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OXIDATIVE STRESS IN HUMAN THYROID GLAND UNDER IODINE DEFICIENCY NODULAR GOITER: FROM HARMLESSNESS TO HAZARD DEPENDING ON COPPER AND IODINE SUBCELLULAR DISTRIBUTION

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Background. *Thyroid disorders are the second most common endocrinopathies found in humans and animals. Determination of their key molecular markers presents a special interest.*

Objective. *We studied iodine and copper accumulation in nodular, paranodular and contralateral (not affected tissue by node) tissues of human thyroid gland in relation to the level of metal-binding proteins, potential antioxidants, and oxidative changes in tissue for this goal. Lower level of organificated iodine and higher level and mass fraction of inorganic iodine and copper in the nodular and paranodular tissue versus contralateral part of thyroid gland was established.*

Results. *The level of both metal-binding and apo-form of metallothioneins was higher. Content of reduced glutathione was lower in node-affected tissue compared to the contralateral part. Signs of oxidative stress (higher activity of superoxide dismutase, catalase, glutathione-transferase and level of oxyradicals) and cytotoxicity (higher cathepsin D activity, higher level of DNA strand breaks and glycolysis activation) in affected tissue were observed. The range of indice variability in paranodular tissue was smaller than in nodule compared to the parenchyma of contralateral part.*

Conclusions. *Excess of copper unbound to metallothionein in goitrous-changed tissue and high level of inorganic iodine could be the reason for elevated DNA fragmentation and increased lysosomal membrane permeability and activation of antioxidant defense. The main criterions of goiter formation were represented by low level of organificated iodine and high level of DNA damage in thyroid gland.*

KEY WORDS: **iodine deficiency nodular colloidal goiter, iodine, copper, metallothioneins, oxidative stress, cytotoxicity**

Introduction

Thyroid disorders are the second most common endocrinopathies found in humans and animals [1]. Determination of the key molecular markers is of considerable interest as they can be used to predict such pathologies. Iodine deficiency in thyroid pathology occurrence rate is rapidly increasing [2]. It has miscellaneous origins as a result of complex interaction of endogenous and exogenous factors and arises in the setting of high level of "nonspecific" goitrogens, among them copper, in the environment. Copper is an essential element for humans and animals, especially for antioxidant defense and the metabolism of the amino acid tyrosine, which is needed for the production of thyroid hormones [3]. Besides these ones, excessive amounts of copper in the body can pose a risk. The mechanisms underlying the acute toxicity effects of copper in humans are not well understood. It is reasonable to speculate that they probably represent a combination of significant oxidative stress at different body areas together with marked perturbations in several components of the endocrine system. It was shown that the increase of copper level in the thyroid gland of patients with colloidal goiter was combined with the prooxidant changes in tissue [4]. It should be noted that Ternopil region with combination of moderate iodine deficiency and high scale of copper water pollution [5] presents an interest to study the relationship between copper accumulation and progress of thyroid pathology.

Free radical-mediated oxidative damage has been implicated in pathogenesis of many diseases. However, the reasons of this phenomenon are disputable under endemic nodular thyroid goiter. It is believed that the prooxidant effect of copper depends on the specific accumulation by metal binding protein, such as metallothioneins [6]. Metallothioneins (MTs) are low-molecular weight proteins of 6–7 kDa, with high content of cysteine (30 %) and complete absence of aromatic amino acids and histidine, capable of binding transition metals with high affinity. MTs synthesis can be induced by a variety of metals, cytotoxic agents, stress-producing conditions, cytokines, and glucocorticoid hormones [7]. Additionally, MTs can function as efficient scavengers of reactive oxygen species to preserve homeostasis of cells. The latest study have indicated a possible role of MTs as a tumor suppressor in papillary thyroid cancer [8] and

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their part in the distribution of metals, thereby optimizing the function of thyroid gland [4].

Thus, the aim of this study was to evaluate the relation between the function of MTs and oxidative stress in node, paranodular and non-affected by node contralateral part tissue of human thyroid gland under iodine deficiency endemic nodular thyroid goiter. Molecular markers of cytotoxicity were used to assess the severity of the pathological process.

Methods

The target population in this study were the people with unilateral euthyroid iodine deficiency nodular thyroid goiter, who had lived in Ternopil Region for at least 22 years. Small samples of nodule, paranodular and contralateral (not affected by node tissue) tissues from thyroid gland were dissected in 25 patients. They were operated at the General Surgery Department of the local Ternopil Emergency Hospital. All experimental studies were conducted in accordance with the approval of the First National Congress on Bioethics (Kyiv, 2000) and the approval of the Bioethics Commission of I. Ya. Horbachevsky Ternopil State Medical University.

Tissue samples were homogenized (1/10 w/v) in 0.1 M phosphate buffer, pH 7.4, containing 100 mM KCl and 1 mM EDTA, as well as 0.1 mM phenylmethylsulfonyl fluoride (PMSF) for proteolysis inhibition for enzymatic measurements. Homogenates were centrifuged for 10 min at 6,000 *g*. Protein concentration in the supernatant (soluble protein) was measured by the method of Lowry et al. (1951), using bovine serum albumin as a protein standard. The absorbance values were measured on an UV/Vis spectrophotometer "Lomo-56" (Russia), and extinction/emission values were measured on the *f*-max fluorescence microplate reader [Molecular Device (USA)].

Reduced glutathione (GSH), 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), glutathione reductase from baker's yeast (*S. cerevisiae*), 2-vinylpyridine, dihydrorhodamine, salmon sperm DNA, Hoescht 33342, serum albumin, phenazinemethosulfate, phenylmethylsulfonylfluoride (PMSF), β-mercaptoethanol, NADH, β-NADPH, and EDTA were purchased from Sigma-Aldrich. All chemicals were of the analytical grade or better.

MTs were determined in parts of thyroid gland tissue after ethanol/chloroform extraction by thiol measurement with DTNB according to the method of Viarengoet al. (1997) [9]. The level of MT-related thiols (MT-SH) was calculated by using following relationship: 1 mol MT-SH = 20 mol GSH. The level of MTs was defined as μg of MTs per gram of fresh weight (FW) tissues. To assess metal concentration in the MTs (MT-Me), they were isolated as thermostable proteins by size-exclusion chromatography on Sephadex G-50 with necessary adjustments needed to avoid their oxidation [10], as described previously [11]. A 5 % homogenate (w/v) was prepared in ice-cold 10 mM Tris-HCl buffer, pH 8.0, containing 10 mM 2-mercaptoethanol for maintaining of the reduced conditions and 0.1 mM PMSF for the inhibition of proteolysis. Fractions of the chromatographic peak with high absorbance at 254 nm and comparative high density ratio $D_{254}/D_{280,}$ identified as MT-containing peak [12], were pooled (total 10 mL) and applied to metal determination.

To determine copper and zinc concentration, fresh tissues (500 mg) and pooled eluate of MTs fraction after the size-exclusion chromatography (10 mL) were digested in 5 mL HNO_3 (Merck) for 3 h at 105 °C for metal analysis using hermetic acid-cleaned Teflon bomb. Concentration of metals was analyzed by the atomic absorption spectrometer with flame detector (C-115, "LOMO", Russian Federation). The metal detection limits were 0.1 $\mu g \cdot g^{-1}$ FW. The analytical methods were validated by external intercalibrations. Quality control was assessed by the Quality Control Sample of trace metal and method of Standard Addition (www.dentalmercury.com/245_1.pdf). Metal concentration in the tissues and MTs was presented as $\mu g \cdot g^{-1}$ FW and $nmol \cdot g^{-1}$ FW. Subcellular distribution of iodine in the thyroid gland was determined by previously developed method [13].

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by the method of Beauchamp and Fridovich (1971) [14] based on the aerobic reduction of NBT at 535 nm by superoxide radicals and expressed as $units \cdot mg^{-1}$ soluble protein; 1 unit of SOD activity is defined as the amount of protein causing 50 % inhibition of the rate of NBT reduction.

Catalase (EC 1.11.1.6) activity was measured by monitoring decomposition of 10 mM H_2O_2 according to Aebi (1974) [15] at 240 nm (ε=40 $M^{-1} \cdot cm^{-1.}$) in buffer containing 50 mM KH_2PO_4 (pH 7.0) and approximately 150 μg of proteins. The results were related to soluble protein.

Total glutathione (GSHt) concentration was quantified by the glutathione reductase recycling assay [16]. To estimate the oxidized glutathione (GSSG) level, the protein free sample was treated with 2-vinylpyridine for 60 min prior to assay at 2 % final concentration [17]. The rate of 5-thionitrobenzoic acid formation was monitored spectrophotometrically at 412 nm. Standards were prepared from GSH, and concentrations were defined as nmol per g wet weight. The redox-index of glutathione (RI GSH) was calculated as the ratio of concentrations [GSHr]/[GSH].

Evaluation of oxyradical formation in thyroid gland tissue (1/10 w/v) homogenates was determined using the non-fluorescent derivative, dihydror-

hodamine, which is converted to the fluorescent dye, rhodamine-123, while reacting with reactive oxygen species [18]. The fluorescence signal was detected by using a*f*-max fluorescence plate-reader [excitation = 485 nm, emission = 538 nm] immediately, and in 20 min.

Glutathione-S-transferase (GST, EC 2.5.1.18) activity was measured by CDNB as the substrate [19]. Enzymatic activity was determined at 25 °C by monitoring changes in absorbance at 340 nm for 2 min. The GST activity was defined as nmol $min^{-1} \cdot mg^{-1}$ soluble protein.

The activity of lactate dehydrogenase (LDH, EC 1.1.1.27) was determined using the UV assay with pyruvate and NADH [20] by determining of the amount of NADH oxidation at 340 nm. Phosphate/ pyruvate solution (3 mL) (50 mM phosphate, pH 7.5, 0.63 mM pyruvate) was pipetted into cuvettes and 50 mL NADH solution (11.3 mMb-NADH) was added. Following this, 100 mL of sample was spiked and mixed. The extinction was checked after very minute interval for a period of 4 min. A molar extinction coefficient of $6.22 \cdot 10^6$ $M^{-1} \cdot cm^{-1}$ was used.

DNA fragmentation was evaluated by the determination of the levels of protein-free DNA strand breaks in the digestive gland by the alkaline DNA precipitation assay [21] using Hoescht 33342. The reduction of possible interference of traces of SDS in the supernatant was measured in the presence of 0.4 M NaCl, 4 mM sodium cholate, and 0.1 M Tris (pH 9). Probe fluorescence signal was detected by using a*f*-max fluorescence plate-reader [excitation = 360 nm, emission = 450 nm]. Quantitation was done using known amounts of salmon sperm DNA in the same mixture without addition of sample and determined as ng of protein-free DNA per mg tissue soluble proteins.

Cathepsin D (EC 3.4.23.5) activity was assayed spectrometrically as described previously [22] in incubation mixtures containing buffered haemoglobin solution [4% (w/v) in 0.25M-sodium acetate buffer, pH5.0].

All measurements were carried out using samples from 25 patients. The results were defined as means ± standard deviation (SD). Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests. Since data were not normally distributed (Lilliefors' test), non-parametric tests (Kruskall–Wallis ANOVA and Mann–Whitney U-test) were performed (significant at $P<0.05$). For detection of correlation, the Pearson's correlation test was also performed at a 0.05 level of significance.

Data were subjected to Multiple regression analysis and Principal component analysis (PCA) with NIPALS algorithm to differentiate the group by the set of their indices and select distinguished criterions. Classification trees were built using Classification and Regression Tree (CART) software on the basis of all determined biological characteristics. All statistical calculations were performed by means of Statistica v 8.0 and Excel for Windows 2000.

Results

The results showed the coherent activation of superoxide dismutase (by 81 %), catalase (up to two times) and glutathione-transferase (by 212 %), decrease of GSH level (by 33 %) and the increase of metallothioneins level (both MT-SH and MT-Me) in affected part of thyroid gland (Fig. 1, 2). Higher level of oxyradicals (by 21 %) and GSSG (up to 41%) has been also detected in these samples. A relation between MT-SH and oxyradical level in thyroid gland was proved. Signs of cytotoxicity, higher free cathepsin D activity (up to 84.6 % and 134.4 % in paranodular tissue and node respectively), and higher level of DNA strand breaks in node (up to 22.6 %), were observed (Fig. 3). Also, activation of glycolysis in the affected part of thyroid gland was observed. The accumulation of reactive oxygen species ($r = 0.72$, $p<0.01$) and initiation of oxidative stress in the cell could be the reason for shifting of

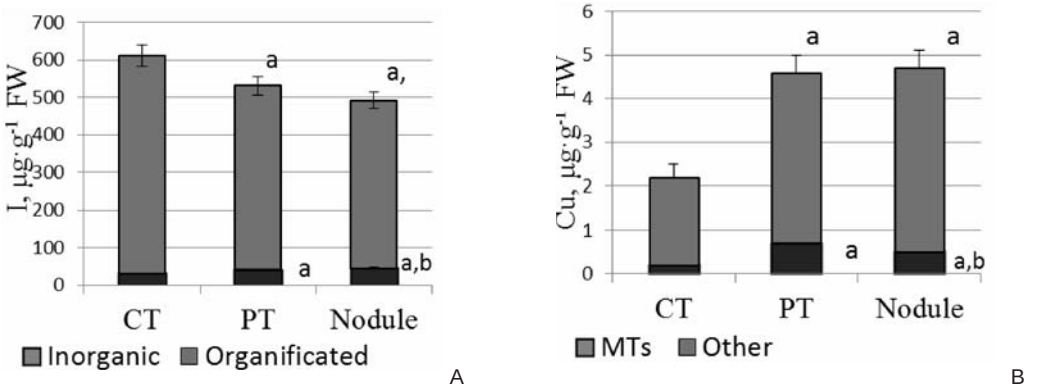

Fig. 1. Subcellular distribution of iodine (A) and copper (B) in the human thyroid gland under iodine deficiency euthyroid nodular goiter, μg×g^{-1} FW, mean ± SD (N=25). In Fig. 1 – 4, the significant differences were regarded as ($p < 0.05$); [a], differences compare to contralateral part; [b], differences between paranodular tissue and nodule.

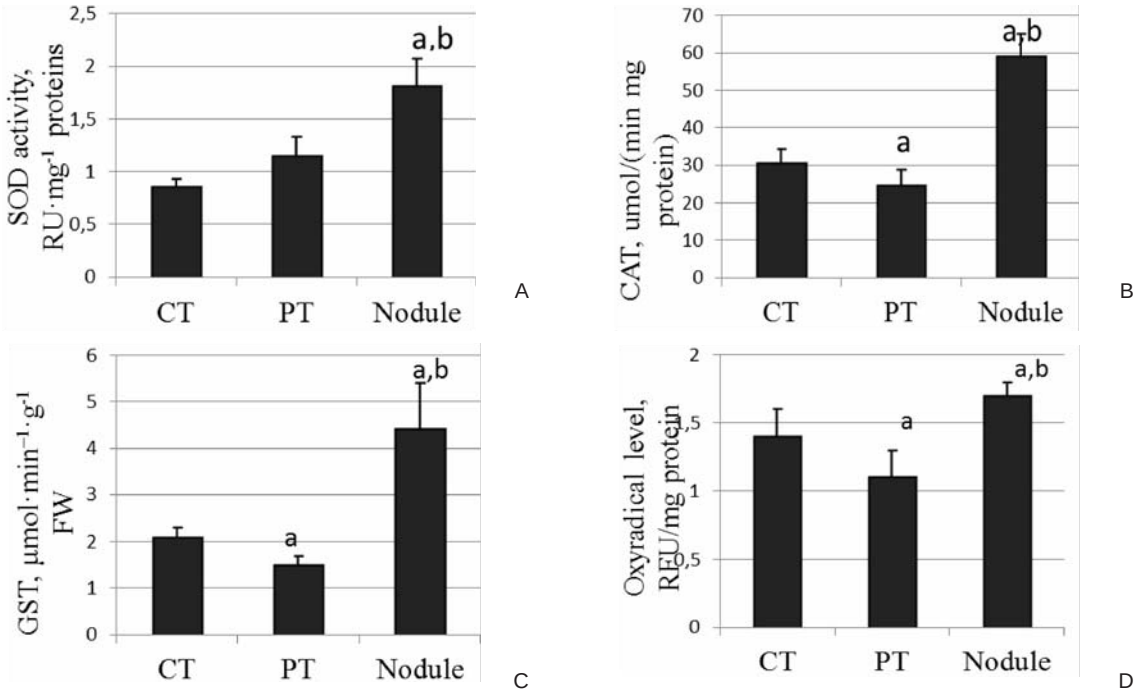

Fig. 2. Oxidative stress parameters in the human thyroid gland under iodine deficiency euthyroid nodular goiter. Data for A: superoxide dismutase, B: catalase, C: glutathione transferase, D: oxyradical level are present as means ± SD (N=25).

energy balance to anaerobiosis [23]. Range of indices variability in paranodular tissue compared to parenchyma of contralateral part was lesser than in the nodular tissue, but had generally the same trend.

The most important finding was lower level of organificated iodine (by 23 and 15 %, respectively) and higher level (by 46 and 32 %, respectively) and mass fraction (by 42% and 82% respectively) of inorganic iodine in the nodular and paranodular tissue *versus* contralateral part of thyroid gland (Fig. 4). The disruption of iodine organification in hyperplastic thyrocytes could be caused by elevated copper level (more than twice) in thyroid gland. Copper excess in the affected tissue partly accumulated in MTs. The binding ability of MTs with copper in the nodule was lesser than in the paranodular tissue.

According to PCA test with NIPALS algorithm (Fig. 5, A), two sets were the most distinguished: nodule was characterized by oxidative stress indices, MTs characteristics, and copper distribution, whereas non-affected contralateral part was located jointly with organificated iodine and GSH level. There was no clear characteristic related to the paranollar tissue. Furthermore, it was important to select the main distinguished criterion for pathological process progress at the nodule formation. We used CART analysis (Fig. 5, B) to achieve this. Followed by analysis of all biochemical parameters of every group, the contralateral part of thyroid gland was singled out by its ability to iodine organification. Paranodular tissue and nodule in affected part of thyroid gland were distinguished by level of DNA fragmentation.

Discussion

It is generally known that nodules develop in pathologically affected thyroid tissue [2]. We established that hyperplastic thyroid epithelium under iodine deficiency nodular colloidal goiter sacrificed its ability for iodine organification and therefore increased of level of inorganic iodine. This pattern designated with higher copper level (r = 0.69, p<0.01), manifestation of oxidative damage (increased of SOD, catalase and GST activity (r = 0.73, r = 0.59 and r = 0.64 correspondingly, p<0.01 and MT-SH level (r = 0.97, p<0.001)) and cytotoxicity (increased DNA fragmentation, r = 0.51, p<0.01) in affected by nodule part of thyroid gland. Therefore, it can be assumed that under deprivation of iodine organification surplus of it has been created in human thyroid gland and determined further formation of iodine toxic intermediates in hyperplastic thyrocytes after its oxidation by thyroperoxidase [24]. This scenario of a stimulating effect on the antioxidant defense system was proved. Similar results, such as increased lipofuscin level, lipid peroxidation, necrosis of epithelial cells, and destabilization of mitochondrial membranes and development of autoimmune processes in the tissue of the thyroid gland were obtained after iodine application in micromolar range into human body [25].

