Competing financial interests

Nil.

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The article should include a brief abstract of no more than 200 words. The abstract should be structured with the following headings: Background, Methods and Results, and Conclusions. The Background section should describe the rationale for the study. Methods and Results should briefly describe the methods and present the significant results. Conclusions should succinctly state the interpretation of the data.

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- ✓ conception or design
- ✓ data collection and processing
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- ✓ writing substantial sections of the paper.

Acknowledgments

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Book: Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. *Medical Microbiology*. 4th ed. St. Louis: Mosby; 2002.

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• Key words

• Text

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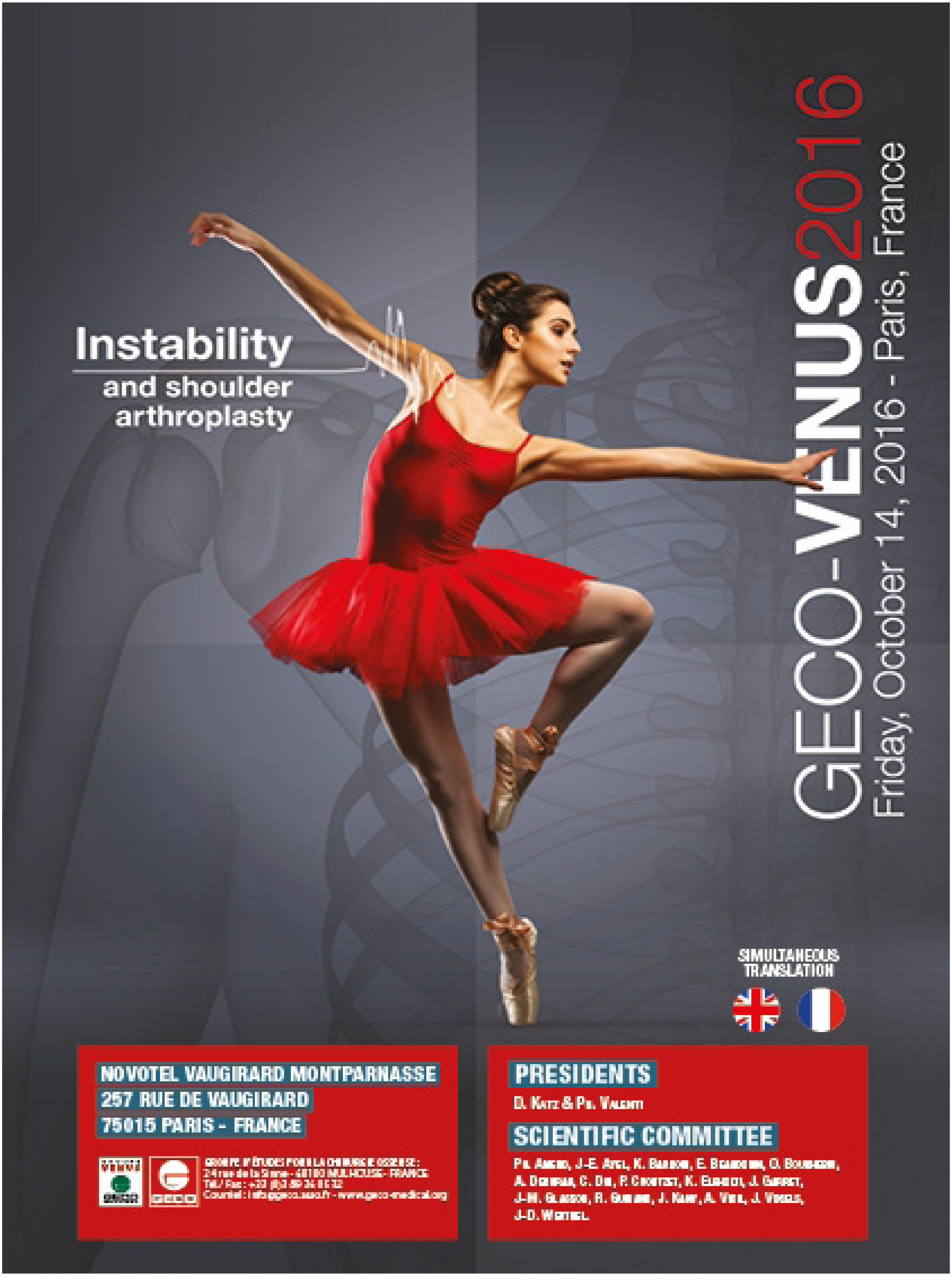
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