It was proved that apoptosis was among the major determinants of pathological conditions progression [24]. Cathepsin D is a lysosomal

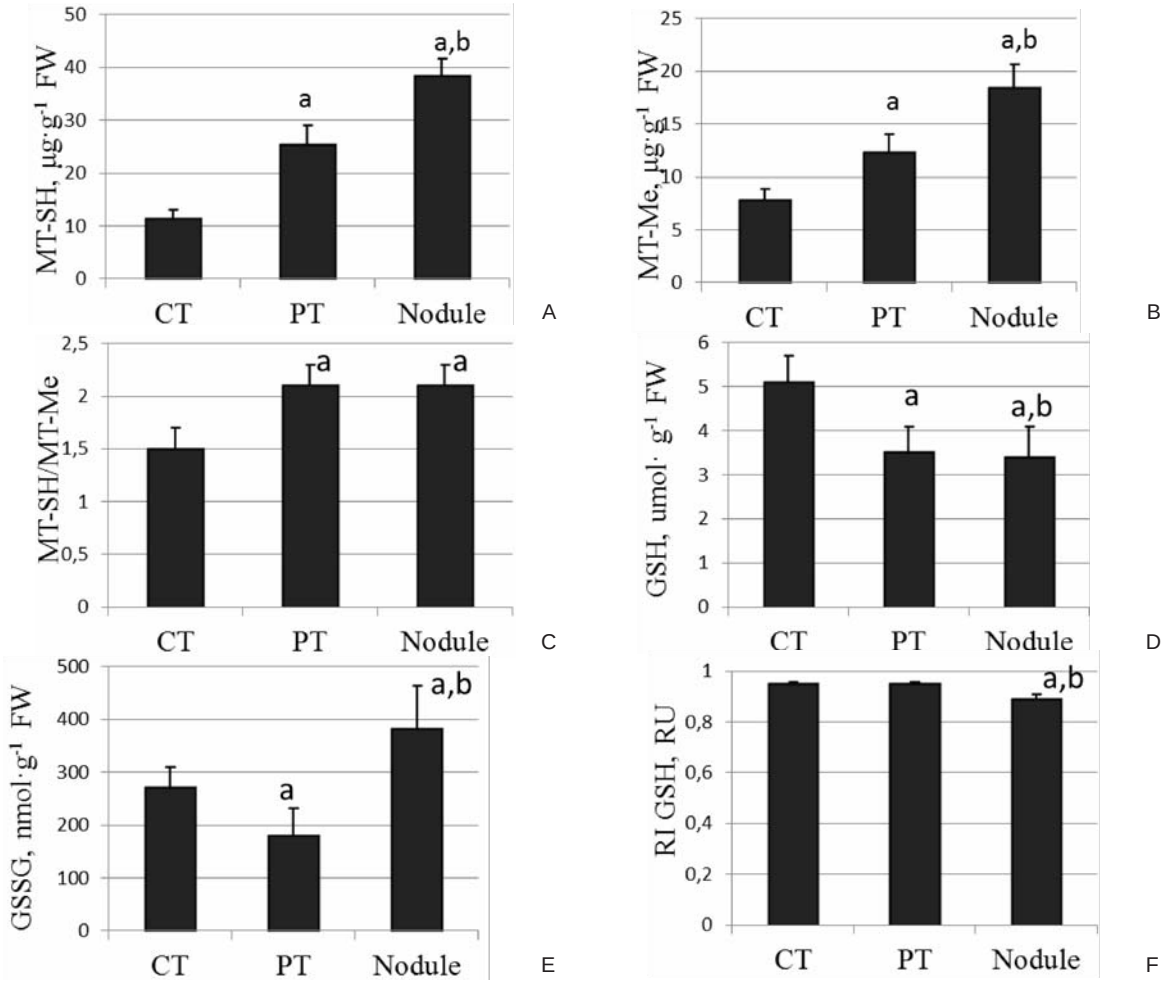

Fig. 3. Characteristics of metallothioneins (A-C) and glutathione (D-F) in the human thyroid gland under iodine deficiency euthyroid nodular goiter. Concentration of MT-SH (A); concentration of MT-Me (B); MT-SH/MT-Me concentration ratio (C); Levels of reduced glutathione (D), oxidized glutathione (E) and redox index of glutathione (RI GSH) (F). The values are expressed as the mean ± SD (N=25).

endopeptidase. It involves in the processing of thyroglobulin, and belongs to mediators of IFN-γ and TNF-α-induced lysosomal programmed cell death pathway [26]. We were observing its increased level in patients with endemic nodular goiter. It was related to the increase of copper level in the thyroid tissue ($r=0.63$, $p<0.01$), and particularly, in potentially toxic, unbound with MTs form ($r=0.67$, $p<0.01$). We can speculate that copper accumulated in lysosomes and consistently caused their swelling, activation of calcium-dependent phospholipase A2 [27], and determined destabilization of lysosomal membranes. The *in vivo* release of the enzymes from the organelles, which happened consequently, has been suggested to play a fundamental role in mediating caspase activation, DNA fragmentation and apoptosis [28].

Recently, it has been shown *in vitro* that MTs and GSH form a part of the pool of cellular thiols and their function in the cell is cohered [29]. We can conclude the same regulation in human thyroid tissue ($r=-0.79$, $p<0.001$) based on our observations. MTs might act as an effective scavenger against reactive oxygen species due to high level of SH-group under weakness of glutathione functional ability, which could indicate a compensatory mechanism. Despite the fact that the MTs level in the cell is lower than GSH, they have exhibited 50x higher antioxidant activity by the molar ratio of thiols[30]. We detected a reverse relation for the MT-SH level and oxyradicals level, which indicated a leading role of MTs in detoxification of oxyradicals: $oxyradicals=-268.4+88.2\times GSSG-4.7\times GSH+405.5\times RI\ GSH+6.4\times GST-2.1\times MT\text{-}SH^{*}$; $R^2=0.45$, $F(5,12)=4.7$, $p<0.03$ (* – indicated significant contribution into the mathematical model). Similar results were obtained by comparing of goitrous-changed and intact human thyroid gland tissue [4].

Conclusions

To summarize, the combination of endemic iodine deficiency with a high environmental copper level increases the risk of node formation and progress of pathological changes. At low level of

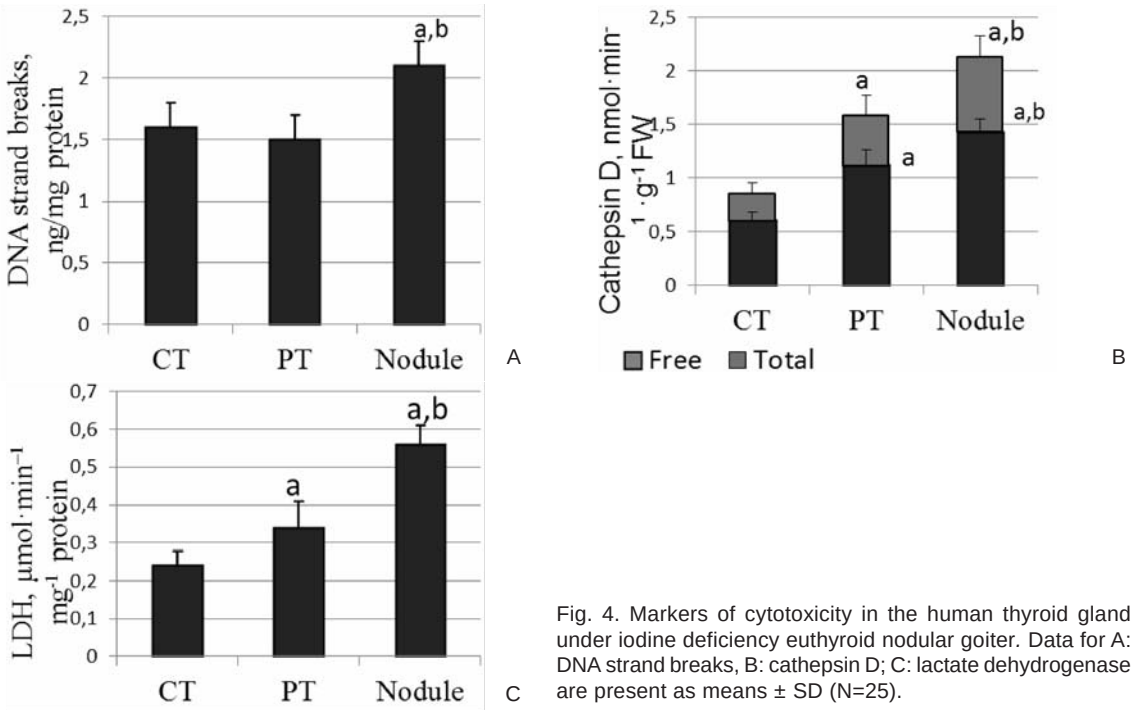

Fig. 4. Markers of cytotoxicity in the human thyroid gland under iodine deficiency euthyroid nodular goiter. Data for A: DNA strand breaks, B: cathepsin D; C: lactate dehydrogenase are present as means ± SD (N=25).

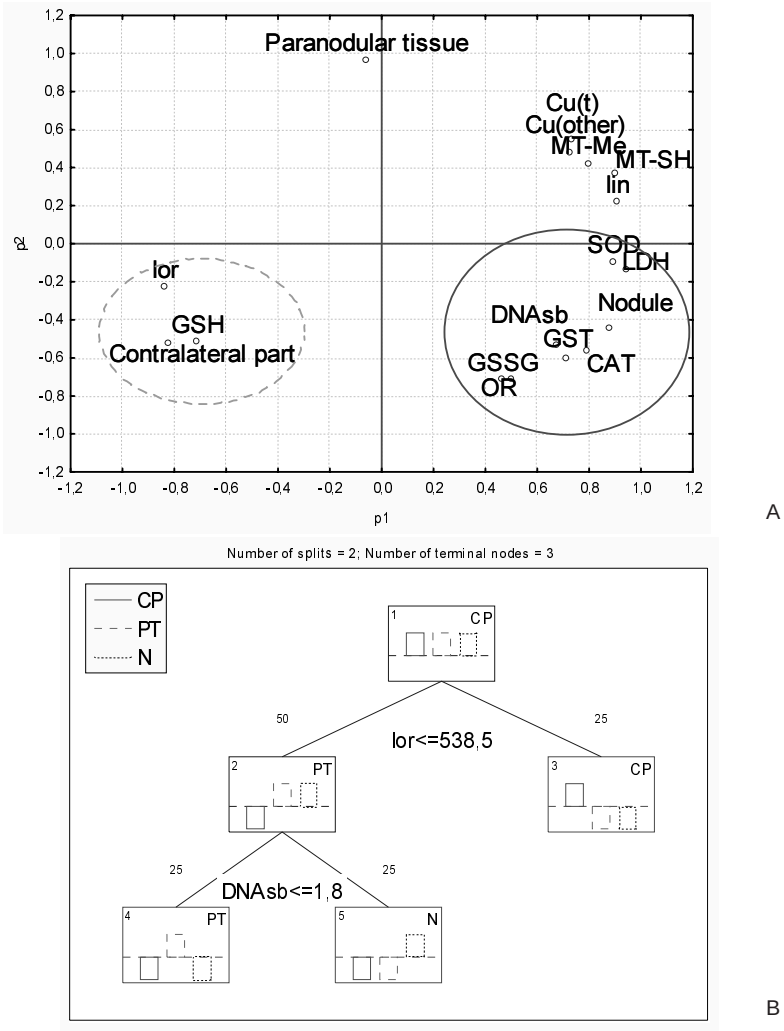

Fig. 5. Principal component analysis with NIPALS algorithm (A) and classification tree models using CART algorithm (B) of total data from human thyroid gland under iodine deficiency euthyroid nodular goiter depending on deepness of pathology processes. Data for B showing node types, split variables, and associated split values of all attributes of tissues.

iodine organification and high copper level in goitrous tissue of thyroid gland, metallothioneins may provide a partial compensatory effect on prooxidative processes. The effects on the secretion of the cathepsin D can depend on the accumulation of copper inside thyrocyte lysosomes. Nodule formation in human thyroid gland has been followed by disruption of iodine organification by thyrocytes (general characteristic of affected by nodule tissue) and increased level of DNA fragmentation (specific character of the nodule).

Acknowledgements

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ASSOCIATION OF FAMILIAL AND ENVIRONMENTAL FACTORS WITH ASTHMA AND ALLERGIC DISEASES IN UKRAINIAN CHILDREN POPULATION

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Background. *Asthma and other allergic diseases as influenced by environmental and familial factors might be targeted using preventive measures. These diseases are a matter of some urgency in Ukraine because of the clinical, social and economic importance in childhood.*

Objective. *The aim of this study was to investigate the relationship between prevalence of asthma, spastic bronchitis, allergic rhinitis, atopic eczema, unspecified hypersensitization and some selected environmental and familial factors in a population sample of 6 to14-yrs old Western Ukraine schoolchildren.*

Methods. *The data set of the study was collected using a questionnaire-based survey, containing the data of 4871 urban and rural children age 6 to 14 years. Correlation of asthma and allergic disease with familial and environmental factors was examined by means of multivariate logistic regression.*

Results. *Increased risk of asthma (1.7 %) was associated with the urban residence (OR=1.8; p=0.04) and high parental education (OR=1.8; p=0.02); spastic bronchitis (6.2%) – with parental allergy (OR=1.3; p=0.03); atopic eczema (6.2 %) – with younger age (OR=1.3; p=0.03), high parental education (OR=1.3; p=0.03), parental allergy (OR=1.4; p=0.02), tobacco smoke at home (OR=0.7; p=0.01) and household density (OR=0.6; p=0.001); diagnosis of unspecified allergic sensitization (11.8 %) was related to high parental education (OR=1.2; p=0.03), parental employment (OR=0.8; p=0.02) and pets at home (OR=1.2; p=0.06).*

Conclusions. *This study identifies that lifeslyle and building factors are associated with an altered prevalence of common childhood allergic diseases. Prevention may need to address the minimization of potential risk-factors.*

KEY WORDS**: asthma, environmental factors, children.**

Introduction

Prevalence of asthma and allergies among children has become an increasing problem during the last few decades. Asthma has become the most common chronic disease among children and is one of the major causes of hospitalization among those younger than 15 years of age. As more people become sensitized to allergens, allergic diseases may increase in Europe in the coming years [1]. Previous ISAAC studies revealed a relatively low prevalence of allergic diseases specifically in Eastern Europe. While the results of the studies from different areas in Eastern Europe have been reported, less is known about the current epidemiology of childhood allergic diseases in different areas of Ukraine [2, 3]. With the prevalence of childhood asthma and allergic diseases increasing worldwide over the past decades, it is widely accepted that environmental factors, in addition to genetics, are closely related to allergic diseases [4, 5]. The role of environmental factors in relation to asthma and allergy increased through the 1990s. There has been widespread public concern that changing patterns of outdoor air pollution underlie the rising burden of asthma, but the professionals are not so sure about such correlations. The indoor environment, in which people spend most of their time, has received less attention [6, 7].

The aims of this study was firstly, to quantify the prevalence of asthma, spastic bronchitis, allergic rhinitis, atopic eczema, unspecified hypersensitization in schoolchildren in Ternopil and Ternopil region, and secondly, to evaluate associations between children's asthma and allergic diseases from one side and housing and familial characteristics from another.

Methods

We used questionnaire which was based on the ISAAC symptom questionnaire in Ukrainian language. The questionnaires were answered by parents or guardians of children, aged 6 to 14 years old from Ternopil and surrounding rural area. Issues and factors affecting the prevalence of physician-diagnosed asthma, spastic bronchitis, allergic rhinitis, atopic eczema, and unspecified hypersensitization

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were included in the list of allergic diseases. The study protocol was approved by the local ethics committee at the Ternopil State Medical University (№ 1 (b) from 07.04.2010). Independent variables of interest were based on questionnaire self-report. Subgroups of subjects defined by place of residence (urban/rural), gender (male/female), age group (<10 years old / >10 years old), parental education (less than high school/high school or above), parental employment (both unemployed/otherwise), any diagnosed parental allergy (at least one parent: yes/no), tobacco exposure at home – smoking adult at home (yes/no), housing (multi-storey house/otherwise), heating of the house (coal or wood stove/otherwise), household density – defined as the ratio of persons living in the house to rooms in the house (≥1 person per room/otherwise), dampness in house defined as moisture stains or signs of mold on the inner surfaces in the house (yes/no), contact with pets inside the house (yes/no).

Statistical analysis was performed by using Statistica 7.1 software. Descriptive analyses were used to examine the prevalence of each outcome with the personal and environmental characteristics of the study population. Statistical significance was assessed using chi^2 test. Multivariable logistic regression analyses with each outcome were used to examine the association with the personal and environmental characteristics and to account for potential confounding. For these analyses, outcomes were the diseases or symptoms of interest. The strength of association was based on the Odds ratio (OR) and 95 % confidence intervals (CI).

Results

Analysis of 4871 completed questionnaires has found the following results: study population was 50.8 % females and 49.2 % males. 54.7 % of children lived in an urban area, 47.3 % – in rural. Groups of children living in urban and rural areas were similar ($p<0.05$) in terms of age (10.4±2.0 years and 10.5±2.0 years, respectively) and gender (51.0 % and 50.5 %, respectively, were females). Descriptive characteristics of the study population by urban and rural status are presented in Table 1.

The prevalence of respiratory and allergic diseases were: childhood asthma – 1.70 %, spastic bronchitis – 6.26 %, allergic rhinitis – 5.79 %, atopic dermatitis – 12.40 %, and current chest wheezing – 11.56 %. Table 2 shows the prevalence of respiratory and allergic outcomes by characteristics of the study group.

Bronchial asthma prevailed in children from urban areas or those with a higher parental education. A higher prevalence of spastic bronchitis was found among those with a parental history of allergy. A higher prevalence of allergic rhinitis was also found among those in an urban area compared to a rural area. There were no statistically significant differences between categories of the characteristics considered for atopic eczema.

As results from logistic regression analysis, we found that an increased risk of asthma was associated with urban residence (p=0.04) and high parental education (p=0.02). Spastic bronchitis was associated with parental allergy (p=0.03). Atopic eczema had statistically significant associations with younger age (p=0.03), high parental education (p=0.03), parental allergy (p=0.02), tobacco smoke at home (p=0.02) and household crowding (p=0.0007). Diagnosis of unspecified sensitization was related to high parental education (p=0.03) and parental employment (p=0.02) (Table 3).

Discussion

This study was designed to assess the relationship between physician-diagnosed asthma, spastic bronchitis, allergic rhinitis, atopic eczema,

Table 1. Descriptive characteristics of the study population in the Ternopil region.

Characteristic	Total N=4871 (%)	Rural N=2206 (%)	Urban N=2665 (%)	p
Age below 11 years	48.0	49.9	46.4	0.01
Male gender	49.2	48.9	49.5	0.7
Higher parental education (both parents)	55.9	49.5	61.2	0.0001
Parental unemployment (both parents)	23.8	27.6	20.7	0.0001
Any allergic disease in mother or father	18.1	13.8	21.7	0.0001
Tobacco exposure at home – smoking adult a home	47.3	48.4	46.4	0.1
Residence in a multi-storey house	56.8	25.6	82.7	0.0001
Coal stove (heating/cooking)	3.3	6.3	0.9	0.0001
Household density >1 person per room	78.4	78.6	78.2	0.7
Dampness in house	35.0	37.9	32.7	0.0001
Contact with pets inside the house	56.5	68.4	46.2	0.0001

Table 2. Frequencies of questionnaire-based variables during the examinatoin of population

Characteristic		Asthma N (%)	Spastic bronchitis N (%)	Allergic rhinitis N (%)	Atopic Eczema N (%)	Allergy (not specifed) N (%)
Residence	Urban	55 (2.06)	156 (5.85)	86 (3.23)	136 (6.17)	300 (11.26)
	Rural	28 (1.27)	149 (6.75)	83 (3.76)	170 (6.38)	274 (12.42)
	P value*	0.03	0.1	0.3	0.7	0.2
Gender	Male	37 (1.54)	151 (6.29)	83 (3.46)	160 (6.67)	293 (12.21)
	Female	46 (1.86)	154 (6.23)	86 (3.48)	146 (5.91)	281 (11.37)
	P value*	0.3	0.9	0.9	0.2	0.3
Parental education	High	57 (2.09)	180 (6.61)	108 (3.97)	193 (7.09)	351 (12.89)
	< High	26 (1.21)	125 (5.82)	61 (2.84)	113 (5.26)	223 (10.38)
	P value*	0.01	0.2	0.03	0.008	0.06
Parental Employment	Yes	62 (1.67)	237 (6.39)	139 (3.75)	236 (6.36)	464 (12.51)
	No	21 (1.81)	68 (5.85)	30 (2.58)	70 (6.02)	110 (9.47)
	P value*	0.7	0.5	0.05	0.6	0.005
Parental allergy – any diagnosed	Yes	19 (2.15)	69 (7.82)	34 (3.85)	72 (8.16)	110 (12.47)
	No	64 (1.60)	236 (5.92)	135 (3.38)	234 (5.87)	464 (11.63)
	P value*	0.2	0.03	0.4	0.01	0.4
Tobacco exposure at	Yes	36 (1.56)	141 (6.11)	78 (3.38)	123 (5.33)	275 (11.93)
	No	47 (1.83)	164 (6.39)	91 (3.55)	183 (7.13)	299 (11.66)
	P value*	0.4	0.6	0.7	0.009	0.7
Housing	Multi-storey house	49 (1.77)	162 (5.85)	91 (3.29)	178 (6.43)	311 (11.24)
	Other	34 (1.62)	143 (6.80)	78 (3.71)	128 (6.08)	263 (12.50)
	P value*	0.6	0.1	0.4	0.6	0.1
Heating of house	Coal/wood	2 (1.23)	9 (5.52)	3 (1.84)	11 (6.75)	15 (9.20)
	Central	81 (1.72)	296 (6.29)	166 (3.53)	295 (6.27)	559 (11.87)
	P value*	0.6	0.6	0.2	0.8	0.2
Household density	1+person /room	67 (1.75)	229 (6.00)	138 (3.61)	213 (5.58)	450 (11.79)
	<1person /room	16 (1.52)	76 (7.22)	31 (2.94)	93 (8.83)	124 (11.78)
	P value*	0.6	0.1	0.2	0.0001	0.9
Dampness in house	Yes	26 (1.52)	105 (6.14)	59 (3.45)	99 (5.79)	199 (11.64)
	No	57 (1.80)	200 (6.33)	110 (3.48)	207 (6.55)	375 (11.86)
	P value*	0.4	0.8	0.9	0.3	0.8
Contact with pets inside the house	Yes	41 (1.49)	175 (6.35)	104 (3.77)	165 (5.99)	345 (12.52)
	No	42 (1.98)	130 (6.14)	65 (3.07)	141 (6.66)	229 (10.82)
	P value*	0.1	0.7	0.1	0.3	0.06

unspecified hypersensitization and various family risk and environmental factors while using representative sample of schoolchildren in the 6 to 14 yrs-old age group from a defined geographical area. It was found that 1.7 % had physician diagnosed asthma, 6.26 % – spastic bronchitis, 5.79 % – allergic rhinitis and 12.40 % – atopic dermatitis. The prevalence of childhood asthma in the Ternopil Region was relatively low comparing to other studies from Eastern Europe and much lower than in the recent findings in children of Kiev (8.1 % in 6–7 years old children and 6.1 % in the 13–14 year old children) [Akopian A. Z. – unpublished report].

There is an evidence to support an underdiagnosis of asthma in this study population. Low prevalence of childhood asthma in the Ternopil Region and frequent occurrence of allergic diseases at the same time, within the range of the published data, suggests an asthma underdiagnosis. Our study revealed a relatively frequent occurrence of spastic bronchitis (6.2 %), which is suggestive of an asthma-like response. However, it is lower then the previous report for central and eastern European children [8].

Using multivariable logistic regression analysis, it was established that urban residence was positively associated with asthma, while parental allergy - with spastic bronchitis in children. High parental education was related to asthma, atopic eczema and unspecified sensitization of children. Parental allergy, tobacco smoke at home, household crowding were positively associated with children's atopic eczema. Keeping pets at home was related with unspecified sensitization of children.

Table 3. Associations of allergic diseases with environmental and familiar factors (multivariate logistic regressions)

Indicator	Odds ratio (95% CI)				
	Asthma	Spastic Bronchitis	Allergic Rhinitis	Atopic Eczema	Allergy (not specified)
Age ≤10	1.14 (0.73-1.76)	1.11 (0.86-1.42)	1.31 (0.96-1.79)	1.29 (1.02-1.64)	1.04 (0.87-1.24)
	p=0.5	p=0.007	p=0.09	p=0.03	p=0.6
Urban Residence	1.79 (1.02-3.14)	0.89 (0.67-1.19)	0.86 (0.59-1.26)	0.95 (0.71-1.27)	0.92 (0.74-1.14)
	p=0.04	p=0.4	p=0.4	p=0.7	p=0.4
Gender – Male	0.82 (0.53-1.27)	1.00 (0.79-1.27)	1.04 (0.75-1.39)	1.10 (0.87-1.39	1.11 (0.93-1.32)
	p=0.3	p=0.9	p=0.8	p=0.4	p=0.2
High Parental education	1.78 (1.08-2.92)	1.11 (0.86-1.42)	1.33 (0.95-1.86)	1.31 (1.02-1.67)	1.22 (1.01-1.48)
	p=0.02	p=0.4	p=0.09	p=0.03	p=0.03
Parental Employment	1.38 (0.81-2.33)	0.92 (0.69-1.24)	0.75 (0.49-1.14)	1.00 (0.75-1.34)	0.77 (0.62-0.97)
	p=0.2	p=0.5	p=0.1	p=0.9	p=0.02
Parental allergy – any diagnosed	1.21 (0.71-2.06)	1.35 (1.01-1.80)	1.10 (0.74-1.62)	1.37 (1.03-1.82)	1.06 (0.84-1.33)
	p=0.4	p=0.03	p=0.6	p=0.02	p=0.6
Tobacco exposure at home	0.90 (0.58-1.40)	0.97 (0.76-1.22)	0.95 (0.70-1.30)	0.75 (0.59-0.95)	1.03 (0.87-1.23)
	p=0.6	p=0.7	p=0.7	p=0.01	p=0.7
Housing–multi-storey house	0.70 (0.41-1.20)	0.88 (0.66-1.17)	0.92 (0.63-1.36)	1.07 (0.80-1.43)	0.91 (0.73-1.13)
	p=0.1	p=0.3	p=0.6	p=0.6	p=0.3
Heating of house – coal or woods stove	0.91 (0.22-3.45)	0.82 (0.41-1.64)	0.48 (0.15-1.52)	1.20 (0.63-2.28)	0.71 (0.41-1.23)
	p=0.9	p=0.5	p=0.2	p=0.5	p=0.2
Household density ≥1+person/room	1.26 (0.72-2.21)	0.86 (0.65-1.13)	1.25 (0.84-1.86)	0.64 (0.49-0.83)	0.99 (0.80-1.23)
	p=0.4	p=0.2	p=0.2	p=0.0007	p=0.9
Dampness in house	0.85 (0.53-1.37)	0.93 (0.72-1.19)	0.93 (0.67-1.30)	0.89 (0.67-1.14)	0.94 (0.78-1.14)
	p=0.4	p=0.5	p=0.6	p=0.5	p=0.5
Pet in house	0.81 (0.51-1.27)	1.04 (0.81-1.33)	1.24 (0.90-1.72)	0.99 (0.77-1.27)	1.17 (0.97-1.41)
	p=0.3	p=0.7	p=0.2	p=0.9	p=0.06

Conclusions

This cross-sectional examination suggests that environmental and family risk factors are associated with prevalence of asthma, spastic bronchitis, atopic eczema and unspecified sensitization in 6 to 14-yrs-old schoolchildren in Ternopil. The determination of potentially preventable environmental and family factors affecting risk of allergic disease is important, considering the apparent world-wide increases in the prevalence of childhood allergic disease. This study identifies lifestyle, building factors which are associated with an altered prevalence of common childhood allergic disease. Prevention may need to address the minimization of potential risk-factors.

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CLINICAL AND LABORATORY DIAGNOSTIC CRITERIA OF IMMUNE RESISTANCE OF HEALTHY AND SICK WITH PNEUMONIA CHILDREN OF DIFFERENT AGE

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Background. *The universality of the general adaptation syndrome and the need to study the impact of general adaptive responses on the occurrence and consequences of pathological processes, as well as detection of early signs of disadaptation, have been proven by numerous works.*

Objective. *The aim of study was to explore the peculiarities of general non-specific adaptive reactions in pediatric population and to discuss the diagnostics effectiveness of general nonspecific adaptive reactions in early detection of health disorders in children of different age.*

Methods. *The paper presents the results of laboratory and clinical tests, conducted in 185 healthy children age 4-12 years and 42 children of the same age with community-acquired pneumonia. The characteristics and prevalence of general nonspecific adaptative reactions in healthy children of different age were given. Determination of adverse adaptative reactions (stress, training and overactivation) based on analysis of the general blood analysis is an early diagnostic criterion of individual health reduction of children.*

Results. *The features of clinical course, the nature and direction of immune disorders of children with community-acquired pneumonia, depending on their adaptive systems, were revealed. The influence of conventional treatment regimens of children with pneumonia on the state of adaptation processes was determined. Clinical diagnostic criteria for prognosis and treatment efficacy based on diagnostics of nonspecific adaptive responses in the dynamic of pneumonia were established. The proportion of morphometrically different blood lymphocytes in healthy children was determined. It was revealed that the blood of preschool age children dominated with type I cells (61.3±1.7) %. The relative content of lymphocytes (morphological type II) (28.7±1.6) %, type III – (6.1±0.6)%, type IV – (3.9±0.6)% was observed.*

Conclusions. *General nonspecific adaptative reactions of mild and increased activation, as the most conducive to children's age, demonstrate a balance of the relative content of different types of lymphocytes according to morphometrical data.*

KEY WORDS: **children, cell-mediated immunity, adaptive reactions, community-acquired pneumonia.**

Introduction

Currently, it is not possible to assess the health of the child without taking into account the processes of adaptation to the external environmental factors, indicators of physical and mental health of an individual [1]. The universality of the general adaptation syndrome and the need to study the impact of general adaptive responses on the occurrence and consequences of pathological processes, as well as detection of early signs of disadaptation have been proven by numerous works [2, 3, 4]. According to the previous studies, poor adaptation processes are the background for the implementation of specific pathogenic factors [5, 6].

Resistance and adaptation are provided with blood components, which serve as a clinical indicator of the organism status, provide immune supervision and act as effectors at various adaptive-trophic effects [7]. Lymphocytes represented by a diverse in form and functions cell population are recognized as central cells of the immune system. Damaging factor may cause activation or depression of the immune system that necessarily is reflected in the number and structure of lymphocytes.

The objective of the study is to explore the peculiarities of general non-specific adaptive reactions in pediatric population and to discuss the diagnostics effectiveness of general nonspecific adaptive reactions in early detection of health disorders in children of different age and to improve the treatment control and prognosis depending on the immune resistance in case of community-acquired pneumonia.

Methods

185 healthy children age four to twelve years were examined. The sample was formed on the basis of pre-schools and secondary schools in Ternopil and Ternopil region. Only children without congenital malformations or chronic disease, during the physical well-being and not less than three weeks after the last acute illness or vaccination were involved into the research. The general totality was formed by probabilistic method (during the general dispensary

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examinations), thus excluding the element of subjectivity. Distribution of surveyed children by age and sex is shown in Fig. 1. Type of the general nonspecific adaptive reactions (GNAR) was determined by the indexes of lymphocytes as white blood count (WBC).

Also, the study involved 42 children of the relevant age with community-acquired pneumonia who were treated in the pulmonological department of Ternopil Regional Children's Hospital (TRChH). All patients in the hospital underwent clinical, laboratory and instrumental examination in accordance with approved Protocols of treatment in the specialty "Children's Pulmonology" (Ministry of Health of Ukraine № 18 from 13.01.2005). In all cases the diagnosis of pneumonia was confirmed radiographically.

The determination of GNAR was done by analyzing the correlation of different types of leukocytes in WBC [2]. The character of GNAR (harmonious or strained) was determined on the basis of adaptation index, calculated as the ratio of relative values of lymphocytes to segmented neutrophils. The level of GNAR reactivity (low, medium, high) was determined by the presence and severity of strain signs at WBC. These strain signs include an increase or decrease in the number of white blood cells, the relative levels of band neutrophils, eosinophils, monocytes, and the emergence of young and immature forms of leukocytes. As a marker of the state of perfect health in children, we accepted GNAR of increased activation at a high level of reactivity.

Studies of the cellular immunity was conducted by determining the number of lymphocytes and their subpopulations (CD3+, CD4+, CD8+, CD16+, CD22+) in venous blood by indirect immunofluorescence using monoclonal antibodies ("Granum", Kharkiv). Indicators of endogenous intoxication were determined by colorimetric method over the serum content of molecules of medium mass (MMM) using the technique of Gabrielian, N.I. and Levitsky, E.R. (1984).

Statistical processing of the research results was performed by parametric analysis with the calculation of Student's t-test using the software package "Microsoft EXCEL 5.0".

Results

We have found that among children of preschools and secondary schools of the Ternopil region and Ternopil only 41 children were healthy (22.2 %) (1st group). In 144 children (77.8 %), who were considered healthy as well (2nd group), some insignificant deviations were found, including functional minor pathology of the respiratory system (61.6 %) and digestive system (42.7 %) at leading positions (Fig. 2).

The GNAR values of increased activation (51.3 %) and over-activation (25.2 %) in preschool children were detected. We have revealed reactions of mild activation (11.3 %) and training (12.2 %) with somewhat less frequency in the same group of children. In children of the 1st group, the reaction of high activation predominated (64.7 %) (Fig. 3).

In the group of younger pupils the GNAR of mild and increased activation (29.4 % and 38.2 % respectively) were predominant. Simultaneoulsy, GNAR of mild activation (50.2 %) was registered in the majority of the 1st group children. In the age group 10-12 years, the same GNAR (high activation (39.0 %), mild activation (25.0 %), and training (25.0 %)) were identified; in the 1st group the prevailing finding was GNAR of training (45.4 %) (Fig. 3).

It was found that general nonspecific adaptive reactions differ in the parameters of the immune response based on the subpopulations of lymphocytes results. Thus, the GNAR of stress and over-activation are characterized by a low content of CD3+ ((37.5±0.3) % and (42.0±1.3) %, respectively) and CD8+ lymphocytes ((11.7±0.2) % and (12.8±0.5) %, respectively), showing a pronounced tendency to immunosuppression. The reactions of mild and increased activation and training are characterized by minor changes of immune cells quantitative indicators that testify to active functioning of all components of the immune system.

Among the clinical peculiarities of children hospitalized in the pulmonary department of TRChH, we detected prevailing focal (91.3 %) forms of community-acquired pneumonia that coincides with the literature data. The prevalence of bilateral forms of community-acquired pneumonia (in 69.0 %) was determined that differs from the reference data

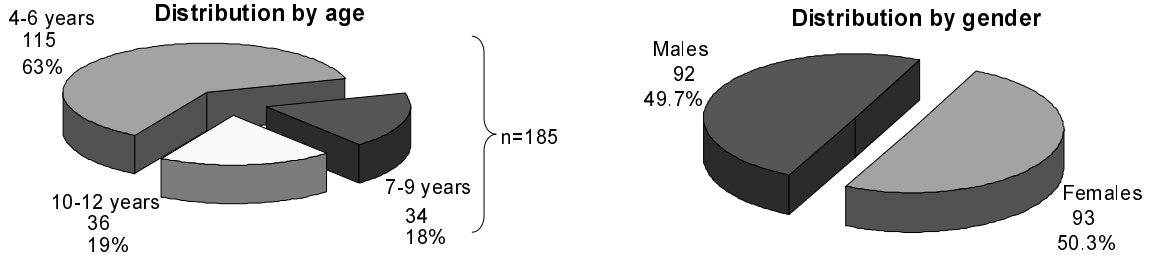

Fig. 1. Distribution of surveyed children by age and gender.

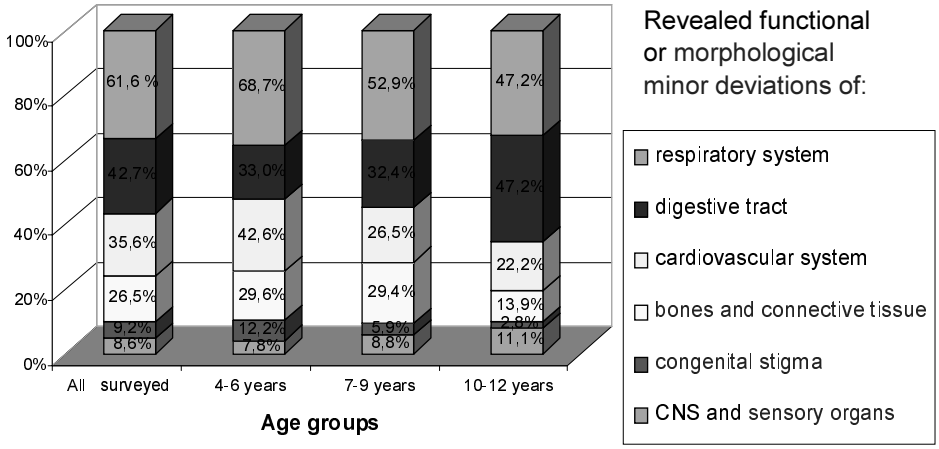

Fig. 2. Minor deviations found in apparently healthy surveyed children

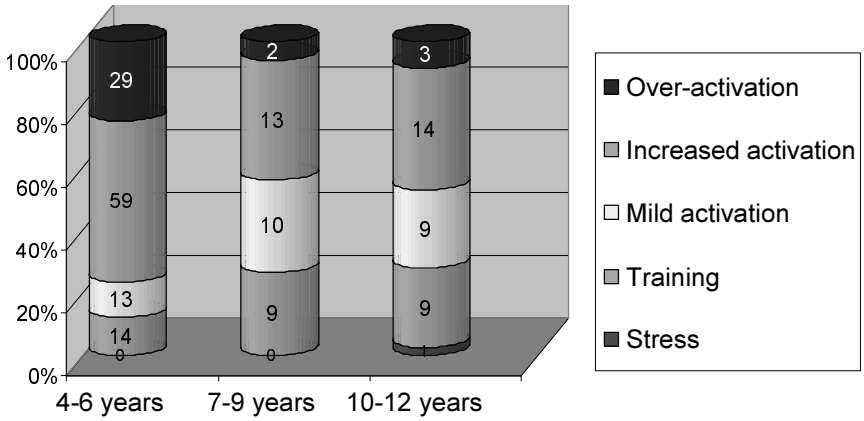

Fig. 3. Types of general nonspecific adaptive reactions in surveyed children from child care institutions of Ternopil region.

testifying the predominance of unilateral pulmonary inflammatory processes in childhood.

Among all analyzed cases, uncomplicated and complicated forms of community-acquired pneumonia were recorded (52.6 % and 58.3 %, respectively). The most commonly observed disorder that complicated the disease was obstructive syndrome (recorded in 26.2 % of cases), with a tendency to increasing the frequency of this complication with age. Allergic history of these patients was not burdened. The most common symptoms of community-acquired pneumonia were cough (95 %), mostly wet and unproductive (50.0 %), respiratory failure events (73,9-78,9 %); objectively dull sound on the lungs percussion in the projection of the inflammatory focus (85.7 %), fine rales (31.0 %) and small-vesicles rhonchi (38.1 %) on auscultation.

Detection of general nonspecific adaptive reactions in patients with community-acquired pneumonia at baseline showed that the GNAR of stress (42.9±1.2) % and training (35.7±1.1) % were recorded more often (Table 1). Consecutive determination of GNAR was conducted on the 7-10th day of hospitalization.

Mean values of endogenous intoxication indices in a group of healthy children were within reference values. The increase of MMM1 and MMM2 was stated at GNAR of stress, training and over-activation; they were significantly lower at activation reaction. In children with community-acquired pneumonia the average values of MMM1 and MMM2 increased by 1.5 and 1.3 times, respectively, in comparison to healthy subjects.

Discussion

It was found that general nonspecific adaptive reactions differ regarding parameters of the immune response according to the subpopulations of lymphocytes. Thus, the GNAR of stress and over-activation are characterized by a low content of CD3+ (37.5±0.3 % and 42.0±1.3 %, respectively) and CD8+ lymphocytes (11.7±0.2 % and 12.8±0.5 %, respectively), showing a pronounced tendency to immunosuppression. The reactions of mild and increased activation and training are characterized by minor changes of immune cells quantitative indicators that testify to active functioning of all components of the immune system.

In children with community-acquired pneumonia GNAR of stress and training prevailed, that was accompanied by insufficiency of T-cell immunity and increased levels of endogenous intoxication. The

Table 1. The frequency of GNAR in children with community-acquired pneumonia

Type of adaptive reaction	At baseline, n=42		After treatment, n=42	
	n	P±m, %	n	P±m, %
Stress	18	42.9±1.2[5]	3	7.1±0.6[2]*
Training	15	35.7±1.1[5]	10	23.8±1.0[1]
Mild activation	5	11.9±0.8[1,2]	16	38.1±1.2[1]*
Increased activation	3	7.1±0.6[1,2]	10	23.8±1.0[1,5]*
Over-activation	1	2.4±0.4[1,2]	3	7.1±0.6[2,4]

Notes: [1] – statistically significant difference (p<0.05) if compared with stress; [2] – statistically significant difference (p<0.05) if compared with reaction of training; [4] – statistically significant difference (p<0.05) if compared with increased activation; [5] – statistically significant difference (p<0.05) if compared with over-activation; * – statistically significant difference (p<0.05) in the treatment process.

reactions of stress and training differ from other adaptive reactions by low levels of all subpopulations of lymphocytes. These changes can be interpreted as immunosuppression. We have identified the peculiarities of GNAR types changing in children with community-acquired pneumonia under the influence of treatment. In particular, there was a reduction in the number of patients with reactions of stress to 7.1 % and training to 23.8 % and increased the frequency of GNAR of mild and increased activation (in 38.1 % and 23.8 % of children, respectively) to the 10th day of hospitalization, which indicates an improvement of adaptive processes and satisfactory mobilization of reserve capacity of the organism. However, we observed the prolonged retention of reactions unfavorable to recovery in 7 patients (16.7 %). Devolution on a scale of adaptation reactions was revealed in 2 children (4.8 %) indicating a reduction in the activity of adaptive processes. The detection of GNAR of stress and training by 7–10 day of treatment, even against the normalization of clinical data, may be regarded as criteria of the bronchopulmonary disease treatment inefficiency. Recovery of these patients should not be considered complete. Prolonged retention of the reaction of training, according to the theory of adaptation reactions, creates conditions for chronic pathology.

Conclusions

1. Considering the level of adaptability and resistance of the organism among children surveyed, the condition of perfect health was diagnosed in 13.5 %, the average health – in 58.4 %, the state of preexisting diseases – in 27.6 %, and the state of the vulnerability to the pathological process – in 0.5 %.

2. It was proved that the most common symptoms of community-acquired pneumonia in children age 4–12 years were cough, mostly wet and unproductive, dyspnea, dull sound on percussion in the projection of the inflammatory focus in the lungs, and fine rales on auscultation. Majority of patients had progressing pathological process occurred despite the reduction of immune resistance that was evidenced by GNAR of stress and training, insufficiency of T-cell immunity and increased parameters of endogenous intoxication.

3. The process of recovery with effective treatment occurs against the transition of GNAR of stress into anti-stress reactions of mild and increased activation of a high level of reactivity through reaction of training. Patients with adverse GNAR types (stress, over-activation) are of high risk of a protracted disease course. Detection of the GNAR type allows to identify the risk group of unfavorable community acquired pneumonia course by identifying adaptive reactions of stress and training or over-activation development.

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COMPARISON OF SHORT-TERM ANALGESIC EFFECTS OF EXTRACORPOREAL SHOCK WAVE THERAPY (ESWT) AND CONSERVATIVE TREATMENT (CT) IN MEN WITH CHRONIC HEEL SPUR (HS)

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Background. *The surgical removal of heel spur (HS) provokes many controversial opinions, but clinical studies show the risk of complication after operation. An alternative to the surgical removal of HS is Extracorporeal Shock Wave Therapy (ESWT), because it is non-invasive method.*

Objective. *The aim of the study is to compare the short-term analgesic effects of ESWT and conservative treatment (CT) in males with chronic HS.*

Methods. *Sixty patients (mean age 54.9±4.3 years; range 42 to 59 years) were examined who had pain associated with HS. In the shockwave group (group 1) patients received 1000–2000 impulses of shocks to the affected heel in a single session. The patients in this group took a series of 5 ESWT in 1 week intervals. Patients in the control group (group 2) at the same time received CT consisting of nonsteroidal anti-inflammatory drugs (NSAIDs), orthotics and a local cortisone injection. The basic method of research was to evaluate pain according to Visual Analogue Scale (VAS) 0–10. The patients of the two groups were tested before the treatment, after the last treatment and 3 months and 6 months after the treatment.*

Results. *A significant decrease of VAS (p=0.000) was seen in the shockwave group. In the control group no significant decrease of VAS was seen.*

Conclusions. *ESWT reduces pain more effectively than CT in men with chronic HS.*

KEY WORDS: **extracorporeal shock wave therapy, conservative treatment, visual analogue scale, heel spur, pain.**

Introduction

Chronic inflammation of the plantar fascia, in the place of its connection to calcaneal tuberosity, can be a major cause of pain in the adult population. Due to a visible X-ray imaging calcification – bone spurs near the heel – the condition is commonly called "heel spur" (HS). Among other causes of the condition, there are: an inflammation of the Achilles tendon associated with inflammation of the Achilles tendon bursitis, inflammation of adipose tissue beneath calcaneal tuberosity by repeated micro damages that cause degeneration of the tissue, fatigue fractures of the calcaneal tuberosity, neuropathy caused by pressure on the plantar branch of the tibial nerve by an overgrown plantar aponeurosis. Risk factors also include excessive body weight, running and jumping, work that entails standing or lifting heavy objects, flat feet, rheumatic changes [1–5]. Originally, the condition may be asymptomatic – pain rarely occurs in the early stages of the condition. Most patients come to the doctor in a fairly advanced stage of the condition, complaining on severe pain in the heel that escalates after they walk on hard ground or carry heavy objects and that usually disappear after they rest.

The treatment of HS and plantar fascia provokes many controversial opinions, many authors point to the need for surgical removal of calcaneal bursitis and resection of HS, but clinical studies show the risk of complication after operation [6–12]. An alternative to the surgical treatment of HS is CT that involves the use of physiotherapy treatments, such as ionophoresis, laser, ultrasound, and recently more and more often ESWT is used [13–16].

ESWT is a very effective method which effectiveness is comparable to surgery. The most important is the fact that it is a completely non-invasive method. It has not yet been fully explained how it works exactly, but it probably involves micro destructions – the application of ESWT causes micro breaks in avascular or poorly-vascularized tissue thus stimulating appropriate revascularization and stem cell growth. Patients may continue to work or train the next day. Given the significant reduction of activity limitations and short duration of the treatment,

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ESWT is not only comparable to surgical treatment, but, in general, it is also less expensive than a few months of CT [17–18].

The aim of the study was to compare the short-term analgesic effects of ESWT and CT in males with chronic HS.

Methods

Continuous research was carried out in the period from January 2010 to July 2012, and involved sixty patients (middle age 54.9±4.3 years; range from 42 to 59 years) who had pain associated with HS. The average BMI was 24.29±0.78. None of the men was obese. The study was conducted in Busko-Zdroj Spa. The patients had unilateral pain. The average duration of pain was 15.2±5.3 months. Inclusion criteria were pain over the X-ray examined HS, unsuccessful CT (iontophoresis, cryotherapy, laser therapy, ultrasound and phonophoresis) during the six months before referral to Spa in Busko-Zdroj. Exclusion criteria were arthritis (rheumatoid arthritis, spondarthritis, crystal induced arthropathies) diabetes mellitus, neurological abnormalities, age under 18 years, infectious or tumorous diseases, skin ulcerations, and bursitis. All patients were informed about the principles of the treatment, and they signed a written permission for treatment and participation in the study.

In the shockwave group (group 1) patients received – during the first treatment 1000 impulses, 1500 impulses in the second, and 2000 impulses in the third, fourth and fifth treatment (the pressure of 2.5 bar, the frequency of 8 Hz, the energy density of 0.4 mJ/mm^2). The patients in the shockwave group took a series of 5 ESWT in 1 week intervals. Apparatus BTL-5000 SWT was used for the treatment. The active engagement between the head of the apparatus with a diameter of 15 mm and skin was gel used in ultrasound scan. The procedure was performed in the area of most intense pain – calcaneal tuberosity. Treatment time did not exceed 10 minutes. Patients in the control group (group 2) at the same time received CT consisting of NSAIDs, orthotics and a local cortisone injection with 0.5 mL of betamethasone (7 mg/mL).

The basic method of research was to evaluate pain according to VAS, where 0 – no pain; 10 – severe (maximum) pain at rest, after walking on awakening, and after normal daily activity. The patients of two groups were tested before the treatment, at the end of the last treatment. The patients were also examined for 3 months and 6 months after the end of treatment. The results observed were classified as excellent improvement (a VAS reduction of over 50 %), good improvement (a VAS reduction of over 30 % to 50 %), slight improvement (a VAS reduction of over 15 % to 30 %).

In order to evaluate the early results of the treatment, the obtained data was subjected to statistical analysis. The statistical distributions of the analyzed characteristics were examined by the use of Kolmogorov-Smirnov's test. It was shown that the tested variables had normal distribution. Basic descriptive statistics was used for the analysis. The mean arithmetic (Δx) and standard deviation (SD) of the studied traits of men in both groups were calculated. In order to evaluate the statistical significance of differences in the intensity of pain before the treatment, after the last treatment and 3 and 6 months later, while at rest, while walking on awakening, and after normal daily activity, one-way analysis of variance (ANOVA) was used in both studied groups. The value of the function F Snedecor was observed in various combinations. The statistical significance of differences in the intensity of pain was assessed at the 0.05 level. Calculations were performed at the Department of Computer Science at Holy Cross University in Kielce with the use of MedCalc software – version 11.4.3.0, licensed to Holy Cross College. The research project was authorized by the Bioethics Committee at Holy Cross College in Kielce, resolution 1/10/KB dated 29.01.2010.

Results

A significant decrease of VAS ($p = 0.000$) was seen in the shockwave group (group 1) just after the end of ESWT and in the period after the end of ESWT 3 months later and in the period from 3 months to 6 months after the end of ESWT at three reference points – at rest, when walking on awakening, and after normal daily activity. No significant decrease of VAS was seen in the control group (group 2) just after the end of CT at rest ($p=0.202$), when walking on awakening ($p=0.367$) and after normal daily activity ($p=0.341$). No significant decrease of VAS was seen in the period after the end of CT 3 months later at rest ($p=0.367$), when walking on awakening ($p=0.630$) and after normal daily activity ($p=0.633$). Also, no significant decrease of VAS was seen in the period from 3 months to 6 months after the end of CT at rest ($p=0.608$), when walking on awakening ($p=0.337$) and after normal daily activity ($p=0.393$). Studies have shown that ESWT reduces pain more effectively than CT in males with chronic HS (Table 1).

The analgesic efficacy of ESWT is confirmed by the detailed analysis of differences in the frequency of occurrence of pain intensity between groups. In the shockwave group (group 1) excellent improvement (a VAS reduction of over 50 %) was seen in 22/30 (73.3 %) patients, good improvement (a VAS reduction of over 30 % to 50 %) was seen in 6/30 (20 %) patients and slight improvement (a VAS

Table 1. The intensity of pain (VAS) in the following terms of research at three reference points

Shockwaves group (group 1)									
Three Reference Point	when at rest			when walking on awakening			after normal daily activity		
Variables	∆x±SD	F-Value	P-Value	∆x±SD	F-Value	P-Value	∆x±SD	F-Value	P-Value
VAS Before the ESWT	7.4±0.9			8.3±0.9			8.5±0.9		
VAS After the last session ESWT	5.8±0.7	59.077*	0.000	6.1±0.8	100.138*	0.000	6.2±0.7	122.077*	0.000
VAS After the last session ESWT	5.8±0.7			6.1±0.8			6.2±0.7		
VAS 3 months after ESWT	3.9±0.8	95.841*	0.000	4.4±0.6	86.700*	0.000	4.6±0.7	78.367*	0.000
VAS 3 months after ESWT	3.9±0.8			4.4±0.6			4.6±0.7		
VAS 6 months after ESWT	2.5±0.7	52.035*	0.000	3.0±0.7	69.176*	0.000	3.5±0.6	42.706*	0.000
Control group (group 2)									
VAS Before conservative treatment	8.4±0.9			8.6±0.9			8.7±0.9		
VAS After the last conservative treatment	8.1±0.9	1.667	0.202	8.4±0.8	0.828	0.367	8.5±0.7	0.923	0.341
VAS After the last conservative treatment	8.1±0.9			8.4±0.8			8.5±0.7		
VAS 3 months after conservative treatment	8.3±0.8	0.828	0.367	8.5±0.8	0.234	0.630	8.6±0.9	0.231	0.633
VAS 3 months after conservative treatment	8.3±0.8			8.5±0.8			8.6±0.9		
VAS 6 months after conservative treatment	8.4±0.7	0.265	0.608	8.7±0.8	0.937	0.337	8.8±0.9	0.741	0.393

reduction of over 15 % to 30 %) was seen 2/30 (6,7 %) patients at three reference points – at rest, when walking on awakening, and after normal daily activity. There was no decreasing in pain intensity in the control group (group 2), as it was unchanged in 24/30 (80 %) patients and worsened in 6/30 (20 %) patients at these points (Table 2).

Discussion

HS is a condition that affects more and more people of all ages. It affects their quality of life, reduces socio-professional activity and becomes the cause of frequent sickness absence. The effectiveness of treatment for HS causes many controversial opinions. There is no consensus on how

Table 2. The analgesic effects of ESWT

	Group 1 (shockwave group)		Group 2 (control group)	
	N	percent	N	percent
VAS>50 % Reducing	22	73.3 %	—	—
VAS>30-50 % Reducing	6	20 %	—	—
VAS>15-30 % Reducing	2	6.7 %	—	—
VAS unchanged	—	—	24	80 %
VAS worsened	—	—	6	20 %

to treat it, conservatively or surgically. Endoscopy is used most often in surgical treatment. This method is cost-effective, non-invasive, with low risk of post-surgery complications and allows patients to return to socio-professional activity after surgical treatment earlier [8, 19–22]. An alternative to surgical treatment is to use ESWT for treatment of chronic heel spur. There are different opinions on this subject, but the prevalent among them are views highlighting the analgesic efficacy of ESWT in the treatment of chronic HS. The analgesic success of ESWT probably depends on the total dose of energy, not on the density of stream of emitted energy [18, 23–25]. Review of subject literature shows that the analgesic efficacy of ESWT with different physical characteristics and over a different period of time concerning was investigated. Hammer, Adam, Kreutz, Kohn, Seil [14] assessed the analgesic efficacy of ESWT in patients with painful chronic inflammation of the plantar fascia. Patients treated with ESWT were given 3000 impulses of shocks with energy density of 0.2 mJ/mm^2 at weekly intervals. Two years after the end of treatment the level of pain on a VAS scale in patients treated with ESWT decreased 94 %. In subsequent studies, the previously mentioned authors used medical ultrasonography to assess the effects of ESWT on changes in thickness of plantar fascia that had been affected by chronic inflammation. During the treatment, 3 sessions of 3000 shock wave impulses with the stream density of 0.2 mJ/mm^2 were applied at weekly intervals. The thickness of plantar fascia was measured 2 cm distally to calcaneal tuberosity. In addition, the level of pain was recorded in the VAS scale. The ultrasound scan 6 months after the end of ESWT showed a significant reduction in the thickness of plantar fascia of the affected foot and the level of patients pain was reduced 79 % in the VAS scale [26]. Metzner, Dohnalek, Aigner [18] also used ESWT in patients with chronic plantar fascia. Each patient received 1000 impulses of shock wave, the density of energy stream was 0.35 mJ/mm^2. The patients were examined 6 weeks, 16 months and 72 months after the end of ESWT. It turned out that pain was reduced in 81% of patients after 6 weeks, in 88 % of patients – after 16 months, and in 96 % of patients in the last examination – 72 months after the end of ESWT. Based on these results, the authors concluded that the applied doses of ESWT effectively reduced pain and the achieved therapeutic effects provide satisfactory long-term results. Yalin, Keskin Akca, Selcuk, Kurtaran, Akyuz [27] used ESWT for analgesic purposes in patients with HS and treated them for 5 weeks with 2000 impulses of shock waves, ranging from 0.05 to 0.4 mJ/mm^2. Clinical results demonstrated excellent results (no pain) in 66.7 % of the cases, good results (50 % of pain reduced) in 15.7 % of the cases, and unsatisfactory outcome (no reduction in pain) in 17.6 % of all cases. At the same time, the study showed no correlation between the reduction of pain and a significant modification of the HS in X-ray picture. Moretti, Garofalo, Patella, Sisti, Corrado, Mouhsine [28] evaluated the analgesic efficacy of low doses of ESWT for foot plantar fascia inflammation accompanying HS in runners – athletes. The subjects received a weekly shock wave of 1000 impulses, 0.06 mJ/mm^2 energy density. ESWT continued for four weeks. The patients were examined after the last session of ESWT. Clinical results were excellent in 59 % of cases, good in 12 % of cases, satisfactory in 21 % and clearly unsatisfactory in 8 %. Ultrasound examination after the end of ESWT showed the disappearance of inflammatory symptoms in 61 % of athletes.

Cosentino, Falsetti, Manca, De Stefano, Frati, Frediani et al. [29] evaluated the analgesic efficacy ESWT at patients with calcaneal enthesophytosis. Patients were randomly assigned into two equal groups. The shockwave group (group 1) received six treatments (one every 7–10 days), each treatment consisting of 1200 shocks with a frequency of 120 shocks/min; the energy density used varied from 0.03 to 0.4 mJ/mm^2 and the control group (group 2) went through the identical process but energy density was simulated (0 mJ/mm^2). The results revealed significant reduction of pain in the shockwave group (group 1). In the control group (group 2) no significant decrease of VAS was seen.

Wang, Wang, Yang, Weng, Ko [30] evaluated patients with an established diagnosis of chronic plantar fasciitis, including patients in the shockwave treatment group and patients in the control group. In the shockwave group, patients received 1500

impulses of shockwaves at 16 kV to the affected heel in a single session. Patients in the control group received CT consisting of orthotics, physical therapy, an exercise program, and/or a local cortisone injection. Before treatment, groups showed no significant differences in the scores for pain. After treatment, the shockwave group showed significantly better pain scores in comparison with the control group. The overall results were 69.1 % excellent, 13.6 % good, 6.2 % fair, and 11.1 % poor for the shockwave group and 0 % excellent, 55 % good, 36 % fair, and 9 % poor for the control group.

Our study confirmed the results of other authors. The doses used in ESWT gradually decreased pain intensity in the shockwave group (group 1) and this trend persisted up to 6 months from the last session of ESWT. The observed differences in this period were statistically significant. In the control group (group 2) no significant decrease of VAS was seen after the treatment and in the period from 3 months to 6 months after the end of CT and in the period from 3 months to 6 months after the end of CT in patients with HS. Furthermore, statistically significant differences in pain were also found between the two groups. Excellent improvement of pain intensity was seen in 73.3 % patients, good improvement was seen in 20 % patients and slight improvement was seen in 6.7 % patients in the shockwave group (group 1) at three reference points: at rest, when walking on awakening, and after normal daily activity. As for the control group (group 1), there was no reduction of pain intensity; it remained unchanged in 80 % of patients and worsened in 20 % of patients at these points. Summing up, the results of short-term studies show that ESWT effectively reduces pain, which, in turn, reduces mental and physical discomfort in patients with chronic HS.

Conclusion

The short-term studies have demonstrated the analgesic effectiveness of ESWT. We achieved a significant reduction of pain, that persisted for 6 months in shockwaves group. ESWT reduces pain more effectively than CT in males with chronic HS. The studies have shown that ESWT is a repeatable and non-invasive treatment, and, therefore, is a valuable alternative therapeutic option for surgical treatment and other CT in patients suffering from pain due to chronic HS.

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APPLICATION OF TRANSURETHRAL MICROWAVE THERMOTHERAPY FOR PATIENTS WITH ACUTE URINARY RETENTION AND SEVERE COMBINED COMORBIDITY FROM BENIGN PROSTATIC HYPERPLASIA

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Background. *The incidence of benign prostatic hyperplasia (BPH) has grown by almost 35 % over the last five years with an incidence rate of 4438.2 per 100 000 of the respective population (147 677 total patients) in Ukrainian men beyond working age in 2007 [3].*

Objective. *The aim of the study was to assess the efficacy of TUMT in men where BPH was complicated by AUR and severe comorbidities.*

Method. *TUMT was performed using the domestically produced equipment 'ALMGP-01' at the frequency of 1300 Hz, rectal temperature of 42.5 °C, and urethral temperature of 44.5 °C. The average session duration is 46-55 minutes.*

Results and Discussion. *Subjective self-assessment: no effect of the procedure was detected (urination not restored) in 9 patients (11.11 %); poor current status with prior temporary improvement (6-9 months of independent urination with repeated AUR) in 14 patients (17.28 %); satisfactory current status with occasional dysuric episodes (however better than pre-TUMT status) in 40 patients (49.38 %). Eight patients (22.22 %) have assessed their status as 'good' and 'excellent'; one patient noted substantial improvement compared to pre-procedure status. The nine patients with lack of success of restoring physiological urination had the following peculiarities: intra-vesical growth of BPH and prostatic volume over 85 cm^3 in majority of these patients.*

Conclusions. *Taking into consideration the minimally invasive nature, favourable tolerability and absence of adverse effects, TUMT can be considered as a method of choice in patients with BPH-triggered AUR and contraindications to major surgical treatments and general anaesthesia. Remote outcomes of TUMT may be evaluated as satisfactory, with good effects in 71.62 % patients. However, in prostatic volumes exceeding 85 cm^3 and pronounced intravesical pattern of BPH growth the efficacy of TUMT is arguable.*

KEY WORDS**: benign prostatic hyperplasia, acute urinary retention, transurethral microwave thermotherapy.**

Introduction

Benign prostatic hyperplasia is a very common condition, found in middle-aged, senior and elderly males, according to epidemiological studies [1]. As for the age, the incidence of this disease is up to 85 % [2]. The incidence of benign prostatic hyperplasia (BPH) has grown by almost 35 % over the last five years which is 4438,2 per 100000 of the respective population (147 677 patients) in 2007 in Ukrainian men beyond working age [3].

The pain- and stress-free urination is known to be an essential component of optimal quality of life. However, there are moments when the perception of the quality of life undergoes a radical change. One of such moments is acute urinary retention (AUR). Occasionally, acute urinary retention occurs due to certain triggering factors, such a surgery under general anaesthesia, excessive fluid intake and medications with sympathomimetic or anticholinergic action [4]. However, the main or principal group consists of over 90% males in whom AUR is a result of natural development of benign prostatic hyperplasia (BPH) [5, 6].

European urological community identified the following risk factors of AUR: age >70 years, prostatic volume >30 cm^3, uroflowmetric results <12 mL/sec, IPSS score >7 points, residual urine >50 mL and PSA levels >1.5 ng/mL [7, 8].

It is generally accepted that the principal methods of AUR relief in patients with BPH are short-term bladder catheterisation, trocar-assisted or suprapubicepicystostomy, transurethral resection or open prostatectomy. However, there are situations when despite a disappointing effect of medical management, it is difficult to decide in favour of radical elimination of AUR due to severe comorbidities. Then the physician faces a difficult question how to solve this problem and which treatment modality is best to spare hold the patient harmless and restore spontaneous micturition.

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Throughout the last decades, there has been a search for new therapeutic approaches in symptomatic BPH [9]. For a technique to be recognized as effective it is essential for it to be less invasive and nevertheless effective, to require no general anaesthesia, to have an outpatient option and to have as few complications as possible. One of the urological technology achievements to meet the above criteria is transurethral microwave thermotherapy (TUMT).

The aim of the study was to assess the efficacy of TUMT in men where BPH was complicated by AUR and severe comorbidities.

Methods

The Ternopil University Hospital has purchased and is currently using (since 2002) a local microwave prostatic hyperthermia device (ALMGP-01), manufactured by the JSC RADMIR State Enterprise Company NDIRV (Kharkov). 516 TUMT procedures have been performed in BPH patients with high surgical risk at the Urology Department between years of 2002 and 2013.

Clinical analysis was performed in medical records of 81 (15.69 %) patients with AUR-complicated BPH.

Patient complaints were assessed using the IPSS symptom scale, developed by the American Urological Association, including the quality of life question in dysuric patients (QOL).The volume of the urinary bladder, the thickness of its walls, prostatic size and volume, as well as residual urine were assessed with transabdominal ultrasound. Upper urinary tract function was assessed using radiological and radionuclide method; the levels of prostate specific antigen (PSA) in all patients were within normal limits (up to 4 ng/mL).

Radical surgical techniques for BPH could not be used due to severe concurrent comorbities in the patients. In the given investigation, twenty-nine patients were diagnosed with coronary artery disease (CAD), diffuse and post-MI cardiosclerosis and Class IIa-IIb heart failure; 34 patients had CAD with complicated arrhythmias; 13 patients had residual post-CVA findings; 32 patients had Stage III hypertension; 17 patients had chronic bronchitis, emphysema and Stage II respiratory failure; 8 patients had severe Type 2 diabetes; 21 patients had varicose leg veins with Stage II–III chronic venous failure; 9 patients had Stage II chronic kidney disease; 2 patients had ankylosing spondylitis (Bechterew's disease) and 3 patients had bilateral coxarthrosis. Each of the TUMT patients had three to five concurrent comorbidities.

TUMT was performed using equipment 'ALMGP-01' (Kharkov, Ukraine) at the frequency of 1300 Hz, rectal temperature of 42.5 °C and urethral temperature of 44.5 °C. The average session duration was 46-55 minutes. According to ultrasound examination, the prostatic volume was within the range of 46 cm^3 to 102 cm^3, with a size of 74.5 cm^3 on average. The patients had a Foley catheter inserted repeatedly and were administered oral antibacterial drugs, usually fluoroquinolones or cephalosporins after completion of the TUMT session. Postoperative hospital stay was 4 to 7 days, 4.92 days on the average. Three days prior to urethral catheter removal, α1a-adrenoblocker tamsulosin (Omnic 0.4 mg/day) was used. The catheter was removed on Week 4 post-TUMT, after completed resorption of cellular necrosis.

Results

Most patients tolerated the TUMT session favourably. Only 5 patients (6.17 %) had a short-term urethrorrhagia; 2 patients (2.46 %) had an acute pyelonephritis and 2 patients (2.46 %) had a single day urethral fever in post operative period. The patients had the following baseline preoperative findings: IPSS 21.62±1.14 and QoL: 4.42±0.36. The following results were obtained in analysis of these findings 9–12 months post-TUMT: the IPSS score decreased to 18.42±1.63 and the quality of life index (QoL) decreased to 3.07±0.24. Physiological urination was restored in 72 patients (88.89 %) after removal of the Foley catheter. Residual urine in the patients where urination was restored was between 15 and 145 mL, 48.25±18.36 mL on the average. PSA levels were subsequently within normal limits.

Discussion

Subjective self-assessment of TUMT efficacy by the AUR patients was the following: no effect of the procedure was detected (urination not restored) in 9 patients (11.11 %); poor current status with prior temporary improvement (6–9 months of independent urination with repeated AUR) in 14 patients (17.28 %); satisfactory current status with occasional dysuric episodes (however better than pre-TUMT status) in 40 patients (49.38 %). Eight patients (22.22 %) have assessed their status as 'good' and 'excellent'; one patient note substantial improvement compared to pre-procedure status. The nine patients with lack of success restoring physiological urination had the following peculiarities: intra-vesical growth of BPH and prostatic volume over 85 cm^3 in majority of these patients.

Within a year's span, post-TUMT surgical treatment was undertaken in 8 patients with lack of independent urination (2 patients had transvesical prostatectomy, 4 patients had TURP and 2 more patients had suprapubic cystostomy placed). One patient refused surgical treatment and was on an indwelling catheter for 3 years already. Concerning the temporary improvement population, 11 patients

had surgeries (4 patients had transvesical prostatectomy, 6 patients had TURP and 1 more patient had suprapubic trocar cystostomy placed). Three patients of this group had repeated TUM session after recurrent AUR with independent urination successfully restored. It is noteworthy that open post-TUMT prostatectomies were associated with difficulty enucleating hyperplastic nodules due to proliferative changes in the nodes and the adjacent tissues. Another peculiar observation was that post-TUMT open prostatectomies, as well as post-TUMT TURP procedures, were associated with less pronounced and storter haemorrhage.

Conclusions

Taking into consideration the minimally invasive nature, favourable tolerability and absence of adverse effects, TUMT can be considered a method of choice in patients with BPH-triggered AUR and contraindications to major surgical treatments and general anaesthesia. Remote outcomes of TUMT may be evaluated as satisfactory, with good effects in 71.62 % patients. However, the efficacy of TUMT is arguable in prostatic volumes exceeding 85 cm^3 and pronounced intravesical pattern of BPH growth.

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INSTRUMENTAL AND DIAGNOSTIC CRITERIA OF HEMODYNAMIC DISORDERS AND ENDOTHELIAL DYSFUNCTION CORRECTION IN PREGNANTS WITH ARTERIAL HYPERTENSION

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Background. *According to the WHO, hypertension is associated with 20-33 % of maternal death during pregnancy within extragenital pathology statistics. There are complications of the fetus and newborn associated with hypertension at 140/90 mm Hg and higher.*

Objective. *A comparative analysis of antihypertensive therapy effectiveness in pregnants with arterial hypertension was performed using modern clinical and instrumental methods of endothelial function diagnostic, central and utero-placental hemodynamic estimation.*

Methods. *We examined 63 pregnant women with hypertension at 28 to 32 weeks of gestation. Women were randomized into three groups:group I (control group – 20 women), that included pregnant women with hypertension treated with basic antihypertensive therapy as recommended by Health Ministry of Ukraine standards (metildofa and nifedipin); group II – 21 pregnant women receiving metildofa and metoprolol; group III (22 pregnant women) receiving metildofa and nebivolol.*

Conclusions. *It was found that the brachial artery ultrasound measuring and occlusive plethysmography procedure by Dietz is an early and safe method of endothelial dysfunction diagnostic in pregnants with hypertension. Doppler ultrasound of blood flow in uterine, umbilical arteries, and middle cerebral arteries of the fetus allows timely diagnosis of the side effect of antihypertensive drugs on the fetus. The therapy of choice for pregnants with Stage II Arterial Hypertension should be based on methyldopa and calcium channel antagonists or selective beta-blockers combination. Highly selective beta-blockers with vasodilative effect (nebivolol hydrochloride) and L-arginine (Tivortin) allow to prevent perinatal adverse effects of antihypertensive therapy, to correct hemodynamic disorders and endothelial dysfunction in pregnants with arterial hypertension.*

KEY WORDS: **arterial hypertension, uterine-placental hemodynamics, endothelial dysfunction.**

Introduction

According to the WHO, hypertension is associated with 20-33 % of maternal death during pregnancy within extragenital pathology statistic. There are complications of the fetus and newborn associated with hypertension at 140/90 mm Hg and higher [1, 5, 7]. Mechanisms of increased blood pressure, leading to hypertension and/or strengthening of symptoms during pregnancy are very diverse. A major role of endothelial dysfunction (ED) in the development of hypertension is shown in the recent years. It is characterized by reduced synthesis of nitric oxide as one of the main vasodilators [3, 8, 9]. Unfortunately, ongoing antihypertensive therapy does not improve pregnancy outcomes for the fetus, and even lead to higher frequency of low weight for gestational age newborns. Overall, high blood pressure decreases due to drug therapy and may improve pregnancy outcomes for the mother but not for the fetus [4, 6, 11]. Antihypertensive drug therapy choice during pregnancy is metyldophamine as it was proved to be safe for the fetus. The second-line drugs include calcium channel blockers. They are recommended for the treatment of high arterial blood pressure in pregnant females when refractory hypertension to methyldopa therapy occurs [2, 12]. The antihypertensive drugs of the second line for pregnant women also include β-blockers, but their use is limited due to the development of adverse effects to the fetus [1, 2, 10]. It is necessary to choose an optimal set of long-term antihypertensive treatment for pregnant women which will not have a negative effect on the fetus. That is why it is very important to perform detailed comparative analysis of differential approaches to the treatment of hypertension during pregnancy and perinatal complications.

The aim is to investigate endothelial dysfunction in pregnants with hypertension using non-invasive instrumental methods and offer an optimal therapy program for pregnants with arterial hypertension and

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to minimize the negative impact of the antihypertensive drugs on fetus.

Methods

We examined 63 pregnant women with hypertension at 28 to 32 weeks of gestation, which were hospitalized to the Ternopil Regional Perinatal Center "Mother and Child" of the Department of extragenital pathology for pregnant.

Clinical research was performed for all pregnant women according to the Ministry of Health of Ukraine standards: clinical analysis of blood and urine, biochemical blood analysis, assessment of urinary albumin excretion to detect microalbuminuria, as well as ECG, echocardiography, ultrasound investigation of renal and peripheral vessels.

We used modified technique by Dietz N. M. (1996) [6, 10], based on the measurement of the diameter of the vessel (by ultrasound) and peripheral vascular resistance (occlusal plethysmography), in order to assess endothelial function and its effect on uteroplacental hemodynamic.

Initially, the brachial artery diameter (distance between opposite walls of the artery) was measured in systole phase via linear 7 MHz sensor. Then arterial blood pressure was measured with classic manual tonometer. The pressure, equal to systolic plus 50 mm Hg, was injected into the cuff for 5 minutes. Measuring of the artery diameter was carried out immediately and again after 10 minutes.

Cardiotocography (CTG) with a score on a Fisher' scale and biophysical profile were used to assess the functional status of the fetus. The development of the fetus was evaluated by comparing ultrasonic fetometry under percentile tables in dynamic survey of 4 weeks after the previous test. Blood flow was assessed at uterine arteries, umbilical arteries, and middle cerebral arteries of the fetus with ultrasonic Doppler.

Women were randomized into three groups: group I (control group – 20 women), which included pregnant women with hypertension treated with basic antihypertensive therapy as recommended by MOH Ukraine standards (metildofa and nifedipin); group II – 21 pregnant women receiving metildofa and metoprolol;group III (22 pregnant women) receiving metildofa and nebivolol. Pregnants of all three groups were treated orally with low-dose aspirin, calcium supplements, and magnesium for the prevention of preeclampsia according to the Ministry of Health of Ukraine standards.

Statistical analysis: data analysis was done and verified using Statsoft STATISTICA 10 at Systemic Statistical Analysis Department of Ternopil State Medical University.

Results

Basic antihypertensive therapy for all groups included dopegit four times a day. Women of the Group I were treated with nifedipin 20 mg per day in four divided doses to stabilize blood pressure. Pregnants of the Group II were treated with metoprolol at a dose of 25 mg per day in two divided doses. Pregnant women of the Group III were prescribed nebivolol hydrochloride 5 mg once a day as a highly selective third generation beta blocker with vasodilative properties and ability to modulate the synthesis of the nitric oxide by the blood vessels endothelium. The endothelial dysfunction treatment should be considered as a strategic line for effective prevention of cardiovascular complications of the arterial hypertension as pregnancy treatment. Therefore, women of the second and the third groups were administered L-arginine aspartate (Tivortin) 1 g (5 ml) orally six times per day for 14 days in addition to antihypertensive preparations.

The gestational term of 28-32 weeks and arterial hypertension stage II were the criterions for randomization. Study groups were homogeneous for age, obstetric history, and the course of the underlying disease. Exclusion criteria were defined as following: hypertension stage III, severe concomitant extragenital pathology (diabetes, diseases of the thyroid gland, other cardiovascular pathology).

We observed different hemodynamic normalization and stability of blood pressure after treatment in each group (Table 1).

Table 1. Blood pressure and heart rate in women of the surveyed groups during dynamic treatment (M±m)

Group	Term of examination	SBP, mm Hg.	DBP, mm Hg.	HR, Bpm
Group I, n=20	Before treatment	156.2±0.39	108.8±1.08	92.0±0.81
	After treatment	137.2±1.42	91.4±1.24	92.0±0.94
Group II, n=21	Before treatment	154.8±0.32	106.4±0.59	91.0±0.81
	After treatment	138.2±0.98	92.2±1.43	68.0±1.50
Group III, n=22	Before treatment	156.8±0.52	108.8±0.92	90.0±0.88
	After treatment	130.2±1.26	82.6±1.12	80.0±1.56

Note: p<0.01 – data accuracy.

Pregnant women of the Group I showed poor tolerability to the therapy. Particularly, we observed sudden changes in blood pressure (hypotension episodes below 100/60 mm Hg or hypertensive crises (blood pressure above 160/110 mm Hg) in three pregnant women; four pregnant women developed swelling and redness of the face, seven women complained about palpitations, dizziness and weakness.

Simultaneously, eight patients of the second group experienced bradycardia (less than 60 bpm), while another five complained about dyssomnia, dizziness, weakness, and increased edema.

The best tolerability to the therapy was observed in the third group, where only two pregnant women noted weakness and headache while the heart rate slowed down below 60 bpm in two women. Also, as it is shown in Table 1, the clinically most effective scheme of arterial pressure normalization was the combination of nebivolol hydrochloride and metildofa on the background intake of L-arginine.

Forty four (70.9 %) of the surveyed women experienced ECG changes before treatment: the deviation of the electrical axis of the heart to the left, ST segment depression, inversions or flattening of T wave in leads II, III and avF. In pregnants of the Group I tachyarrhythmia was observed, including seven women (36.8 %) with arrhythmias. Bradyarhythmia and conduction disturbances (different degrees of blockages) were observed in nine pregnants of the Group II (42.9 %). Conduction disorders and slowing heart rate were observed in six women of the third group (27.2 %).

As for results of the sonogram of the heart, ejection fraction in all three groups before treatment was 63.3 %±3.4 %, and it decreased insignificantly after the treatment only in the second group. All females in all three groups (38 females, 62.1 %) were equally affected by end-diastolic increase in the size of the right ventricle, and an increase of the index of left ventricular mass by 10–15 %. Ten pregnant women (16.1 %) showed increase of the index of the left ventricular mass from 15.1 to 27.8 %, which may be classified as excessive. Combined preeclampsia developed in seven females (two pregnants from the first group, three from the second group, and two from the third group).

Data for the indicators of the endothelial function are presented in Table 2.

In all three groups, the diameter of the brachial artery before treatment should be assessed as narrowed. Therefore, an endothelial dysfunction is observed in pregnants with hypertension, which is manifested by spasm of the arteries. There was a significant dilatation of the brachial artery with slow normalization of arterial tonicity for the Group I after the treatment (diameter 4.4 mm±0.2 mm and endothelium-dependent dilatation was more than 22 %, which is relatively higher than in healthy pregnant women). Pregnants of the second group experienced spasm of the arteries, and this condition did not normalize. As for the third group, the diameter of the brachial artery was significantly restored to the values close to the healthy pregnant women and endothelium-dependent vasodilation (18.4 %) showed a normal tone of arteries and their ability to respond adequately to stimulation. Therefore, the endothelial function was restored in the Group III of patients to the highest level.

We have also established a relationship between fetometric performance relative to the gestational

Table 2. Indicators of the endothelial function (M±m)

Group	Term examination	Initial brachial artery diameter, mm	Endothelium dependent vasodilation, %.
Group I	Before treatment	3.41±0.04	8.0±0.31
	After treatment	4.14±0.04	22.4±0.51
Group II	Before treatment	3.33±0.05	8.2±0.32
	After treatment	3.38±0.05	5.0±0.47
Group III	Before treatment	3.37±0.04	8.1±0.29
	After treatment	3.80±0.03	18.4±0.47

Note: p<0.01 – data accuracy.

Table 3. The main parameters of fetometry during treatment of pregnants with hypertension of 32–34 weeks term (M±m)

Group	Term examination	Biparietal diameter (BPD), mm	Abdominal diameter (AD), mm	Femur length (FL), mm
Group I, n=20	Before treatment	79.06±0.17	79.64±0.89	48.56±0.48
	After treatment	84.42±0.95	87.36±1.19	62.05±0.99
Group II, n=21	Before treatment	78.27±0.21	79.15±0.38	48.16±0.28
	After treatment	82.30±0.86	84.24±0.90	60.03±1.23
Group III, n=22	Before treatment	78.01±0.22	79.10±0.19	48.30±0.45
	After treatment	86.30±0.74	91.20±0.84	65.24±0.77

term and the option of antihypertensive therapy on the basis of surveys (Table 3).

The size and weight of the fetus is less than average in the general population according to the gestational age in women with hypertension, which is often due to the disruption of hemodynamics in the mother-placenta-fetus system. Therefore, according to the ultrasound examination of the surveyed patients, the average biparietal diameter decreased for a given gestational age and evidence about disruption of uteroplacental hemodynamics; moreover, after the treatment, biparietal diameter was the biggest in the third group, followed by the first group and the second group. As for indicative parameters such as average diameter of the abdomen and femur length, best values were observed in the third group that explains normal fetal fatness and skeleton development, and therefore the optimal normalization of utero-placental hemodynamics, which correlated with indicators of endothelial function. Thus, the maximum normalization of the biometric parameters was observed in the third group of pregnant women.

Doppler velocimetry is helpful to evaluate blood circulation in the placenta and detect early signs of poor circulation. To assess the blood circulation, the following indices are used: systolic-diastolic ratio (SDR), pulsation index (PI) and index of resistance (IR), all of those are shown in Table 4.

The degree of hemodynamic disorders in the mother-placenta-fetus system is evaluated according to the Medvedev classification: 1A – isolated violations of the utero-placental blood flow; 1B – isolated violations of the feto-placental circulation; II – violation of utero- placental and feto-placental circulation does not reach the critical meanings; III – decompensated disorders and circulatory centralization.

Based on our results, we can conclude that pregnant women of the Group I experienced slight improvement of the parameters of the uterine artery blood flow, but no significant improvement of blood flow in the umbilical artery and middle cerebral artery of the fetus. Pregnant women of the second group showed insignificant worsening of the blood flow in the uterine arteries, umbilical artery and middle cerebral artery. Pregnant women of the third group were characterized by a slight improvement in blood flow of the uterine artery and nonsignificant improvement in umbilical artery and middle cerebral artery of the fetus.

Table 4. Doppler velocimetry in surveyed groups of pregnant women after 30 weeks of gestation

	Indicators	Uterine artery	Umbilical artery	Fetal middle cerebral artery
Group I, n=20	SDR	2.10±0.011	2.98±0.017	5.6±0.017
	PI	0.93±0.008	0.84±0.015	1.68±0.020
	IR	0.68±0.004	0.57±0.006	0.62±0.006
Group II, n=21	SDR	1.78±0.016	2.88±0.016	5.41±0.030
	PI	0.83±0.015	0.72±0.016	1.46±0.020
	IR	0.78±0.006	0.67±0.006	0.72±0.005
Group III, n=22	SDR	1.76±0.001	2.82±0.006	5.40±0.024
	PI	0.81±0.015	0.72±0.015	1.44±0.020
	IR	0.54±0.006	0.55±0.006	0.64±0.006

Note: $p<0.01$ – statistical significance and data accuracy.

Discussion

Summing up the results of the abovementioned surveys, we can assume that in order to minimize side effects during gestation, the cardioselective β-blockers with vasodilation properties should be preferred. These drugs will prevent the increase in total peripheral vascular resistance and can smooth the negative effects of classical beta blockers (delayed intrauterine development, bradycardia, hypotension, hypoglycemia, respiratory depression of the fetus).

Conclusions

1. It was found that the brachial artery ultrasound measuring and occlusive plethysmography procedure by Dietz is an early and safe method of endothelial dysfunction diagnostic in pregnants with hypertension. Doppler ultrasound of blood flow in uterine arteries, umbilical arteries and middle cerebral arteries of the fetus allows timely to reveal the side effect of antihypertensive drugs on the fetus.

2. The therapy of choice for pregnants with Stage II Arterial Hypertension should be based on methyldopa and calcium channel antagonists or selective beta-blockers combination.

3. Highly selective beta-blockers with vasodilative effect (nebivolol hydrochloride) and L-arginine (Tivortin) let us to prevent perinatal adverse effects of antihypertensive therapy, to correct hemodynamic disorders and endothelial dysfunction in pregnants with arterial hypertension.

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ULTRASTRUCTURAL RESEARCH OF THE ENDOMETRIUM RECEPTIVITY IN THE CONDITIONS OF PRE-CONCEPTIONAL PREPARATION IN REFRACTORY PREGNANCY LOSS

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Background. *Refractory pregnancy loss (RPL) is a multifactoral pathology in women of reproductive age which treatment represents great difficulties. Great role in RPL pathogenesis is stipulated by chronic endometritis resulting from disturbances of implantation followed by gestational sac malfunction, which is not less significant than progesterone deficiency.*

Objective. *The aim of research was to study the dynamics of ultrastructural changes of endometric epithelial cells in the conditions of pre-conception preparation in refractory pregnancy loss.*

Methods. *63 samples of endometrium bioptates obtained from female patients of reproductive age with insufficient middle luteal phase of the cycle were studied. Light and electronic microscopy was used to study the morphological changes in endometric epithelial cells.*

Results. *Pre-conceptional cyclic hormone therapy (1 mg 17β-estradiol and 20 mg of didrogesteron) in comparison with monotherapy of 20 mg of didrogesteron during 3 months contributed to intracellular regeneration and restoration of secretor phenotype of endometrium epithelial cells corresponding to the status of receptivity with "opened window of implantation".*

Conclusions. *Cyclic hormonotherapy in combination with complex metabolic therapy as a pre-conceptional preparation contributing to pregnancy illustrates much higher effectiveness of pathogenetically proved pre-conceptional hormonotherapy*

KEY WORDS: **refractory pregnancy loss, endometrium receptivity, hormone therapy, ultrastructure.**

Introduction

Refractory pregnancy loss (RPL) is a multifactoral pathology in women of reproductive age, which treatment represents great difficulties [1, 3, 4]. In the majority of cases the termination of pregnancy in PRL is due to inadequate reaction to endogenic progesterone and disturbance of secretory transformation of endometrium that results in the disturbance of blastocyst receptivity.

Great role in RPL pathogenesis is stipulated by chronic endometritis resulting from disturbances of implantation followed by gestational sac malfunction, which is not less significant than progesterone deficiency [11]. Weak receptive sensitivity of the endometrium in maintaining normal level of hormones is connected with a wide prevalence of chronic infectious and inflammatory processes accompanied by high content of cytotoxic cells CD16+ and CD56+ and their products such as anti-inflammatory cytokines that, in their turn, contribute to activation of prothrombinase with following thrombosis and placental infarctions [5, 8]. Besides of all mentioned causes of RPL and chromosomal fetus pathologies, there are idiopathic misbirths (approximately 15–25 %), pathogenesis of which is not clear and empirically progesterone is used [4].

Hormone therapy, as well as drugs possesing direct or indirect action on the steroid metabolism, are used to recover endometrium receptivity in case of correction of luteal insufficiency in RPL [12]. However, a great percentage of misfortune in the recovery of endometrium receptivity and achievement of fertility in RPL needs development of new approaches to the therapy and improvement of schemes of pre-conceptional preparation [9].

Aim of the research: the research was aimed to study the dynamics of ultrastructural changes of epithelial cells of the endometrium in the conditions of pre-conceptional hormone therapy in RPL.

Methods

Morphological studying of endometrium sampling bioptates in 63 women of reproductive age with RPL was performed. The patients were empirically divided into 2 groups (31 and 32 female patients 27.2±1.2 and 28.3±0.9 years old, respectively) depending on the scheme of pre-conceptional

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hormone therapy performed during 3 menstrual cycles.

The first scheme is a cyclic hormone therapy, in which femostin was administered 1/10 (1 mg 17β-estradiola and 10 mg didrogesterone) from the 1st to the 28th day of the cycle with additional supplementation of dodrogesterone (dufastone) in the dosage 10 mg per 24-hours from the 16th till 25th day. It's important to note that in comparison with recommended schemes [4], the 17β-estradiol dosage was decreased twice to stimulate insignificantly proliferative and differentiated activity of cellular endometrium populations. The first scheme included administration of 20 mg of didrogesterone (dufastone) per 24-hours from the 16th to 25th days of cycle. In both groups hormone therapy was used on the background of cyclic metabolic therapy including 100 mg of vitamin E in combination with 0.012 g of lipoic acid and 0.1 g of calcium pantothenate from the 5th to 15th days of the cycle, and with 1 mg of folic acid and 0.5 mg potassium orotate from the 16th to 25th days, respectively.

Sampling bioptates of endometrium were obtained according to the indications on the 22-24th day of the menstrual cycle till pre-conceptional preparation. Bioptates were fixed in 4 % solution of paraform. Paraffin sections colored with haematoxylin and eosin, as well as semithin sections colored with Schiff's reagent and toluydine blue, were studied using microscope Axio Scope.A1 with camera AxioCam MRc5 and software ZEN blue (C.Zeiss). Ultrathin sections of epithelial cells of endometrium contrasted with uranyl acetate and lead citrate were studied with electronic microscope JEM-100S. Written agreement on performing examination and treatment of RPL was taken in female patients.

Results

Before pre-conceptional hormone therapy the complex of structural changes of endometrium in female patients with RPL in the middle secretory stage of menstrual cycle in light-optical studying of paraffin and semithin sections corresponded to general pathological agreement about severe dystrophy and atrophy. Endometrial glands were reduced in the amount, being shortened and polymorphic according to the maturity: secretory glands did not prevail and were desynchronized with the phase of menstrual cycle, with dystrophy and atrophy of epithelium. Subepithelial stroma of endometrium was characterized by weak signs of decidualization of cellular elements and the presence of simple subepithelial lymphatic cells (fig. 1), that didn't correspond to the severe inflammatory changes in chronic endometritis.

Electronic microscopic examination of epithelial cells of endometrium revealed ultrastructural signs of a considerable reduction of the level of biosynthetic processes. The decreasing of height of epithelial cells, apical membrane of which didn't form pinopods, associated with severely decreased amount of nucleolus and components of canalicular system in nucleus and was accompanied with the reduction of elements of granular cytoplasmatic net in the absence of mega mitochondria. The decrease of synthesized protein and energetic potentials of epithelial cells was due to the accumulation of only singular glycogen grains.

Thus, ultrastructural equivalents of the disturbance of endometrium receptivity in RPL were insufficiently expressed by elements of protein synthesized complex of the nucleus and cytoplasm that in combination with little content of glycogen and absence of mega mitochondria confirmed insufficiency of plastic and energetic material for providing of secretory transformation. Disbalance of hormone-dependent cellular differentiation of epithelial cells of endometrium in cyclic stage in female patients with RPL is possible to be interpreted as systemic manifestation of universal syndrome of regenerative and plastic insufficiency [7], which essence consists of the disturbance of processes of intracellular regeneration and forming of plastic deficiency in different organs and tissues undergoing chronic unfavorable influences.

The use of the 1st scheme of hormone therapy during 3 menstrual cycles has stimulated intracellular regenerative reactions of epithelial cells since an adequate stage of the cycle by means of differentiation and expression of secretor phenotype of endometrium (fig. 2) more than proper level of reproductive health with "opened window of implantation" in comparison with the 2nd group (fig. 3). Gigantic mega mitochondria contributing to hormone-induced cytological differentiation were revealed in the epithelial cells of endometrium. There were large karyosomes with clasters of regular tubular structures, those membranes were appropriate to the tubules of granular cytoplasmatic net with numerous polysomes in the nucleus of the majority of cells (fig. 4); gross deposits of glycogen were localized in the cytoplasm diffusively and like super nuclear aggregates. High polymorphic protrusions of apical plasmolemma as pinopods were formed in 47-63 % of epithelial cells (fig. 5), other cells had polymorphic microvillus and cilia on their apexes.

Discussion

As a result of using the 2nd scheme of pre-conceptional preparation during three cycles, it was noted the growth of proliferative activity of epithelial cells which corresponds to the luteal phase of the menstrual cycle – the glands of stellar and serrated forms with wide lumens, and folded counters lined

by prismatic epithelium with ultrastructural signs of adequate cytodifferentiation. However, taking into account the complex of clinical data we can observe that the given scheme in most cases did not provide the stimulation expression level of plastic and energy potential of endometrial epithelial cells necessary to "open a window to implantation".

Normally the epithelium and the endometrium stroma are rapidly renewing tissues under the influence of a particular combination of reproductive hormones and should be exposed to remodeling for the normal receptivity of blastocytes. The basis of luteal phase deficiency is a reduced level of progesterone receptor expression as a consequence of inadequate estrogen content [6]. However, the morphological "immaturity" of endometrium can be observed even during the insufficient progesterone production as a result. What is important is not only the level of steroids in the organism, but also the adequate realization of hormonal effect [8, 10].

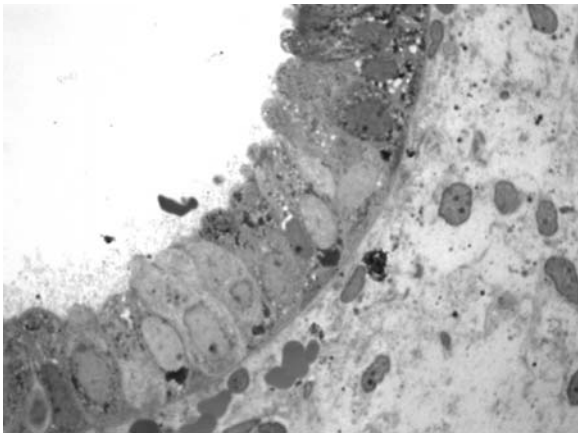

Fig 1. Refactory pregnancy loss. Endometrial biopsy sample on the 23rd day of menstrual cycle. Dystrophy of epithelial cells, absence of pinopods; isolated stromal cells. Semifine section, coloring with Schiffs reagent and toluidine blue. Magnification 1050.

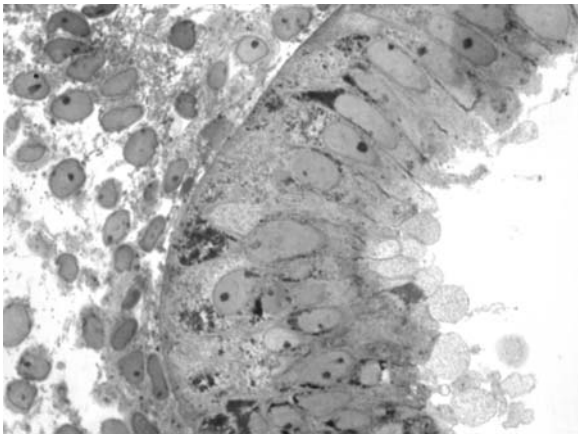

Fig. 2. Refactory pregnancy loss after cyclic hormonotherapy during 3 months. Endometrium biopsy sample on the 23d day of menstrual cycle. Numerous pinopods on the apical pole of epithelial cells. Semifine section, coloring with Schiffs reagent and toluidine blue. Magnification 1050.

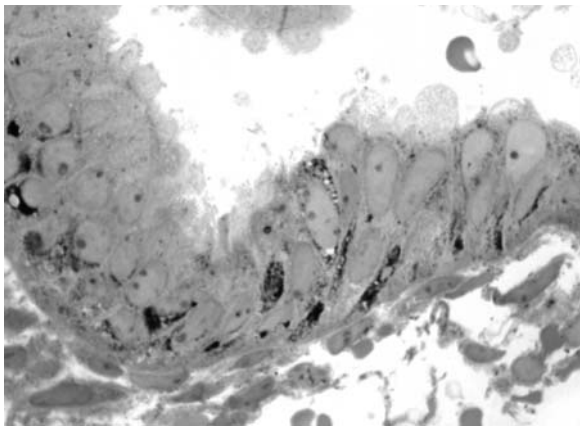

Fig. 3. Refactory pregnancy loss after dydrogesterone hormonotherapy during 3 months. Endometrium biopsy sample on the 24th day of menstrual cycle. Isolated pinopods on the epical poles of epithelial cells. Semifine section, coloring with Schiffs reagent and toluidine blue. Magnification 1050.

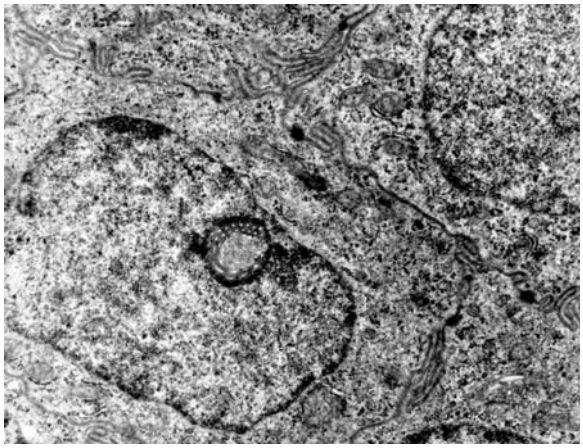

Fig. 4. Refactory pregnancy loss after cyclic hormonotherapy during 3 months. Fragments of endometrium of granulocytes on the 23rd day of menstrual cycle: the nucleolus in the nucleus is with the clusters of granular tubular structures; the cytoplasm contains free and attached polysoms. Electron-diffraction pattern. Magnifying power 10 000.

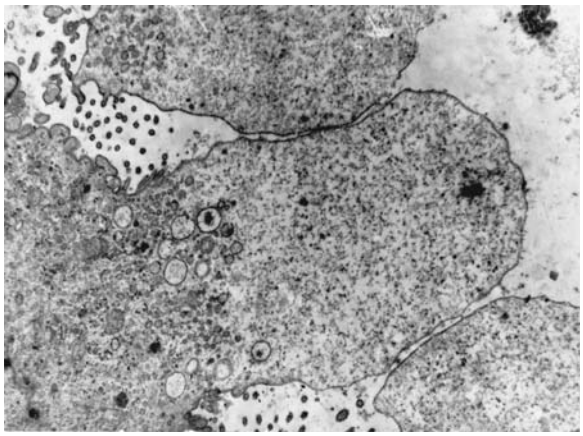

Fig. 5. Refactory pregnancy loss after cyclic hormonotherapy during 3 months. High pinopods on the apical poles of endometrium epithelial cells on the 23rd day of menstrual cycle: numerous exosomes and vesicles. Electron-diffraction pattern. Magnifying power 15 000.

In our trial the low doses of estrogens at the stage of pre-conceptional preparation of patients with refectory pregnancy loss contributed both to proliferation and hormone-mediated epithelium differentiation with receptors expression both to estrogens and to progesterone with the increased dosages to the 16th day of a cycle inducing secretory transformation. In other words, the cyclic hormonotherapy, providing the adequate supply of reproductive steroid hormones, consequently induced the intracellular regeneration of endometrium epithelial cells with the restoration of the necessary level of the receptor apparatus to estrogens and progesterone. The result of this process was the realization of status of endometrium receptivity corresponding to the indexes of reproductive health.

Mucous membranes including endometrium play a leading role in the adaptation processes; complex morphological studies of mucous membrane based on endoscopic biopsies demonstrate the permanence in their reaction that allows to formulate the conception of "reaction unity of mucous membranes" frequently realized by the syndrome of regenerative and plastic insufficiency based on the reducing of the processes of intracellular regeneration [2]. This syndrome can become a fundamental basis to create principally new approaches to the therapy of chronic diseases especially of endometrium by means of remedies inducing regenerative reactions in opposition to aggressive antibiotic therapy [3].

Conclusions

Thus, the obtained results allow us to distinguish a new aspect in morphogenesis of endometrium pathology in case of refractory pregnancy loss. In the complex of interrelated pathologic phenomena, there are the endocrine disorders, thrombophilia, and chronic endometritis. One of the important positions is concerned with endometrium dystrophy, which is evident in the reduction of intracellular regenerative epithelium reactions. The ultrastructural equivalents of reducing the endometrium receptivity is the reduction of protein synthesizing nuclear and cytoplasmic organelles in epithelial cells that underlie the discrepancy of the structure of luteal phase cycle with a reduction of secretory activity and «closed door to implantation».

The strategy basis of refractory pregnancy loss treatment is the intensification of regenerative reactions in cellular endometrium populations that contributed to the induction of biosynthetic reactions with endometrium receptivity and fertility. Cyclic hormonotherapy in combination with the complex metabolic therapy as a pre-conceptional preparation contributed to pregnancy in 16 (51.6 %) women of the 1st group and 13 (40.6 %) women in the 2nd group; full-term children with Apgar scores 8-9 were born in the 1st group – in 10 women (32.3 %), in the 2nd group – in 8 women (25.0 %) demonstrating higher effectiveness of pathogenetically proved pre-conceptional hormonotherapy.

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PROGNOSTIC SIGNIFICANCE OF CD56 EXPRESSION IN ACUTE LEUKEMIAS

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Background. *CD56 expression was extensively investigated in cases of acute leukemia. Many studies associated it with short overall survival, unfavorable outcome, lower rates or short complete remission, however the results remain controversial.*

Objectives. *The aim of this study was to investigate the frequency and prognostic relevance of CD56 expression in patients with acute leukemia and to compare its value with other standard prognostic factors, such as age, gender, leukocytosis, morphologic subtypes, extramedullary invasion, cytogenetic abnormalities and performance status.*

Methods. *Forty cases of acute leukemia treated at Ain Shmas University hospitals were investigated. They were classified by the French-American-British group (FAB) criteria, flow cytometry, and cytogenetics data. They included twenty cases of acute myeloid leukemia (AML) and twenty cases of acute lymphoblastic leukemia (ALL).*

Results. *CD56 positive expression was detected in nine cases of AML (45 %), and only in two patients with ALL (10 %). The highest incidence of CD56 positivity was in FAB subtypes M1 (35 %) and M2 (35 %).Association studies between CD56 expression and other prognostic factors in AML cases showed no significant association with age, gender, clinical presentation, hematological data or cytogenetic risk groups. Incidence of relapse was higher in AML patients expressing CD56 than those who did not (66.7 % vs 10 %, P=0.01). Higher death rates were encountered in AML cases with CD56 expression than those without (55.6 % vs 10 %, P=0.032).*

Conclusions*. CD56 antigenic expression in AML cases represents an adverse prognostic factor. It should be regularly investigated in cases of AML for better prognostic stratification and assessment.*

KEY WORDS: **CD56; leukemia, myeloid; prognosis**

Introduction

Acute leukemia is a heterogeneous group of disorders. They have various morphological, immunophenotypic and cytogenetic patterns. Identifying these characteristics is useful for prediction of therapy responses and prognosis of the disease [1]. Several phenotypic markers demonstrated to have clinical significance including detection of minimal residual disease [2, 3] rate and duration of complete remission (CR), disease-free survival, and overall survival (OS) [3].

CD56, a neural cell adhesion molecule (NCAM), is an early described natural killer cell-associated antigen. It mediates cell-to-cell interaction and is possibly involved in cell-mediated cytotoxicity. This antigen is also found in a subset of CD3+ cytotoxic T-cells and a small population of CD4+ T-cells and monocytes [4]. It is expressed in various hematopoietic neoplasms, including acute myeloid leukemia(AML) [5], acute lymphoblastic leukemia (ALL) [6], lymphoma [7], and myeloma cells [8]. Being a cell adhesion molecule, CD56 expression on tumor cells is believed to play a role in their localization with involvement of extramedullary metastasis [9].

The prognostic value of CD56 expression in cases of acute leukemia has been extensively investigated but with few consistent results [3]. Some investigators associated CD56 expression with short OS [5, 10–12], and lower CR rates [10] in AML patient. Although Ferrara et al.[12] reported short OS in AML cases expressing CD56; they could not detect association of this marker with the rate of CR in M3 AML cases. Moreover, Chang et al. studied 379 cases of AML including all subtypes except M3 and reported that the CR rate was not associated with CD56 expression, but with CD34 and HLA-DR expression [13].

The aim of this study is to evaluate the prognostic role of CD56 expression in newly diagnosed acute

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leukemia including AML and ALL cases and correlate the results with other prognostic factors.

Methods

Patients

This study was carried out on 40 newly diagnosed cases of acute leukemia. It included 20 AML and 20 ALL patients presented at the Hematology/ Oncology Clinics in the Internal Medicine and Pediatric Hospitals, Ain Shams University, Cairo, Egypt.

Diagnosis and classification of acute leukemia was based on WHO (2008) [14] and FAB [15] criteria. Bone marrow and peripheral blood samples obtained at presentation were examined for morphology, immunophenotyping and complementary cytogenetics. Another bone marrow samples were taken on the 28th day to evaluate response to induction therapy. Response to treatment was considered ineffective if more than 5 % blasts were detected in BM on the 28th day.

Immunophenotyping

Flow-cytometric analyses were performed as previously described [16]. The reactivity for the following markers was analyzed: a panel of fluorescein isothiocyanate (FITC)/phycoerythrin (PE) conjugated monoclonal antibodies to B-lineage markers (CD19, CD10, CD20, CD79a), T-lineage markers (CD2, CD3, CD5, CD7, cytCD3), myeloid markers (CD13, CD33, CD15, MPO, CD61, glycophorin, CD117), monocytic markers (CD14), and common progenitors markers (CD34, HLA-DR), supplied by Beckman Coulter, France. In addition, MoAb NCAM-PE (clone NCAM) (MiltenyiBiotec, Germany) for detection of CD56 was used. Cells were considered positive for a certain marker when ≥20 % of blasts expressed it, except for CD56, CD34 and intracellular MPO, where expression of ≥10 % of blasts was reported as positive.

Cytogenetic analysis

Leukemic cells were cultured, and the chromosomes were banded. Cytogenetic abnormalities were determined according to the International System for Human Cytogenetic Nomenclature [17]. Cytogenetic results were categorized into favorable, intermediate and unfavorable risk group.

Statistical analysis

Statistical analysis of the data was performed by using SPSS 15 software package and Windows 7® operating system. Categorical data parameters were presented in terms of frequency and percent age. Comparisons and associations that involve categorical variables were done by chi square test or Fisher exact test depending on the nature of the data. Continuous data parameters were described as middle, standard deviation (SD), median and interquartile range (IQR).

Results

Forty acute leukemia patients (20 AML and 20 ALL) were enrolled in this study. Table 1 summarizes age, gender, and clinical findings in the studied AML and ALL cases.

According to FAB criteria, AML patients were classified into: 7 (35 %) M1, 7 (35 %) M2, 4 (20 %) M3, one (5 %) M4 and one (5 %) M5 case. ALL were classified into 17 (85 %) B-ALL and 3 (15 %) T-ALL cases.

Successful mitosis was encountered in 18/20 (90 %) AML cases. Ten of these cases (55 %) were categorized as favorable [t(8:21), inv(16),t(15:17)], 6 (33.3 %) were intermediate (+8, normal) and 2 (11 %) cases were poor risk group [t(9:22), 11q23]. Successful mitosis was obtained in 12(60 %) of ALL cases. Three ALL cases (25 %) were classified as favorable risk (del 6q, hyperdiploidy>50), 5(41 %) were intermediate risk (normal), and 4(33 %) were poor risk group (t(9:22), hypodyploidy<45).

Our follow up of AML cases during induction period showed that 12 (63.2 %) AML cases showed good response to chemotherapy and achieved complete remission, while 7 patients (36.8 %) relapsed, and one case was missed. Six of the relapsed patients died. Meanwhile, 14 (70 %) ALL patients showed good response to chemotherapy and achieved complete remission, while five cases (25 %) relapsed, 3 of them died.

CD56 expression and association studies:

Nine AML cases (45 %) were positive for CD56, while 11 (55 %) were negative. CD56 and CD34 coexpression was found in 2 cases (10 %). None of our AML cases co-expressed CD56 and CD7. Only 2 ALL cases (10 %) expressed CD56. One ALL case co-expressed CD56 and CD34. There was a statistically significant positive association between CD56 positive expression and CD117 in AML ($p<0.05$). No significant association was found between CD56 positive expression and immuno-

Table 1. Demographic and clinical findings in AML and ALL cases

	AML n=20	ALL n=20
Age (years)		
Median	30.5	20
Range	13-65	10-50
Male (M) %(n)	55 (11)	55(11)
Female (F) %(n)	45 (9)	45 (9)
M:F ratio	1.2:1	1.2:1
Hepatomegaly %(n)	15% (3)	60 (12)
Splenomegaly %(n)	35% (7)	45% (9)
Lymphadenopathy %(n)	20% (3)	50% (10)
Pallor %(n)	60% (12)	40% (8)
Fever %(n)	40% (8)	50% (10)
Bleeding % (n)	25% (5)	30% (6)

phenotype profile of ALL (*P*>0.05). Because of the small ALL sample, expressing CD56, the analysis of results of these cases questions its statistical reliability.

No significant association was detected between CD56 positive expression and age (*P*=0.806) (Table 2), gender, clinical or hematological data in AML patients (Table 3). Similarly, we could not associate this marker expression with cytogenetics risk groups *(P*=0.118) (Table 4).

AML cases showed a statistically significant association between CD56 positivity and poor outcome. We missed one case and only 19 patients were evaluated including nine CD56 positive and

Table 2. Association studies between CD56 and age in AML patients

	Negative CD56 n=11	Positive CD56 n=9	X^2	*P* value	Significance
Mean age (years)	37.3	30.44	0.06	0.806f	NS

n = number of cases, NS: non-significant.

Table 3. Association studies between CD56 expression and gender, clinical and hematological data in AML patients

		Negative CD56 (n=11)		Positive CD56 (n=9)		x^2	*P*	Sig
		n	%	n	%			
Sex	M	6	54.55	5	55.56	0.002	1.000f	NS
	F	5	45.45	4	44.44			
Liver	-	10	90.91	7	77.78	0.669	0.566f	NS
	+	1	9.09	2	22.22			
Spleen	-	9	81.82	4	44.44	3.039	0.160f	NS
	+	2	18.18	5	55.56			
LNs	-	8	72.73	8	88.89	0.808	0.591f	NS
	+	3	27.27	1	11.11			
Pallor	-	5	45.45	3	33.33	0.303	0.670f	NS
	+	6	54.55	6	66.67			
Fever	-	7	63.64	5	55.56	0.135	1.000f	NS
	+	4	36.36	4	44.44			
Bleeding	-	9	81.82	6	66.67	0.606	0.617f	NS
	+	2	18.18	3	33.33			
Hb	<10	9	81.82	8	88.89	0.194	1.000f	NS
	≥10	2	18.18	1	11.11			
WBC	<50	8	72.73	4	44.44	1.650	0.362	NS
	≥50	3	27.27	5	55.56			
PLT	<100	9	81.82	7	77.78	0.051	1.000f	NS
	≥100	2	18.18	2	22.22			
PB Blasts	<60	5	45.45	4	44.44	0.002	1.000f	NS
	≥60	6	54.55	5	55.56			
BM Blasts	<70	6	54.55	4	44.44	0.202	1.000f	NS
	≥70	5	45.45	5	55.56			

n: number of cases, Hb: hemoglobin, WBC: white blood counts, PLT: platelets count, PB: peripheral blood, BM: bone marrow
Sig: significant, NS: non-significant.

Table 4.Association between cytogenetics risk groups and CD56 expression in AML patients

		Negative CD56 (n=9)*		Positive CD56 (n=9)		x^2	*P*	Sig
		n	%	n	%			
Cytogenetic Risk Group	Good	3	27.27	7	77.78	4.267	0.118f	NS
	Intermediate	4	36.36	2	22.22			
	Poor	2	18.18	0	0			

*2 cultures failed, n: number, Sig: significant, NS: non-significant

10 negative cases. Six patients out of the 9 positive CD56 cases (66.7 %) relapsed and only 3 (33.7 %) developed remission. Five (55.6%) of the CD56 expressing patients died. On the other hand, the CD56 negative patients group showed one (10 %) case relapse and death, while nine (90 %) patients developed remission.

Statistical comparison between those patient with CD56 expression and those without showed significant increased incidence of relapse (*P*=0.01) (Table 5, Figure 1) and deaths (*P*=0.032) among patients expressing CD56 (Table 5, Figure 2).

Discussion

Acute leukemia comprises a heterogeneous group of diseases that differ in their etiology, pathogenesis, and prognosis. Our study investigated the prognostic significance of CD56 expression in these cases to evaluate its association with other prognostic factors, and its influence on the outcome during induction therapy.

CD56 positivity was observed in 45 % of AML cases, and 10 % ALL cases. These results are in concordance with Fischer et al. [18] who reported that CD56 expression was not restricted to AML samples and could be detected in both B-cell and T-cell ALLs (14 %) as well. They concluded that it is not reliable for lineage distinction between AML and ALL. However, Montero et al. [19] reported expression of this marker in only 4 patients (2 %) out of 200 ALL cases. These differences could be explained by lower number of cases in our study as compared to 200 cases studies, or due to methodology variation. The study of Fischer et al. [18] investigated CD56 in 452 newly diagnosed T-ALL patients included in the GMALL trial. The marker was expressed in 13.9 % of patients, predominating in the non-thymic subtypes, whereas thymic T-ALL was most common in the CD56 negative group. In addition, the authors reported that CD56 expression was associated with higher resistance to therapy.

Our statistical analysis showed that CD56 expression in AML cases was not associated with age (Table 2), sex, or any laboratory variables including blasts counts, WBC, hemoglobin concentration or platelets counts (Table 3). Similarly Yang et al. [20] could not associate CD56 expression with any laboratory variables in AML cases. However, other investigators [1] reported a higher proportion of CD56 expression in men.

The presence of lymphadenopathy, hepatomegaly, and splenomegaly provide an indirect measurement of leukemic cell burden. We could not detect significant association of positive CD56 and presence of any of these clinical conditions in AML cases. Also, no significant association was found with other clinical features as presence of fever, pallor or bleeding tendency (Table 3). These findings are compatible with other published data (Table 18, 21).

Our work detected only two cases of ALL expressing CD56, these were of B-ALL subtype and were not characterised by hepatomegaly or splenomegaly. Although Ravandiet al. [6] considered that

Table 5. Association between treatment outcome, fate and CD56 expression in AML patients

		Negative CD56 n=10*		Positive CD56 n=9		X^2	*P*	Significance
		n	%	n	%			
Outcome	Remission	9	90	3	33.3	6.5369	0.010f	Significant
	Relapse	1	10	6	66.6			
Fate	Alive	9	90	4	44.4	4.5497	0.032f	Significant
	Died	1	10	5	55.6			

n = number of cases, *= one case was missed.

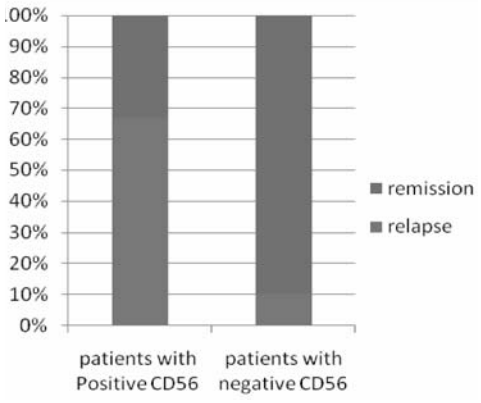

Fig. 1: Association between CD56 expression and treatment outcome in AML patients.

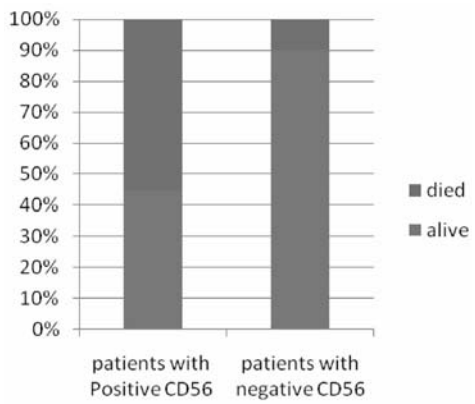

Fig. 2. Association between CD56 expression and mortalityin AML patients

CD56 expression predicts occurrence of CNS disease in ALL, none of our positive CD56 ALL cases had CNS involvement. In addition, no association was found between CD56 expression and demographic, clinical presentation or laboratory tests (P>0.05). However, the low number of ALL cases expressing CD56 incapacitated our statistical and association analysis and doubts these results.

CD56 expression was heterogeneous in different FAB subtypes. The more frequent CD56 expression was observed in M1 (35 %) and M2 (35 %). Allegrettiet al. [1] detected highest incidence of CD56 positivity among FAB subtypes M4 and M5, while Di Bona et al. [22] found more CD56 expression in M5 patients. These differences could be attributed to different number of patients in sample.

To elucidate the prognostic value of CD56, its expression was studied in relation to treatment response in our AML patients. CD56 expression was shown to be significantly related to response to chemotherapy with higher relapse (66.6 % vs 10 %, P=0.010) and death rate (55.5 % vs 10 %, P=0.32) among patients with positive CD56 expression as compared to patients without it. The investigation [1] in a single center in Brazil that investigated cohort of 48 AML patients supported our results and documented the association of CD56 expression with worse prognosis. The authors observed higher death rate during induction in the CD56 positive cases. Their study detected short OS among cases with positive CD56 expression compared to CD56 negative patients. Similarly, Baer et al. stated that CD56 expression was significantly correlated with a short complete remission (CR) period and low OS [21].On the contrary, Ferrara et al. reported that there was no association between CD56 expression and the rate of CR in patients with AML [12]. Moreover, Di Bona et al. failed to demonstrate the influence of CD56 positivity on CR duration or OS in 171 studied cases of AML [22].

AML with t(8;21) is considered a group with favorable outcome, usually marked by high CR rate and prolonged disease-free survival. We encountered five cases with this subtype (25 %), three of these patients (60 %) expressed CD56 and they relapsed and died. Although the small number of our cases (n=5) questions the significance of these results, the fatal outcome is too striking to be ignored. A previous large-scale studies investigated 144 AML patients with t(8;21) in the JALSGAML97 study. Univariate analysis showed that increased white blood cell counts (WBC$\geq 20\times 10^9$/L),CD19 negativity, and CD56 positivity were critical adverse factors for relapse after CR. Multivariate analysis showed that WBC count and CD56 expression were independent adverse risk factors. The authors concluded that CD56 expression has a possible role in risks tratification for patients with AML with t(8;21) [23].

Our study included only four cases of promyelocytic leukemia (APL), three of these patients expressed CD56 (75%). Relapses were evident in two (50%) of those cases expressing this marker. The clinical significance of CD56 expression was studied in a large series of patients with APL, treated with all-trans retinoic acid and anthracycline-based protocols. The authors documented that expression of CD56 is an independent adverse prognostic factor for relapse in these patients and suggested implementing this marker as a risk-adapted therapeutic strategies in APL [24].

Conclusions

The association between CD56 expression and the poor response of AML to current treatments is not fully understood. Raspadori et al. [25] hypothesized that $CD56^+$ AML blasts might overexpress p-glycoprotein (PGP), a multidrug-resistance (MDR) related protein, and reduce responsiveness to chemotherapy. Their data underlined the independent negative prognostic role of CD56 and PGP expression in cases of acute myeloid leukemia. In contrast, other investigators [26] showed that CD56 expression did not correlate with PGP expression, function, or with expression of the other MDR proteins. Other authors [27] have shown that CD56 expression by AML cells is positively regulated by RUNX1 p48 and is negatively regulated by other splice variants. Their findings suggest that p48 and RUNX1-driven NF-B and bcl2 pathways are new elements for targeted treatments in high-risk CD56AMLs.

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METHOD OF ONCOPLASTIC BREAST RESECTION

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Background. *In women with breast cancer the significant postop lateral deviation of the nipple-areola complex may occur if the partial breast resection with lymphadenectomy was performed through one-line straight incision from the middle axilla to the edge of areola.*

Objective. *To describe radical oncoplastic breast resection with avoiding of nipple-areola deviation and preservation of the symmetry of breasts.*

Methods. *The study included 16 women, age 32 to 67 years. Breast cancer at the stage I was found in 5 patients, stage IIA – in 4, stage IIB – in 2, and stage IIIA – in 5 patients. We proposed to use curved (broken line) incision with 120° angle instead of linear incision. Oncoplastic breast resection began with incision of skin and soft tissues in axilla, along the pectoral muscle downward to lateral contour of breast, and then direction was changed at an angle of 120° towards areola. The tumor projection was encircled by two semi-oval incisions, and then partial breast resection with axillary lymphadenectomy was done. After morphological confirmation of "clear" surgical margins the wound was sutured along the trajectory of the incision.*

Results. *All women rated the postop cosmetic result as "good" and "satisfactory". The selected configuration of tissue incisions provided physiological position of the operated breast.*

Conclusions. *Method of "broken line" incision of skin and soft tissue at an angle of 120° allows performing the radical breast resection with axillary lymph node dissection and prevents postoperative lateral deviation of nipple-areola, and maintains symmetry of the breasts.*

KEY WORDS: **breast cancer, oncoplastic resection.**

Introduction

In Ukraine, breast cancer of I-II stages is diagnosed in 76.9 % of patients according to the National Cancer Registry [1]. Thus we can expect increasing the likelihood of organ-sparing operations in patients with breast malignancies.

In the world, breast-conserving surgery (BCS) with postoperative whole breast irradiation is generally accepted as a routine method for most patients with early-stage breast cancer [2]. The proportion of patients treated with BCS has increased from 37 % between 1990 and 1992 to 62 % between 2002 and 2004 [3]. Nowadays 70 % of breast cancer patients are treated with BCS, 27 % with mastectomy alone, and 3 % – with mastectomy plus radiation [4]. BCS of early-stage cancer has been proven to be as equally effective as mastectomy in term of local control, distant disease, breast-cancer-specific and overall survival [3, 5]. BCS depends on the quadrant and distance from the nipple-areolar complex (NAC). They may require an oncoplastic approach to avoid the nipple or cutaneoglandular retraction with aesthetic sequelae [6].

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Objective: to present a method of oncoplastic breast resection with avoiding of nipple-areola deviation and preservation of symmetry of breasts.

Methods

The study included 16 women, age 32 to 67 years (mean 51.7±2.5 yr.). Breast cancer at the first stage (T1N0M0) was found in 5 patients, stage IIA (T2N0M0, T1N1M0) – in 4, stage IIB (T2N1M0) – in 2, and stage IIIA (T1N2M0) – in 5 patients. Category T1 tumor was diagnosed in 12, and T2 – in 4 patients. In 11 patients the tumor was located in the upper outer quadrant, and in 5 cases – in the inferolateral quadrant.

Staging procedures included: clinical examination of breasts and regional lymph nodes, ultrasound of breasts and ways of lymphatic drainage, mammography, lung X-ray or CT scan, liver ultrasound, fine needle aspiration or core biopsy of tumor, blood analyses and CA-15.3. Histological and biological evaluation results: tumor size, histologic type, grading, margin histology, receptors (ER, PR, Her2neu) and Ki-67 status.

The general scheme of treatment consisted of neoadjuvant chemotherapy, 2–3 cycles (6 patients) or chemoradiation therapy (10 patients). All patients underwent sectoral (or partial) resection of breast that included 2–3 cm of normal tissue surrounding

the malignant tumor; also the overlying skin and underlying fascia had to be removed. These radical sectoral resections were performed with axillary lymph nodes dissection by using the suggested oncoplastic technique [7]. In postoperative period the correction of anticancer treatment was done with regard to molecular subtypes of breast cancer. Patients' quality of life was assessed by EORTC scale QLQ-C30 BR23.

Oncoplastic breast resection.

Technical consideration. Radical sectoral resection in breast cancer patients consists of removing of the outer breast sector with simultaneous axillary lymphadenectomy. Cosmetic effects of such operations are not always satisfactory and mostly because resection of the breast and axillary lymph node dissection are performed on one-line tissue incision from the middle axilla to areola (Fig. 1 – AC). Postoperative linear scar causes significant lateral deviation of the areola and nipple (Fig. 1 – D). To avoid deviation and preserve symmetry, surgeon must perform the extra step operation – the centralization of nipple-areola complex (NAC), i.e., medial transposition of the NAC (Fig. 1 – C^1). We propose, instead of linear incision (AC), to apply the curved (broken line) incision with 120° angle between two lines AB and BC (Fig. 1). Because of this incisional geometry, when ABC>AC, the postoperative sutures (or scars) have discordant power vectors that provide physiological position of the operated breast.

Clinical case. In patient N., 41 years old, the 10 mm tumor in upper quadrant of right breast was found by ultrasound and mammographic examination. Fine needle aspiration biopsy confirmed moderately differentiated cancer. Axillary lymph nodes were 18 mm with margin hyperplasia according to the ultrasound findings. Diagnosis: cancer of the right breast cT1NxM0. Treatment scheme: neoadjuvant chemotherapy (Doxorubicin–Cyclophosphamide, ×2 cycles) and preoperative Co^{60} external radiation therapy on breast and axilla (4.5-5.0 Gy per fraction, 5 days, doses on axilla 34.2, on breast 38.2 Gy equivalent); surgery – radical partial breast resection with axillary dissection; postoperative radiation therapy on the breast only (2.2 Gy×10 days, total dose on breast 60.2 Gy) and chemotherapy (Cisplatin – Paclitaxel).

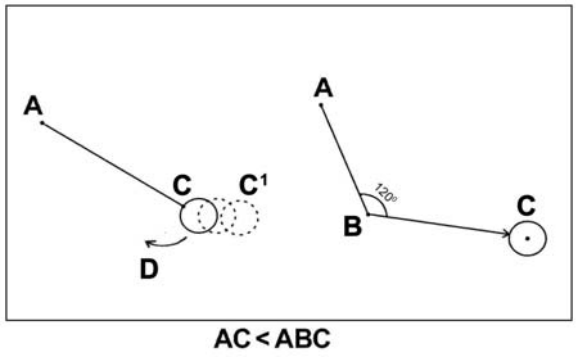


Fig. 1. Geometry of incisions: A – axilla, C – nipple-areola complex (NAC), B – lateral border of breast, D – direction of deviation, C^1 – transposition of NAC.

Surgery. The operative designs made in upright position of patient marking tumor's projection on skin with help of breast ultrasound. Oncoplastic breast resection was started with incision of the skin and soft tissues in axilla along the pectoral muscle downward to the lateral contour of breast, and then changed direction at an angle of 120° towards areola (Fig. 2). The tumor projection was embraced by two semi-oval incisions, and then partial breast resection with axillary lymphadenectomy was done (Fig. 3). After morphological confirmation of "clear" margins in surgical specimen the wound was sewn along the trajectory of the incision (Fig. 4). Surgical specimen: a tumor size of 5×7 mm at a distance of 2.0 cm to the margins of resection, with adequate volume ratio "tumor – to – breast tissue". The 11 lymph nodes ranging in size from 5 to 15 mm were present in axillary fat (Fig. 5). Histology: invasive ductal carcinoma, grade G2; lymph nodes with hyperplasia and angiomatosis; margin tissues histologically negative. Immunohistochemistry: ER–, PR–, Her2-

Fig. 2. Clinical case: trajectory of "broken line" incision on axilla and breast.

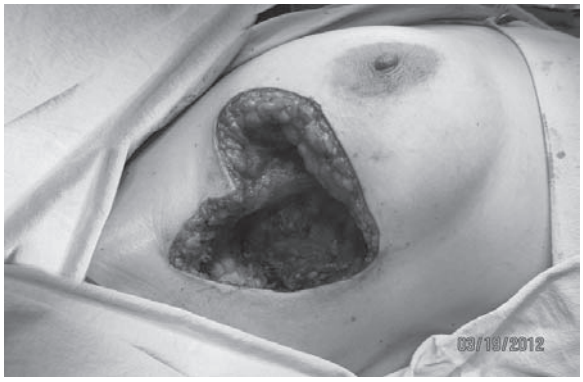


Fig. 3. On-table result of radical breast's resection with axillary lymphadenectomy.

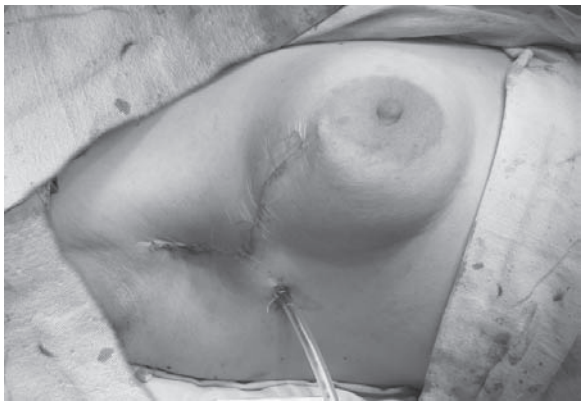


Fig. 4. Completed of oncoplastic breast's resection.

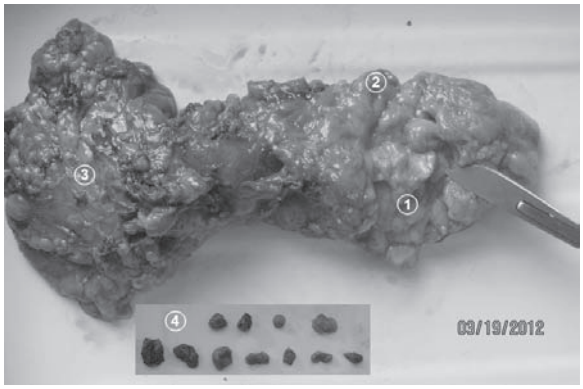


Fig. 5. Surgical specimen (1 – cancer, 2 – breast tissue, 3 – axillary fat, 4 – axillary lymph nodes).

neu++. According to the clinical and morphological data, final diagnosis was determined as: cancer of the right breast *p*T1bN0M0G2, stage I, ER/PR-, Her2-neu++.

In postoperative period wound healing was without any complication. Patient completed treatment by radiation therapy on operated breast and systemic chemotherapy. Two years follow-up with clinical examination, breast ultrasound (every 3 months) and annual X-ray mammography revealed no signs of relapse. Outcome of oncoplastic surgery is satisfactory; breasts are located symmetrically with good cosmetic effect (Fig. 6).

Results

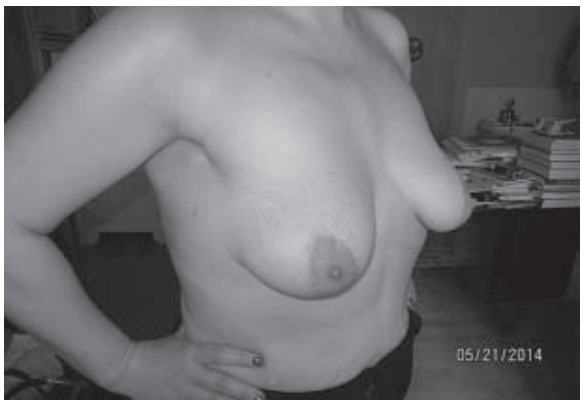
Complications. Swelling of the operated breast was observed in 11 (69.0 %) women, and it lasted up to 6 months; lymphedema of upper extremity was noted in 4 (25.0 %); seroma around postoperative suture was found in 7 (44.0 %) of patients; pain and stiffness in the shoulder joint occurred in 6 (38.0 %) women. The postoperative cosmetic result all patients rated as "good" and "satisfactory". The selected configuration of tissue incisions provided a physiological position of the operated breast.

During the 1-4 years of follow-up, 14 patients (88.0 %) were without evidence of recurrence; one patient died due to metastases into meningeal membranes; juxtaregional lymph node metastases developed in one patient after two years .

The organ-sparing surgery was performed in cases of tumor placing at a short distance from the regional lymph nodes in the outer quadrants of the sufficient developed breasts, when the proportion "tumor-to-breast volume" was more than 1:75-100. It was noted that neoadjuvant anticancer chemo- and radiation therapy reduced the volume of primary breast tumor and lymph nodes and by these enabled to do breast-conserving surgery. On the other side the organ-sparing surgery can be safely performed after neoadjuvant chemo- and radiation therapy in patients with breast cancer.

Discussion

Breast-conservative surgery is considered today as equivalent, if not superior, to mastectomy in terms of breast-cancer-specific survival. As shown in Agarwal et al. analysis of 132,149 women with early-stage invasive breast cancer (tumor $\leq$4 cm; $\leq$3 positive lymph nodes) that underwent BCS have a higher rate of disease-specific survival than those who undergo mastectomy. The 5-year breast cancer-specific survival rates of patients who underwent BCS, a mastectomy alone, or a mastectomy with radiation were 97 %, 94 %, and 90 %, respectively (p<0.001) [4].



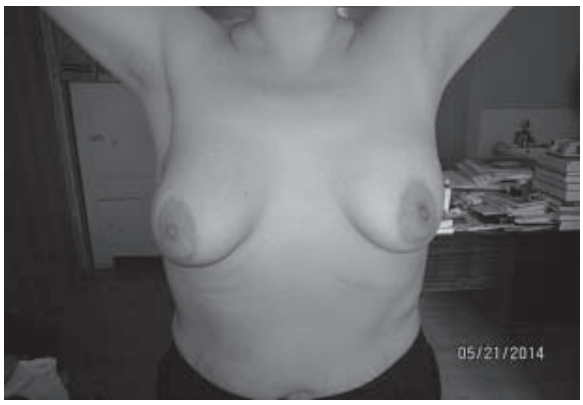


Fig. 6. Cosmetic results 2-years after oncoplastic resection of right breast.

The comparative study of 112,154 eligible women (T1/T2, stages I or II disease) included 61,771 who received lumpectomy (BCS) and radiation, and 50,383 who had mastectomy without radiation was published recently [3]. The 51 % of women under the age of 40 underwent BCS compared with 59 % of women age 50 to 59 years and 51 % of women age 70 to 80 years. The group achieving greatest benefit in overall survival with BCS relative to mastectomy was women at the age of 50 and older who were diagnosed with HR-positive tumors. The smallest benefit was seen among women who were under age of 50 diagnosed with HR-positive tumors. Kaplan-Meier survival estimation showed significantly greater overall and breast cancer-specific survival favoring BCS over mastectomy [3].

In another prospective, randomized study of 749 elderly patients with tumor less than 25 mm and pN0, ER+, G1-2, Ki-67<20 %, concluded that whole breast irradiation after BCS can be omitted in selected patient. After 9 years of median follow-up no significant statistical difference was found regarding overall survival and distant disease free survival between both groups with wide resection of the breast tumor (BCS) and BCS plus 50 Gy breast radiotherapy [5].

Thus, these data and our findings provide confidence that BCT remains an effective alternative to mastectomy for early stage disease regardless of age or HR status.

Conclusions

Method of "broken line" incision of skin and soft tissue at an angle of 120° allows performing radical breast resection with axillary lymph node dissection and prevents postoperative scar's lateral deviation of nipple-areola, and maintains the symmetry of the breasts.

Suggested method of oncoplastic resection is advisable to apply in the case of lateral localization of tumor in the breast.

Organ-sparing surgery can be safely performed after neoadjuvant chemo- and radiation therapy in patients with breast cancer.

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ANTHROPOMETRIC PROFILE, BODY COMPOSITION AND VERTICAL JUMP SCORE IN BOXERS AND SWIMMERS

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Background. *Vertical jump test (VJT) is used in some sport disciplines to evaluate an individual's lower extremity power. However, VJT data is unavailable in young Indian boxers and swimmers.*

Objective. *The given study was aimed to evaluate the VJT, anthropometric profile and body composition in young male Indian swimmers and boxers and compare the data with sedentary control group. The study also explored the relationship of VJT with anthropometric parameters and different components of body composition in the studied groups.*

Methods. *Male boxers (n=40), swimmers (n=40) and sedentary subjects (n=40) with similar socio-economic background with age ranging between 21 and 25 years were sampled for the study from Kolkata, India. Body composition was determined by skinfold measurements and VJT was evaluated by Sargent Jump Test.*

Results. *One way ANOVA shows significant ($p<0.001$) difference in body mass, %Fat, total body fat (TF), lean body mass (LBM) and VJT score in boxers, swimmers and sedentary groups. Significant positive correlation of LBM with VJT score was associated with a greater jumping height in swimmers. On the other hand, VJT had significant negative ($p<0.05$) correlation with sum of all the skinfolds, individual skinfold and total fat in both athlete group as well as in the sedentary group.*

Conclusion. *Significant intergroup difference was found in the studied parameters. VJT scores obtained in all the groups were well comparable. Higher value of body %Fat imposed the unfavourable effect towards achieving higher jumping height mainly in sedentary group.*

KEY WORDS: **VJT, boxers, swimmers, %Fat, LBM.**

Introduction

Vertical jump test (VJT) is used to evaluate the leg power or leg strength which is an important component of fitness testing in athletes as well as in sedentary population [1, 2]. Muscular strength is also assessed by vertical jump testing procedure [3]. Plyometric exercise training improves vertical jump performance and leg strength [4]. Vertical jumping and lower extremity power are significant in achieving success in volleyball players [5]. Higher value of VJT of international male volleyball players showed a better performance of explosive strength and a better use of arms during jumping activities [6]. Low fat percentage (%Fat) appeared to coincide a better performance in VJT score also in elite male handball players [7]. To the opposite, higher %Fat and BMI values exhibited poor physical fitness and lower value of VJT score in volleyball players [8]. Swimming performance depends on muscular power [9], muscular endurance [10], anthropometric characteristics, as well as on body composition in relation to VJT score. Swimmers need high muscle power functioning in lower limbs to achieve a sound performance [11]. The swimming start can be seen as an explosive event (jump), which requires high force production over a short period of time. There are, however, a few citations in the literature regarding the jump performance of swimmers [12]. Previous study showed that amateur boxers had lower VJT score, compared to wrestlers [13]. There is no data concerning jump performance (VJT) in Indian boxers and swimmers. Moreover, the relationship of VJT with anthropometric parameters and body composition in swimmers and boxers has not yet been investigated in Indian context. Therefore, the purpose of the present study was to: evaluate the VJT, anthropometric profile and body composition in young male Indian swimmers and boxers; compare the VJT, anthropometric profile and body composition between the boxers, swimmers and with sedentary control group; explore the

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relationship of VJT with anthropometric parameters and different components of body composition in the studied population.

Methods

Selection of Subjects

Young male swimmers (n=40) and boxers (n=40) belonging to 20–24 years age group with an average of five years training experience were sampled for the study from different sports academies in Kolkata, India. The sedentary control group (n=32) was randomly selected from the postgraduate section of the University of Calcutta, Kolkata, India. All participants had similar socio-economic background, and were disease-free, took no medication during the study period, and had no history of previous bone fracture or heavy injury. Written informed consent was obtained from each participant in accordance with the policy of the University of Calcutta Ethics Board. The study was approved by the Human Ethics Committee of the Department of Physiology, University of Calcutta.

The study was conducted at a room temperature ranging between 20–23 °C and relative humidity ranging between 40–45 %. After coming in the laboratory, subjects took rest for half an hour. Body height was measured with the subject standing barefoot with an accuracy of ±0.50 cm whereas the body mass was measured to an accuracy of ±0.1 kg by using the weight measuring instrument fitted with height measuring rod (Avery India Ltd., India) with the subject wearing minimum clothing.

Determination of Body Composition [14]

Body composition was determined by skinfold measurement using the following formulae:

Body density (BD; $gm \cdot cc^{-1}$) was determined by means of the following equations:

$BD = 1.10938 – 0.0008267X_1 + 0.0000016X_1^2 – 0.0002574X_2$

(X_1= sum of chest, abdominal, thigh skinfolds, X_2= Age in nearest yrs)

%Fat = 495/BD – 450

Percentage of lean body mass (%LBM), total body fat, and total LBM were calculated using the following equations:

- % Lean Body Mass (%LBM) = 100 – %Fat
- Total Fat or TF (kg) = %Fat/100 × Body Mass (kg)
- LBM (kg) = Body Mass (kg) – Total Fat (kg)

Determination of VJT Score

The VJT Score of each subject was measured according to the protocol elaborated in earlier reports [15].

Statistical analyses

Data have been presented as mean±SD. One way ANOVA was conducted to determine the significance of difference in the undertaken parameters among the studied groups. Pearson's product moment correlation was conducted to calculate the relationship between physical parameters and VJT score. Regression analysis was adopted to calculate the prediction norms for predicting VJT score from different physical parameters.

Results

Values of physical and anthropometric parameters and vertical jump score in boxers and swimmers and their sedentary counterparts are shown in table 1. Table 2 represents the values of correlation coefficient of VJT scores with different anthropometric parameters and body composition in boxers, swimmers and sedentary group. Simple and multiple regression norms for the prediction of VJT score in Indian boxers and swimmers and sedentary groups are shown in table 3 and table 4, respectively.

Discussion

Mean values of age, body weight, and body height, BMI, %Fat, TF, LBM and VJT score are displayed in table 1. Among the three groups, one way ANOVA shows significant inter-group difference ($P<0.05$) in the parameters (body height, BMI, %Fat, TF, LBM and VJT score), except the body height (table 1). Bonferroni correction was performed in the cases when it was reasonable. According to the classification of Arkinstall VJT scores, obtained in boxers and swimmers, were below average level [16].

Table 1. Values of physical and anthropometric parameters and vertical jump score in boxers and swimmers

	Age (yrs)	Height (Cm)	Weight (Kg)	BMI ($kg \cdot m^{-2}$)	%fat (%)	Total Fat (kg)	LBM (kg)	VJT Score (cm)
Sedentary (n=32)	22.53± 1.5	167.04± 3.2	66.01± 7.7	23.64± 2.56	14.58± 3.29	9.8± 3.06	56.2± 5.29	35.5± 2.7
Boxer (n=40)	22.12± 1.11	166.16± 3.33NS	53.32± 2.81*	19.33± 1.51*	6.25± 1.47*	3.37± 0.95*	4.95± 2.06*	37.31± 4.28
Swimmer (n=40)	22.95± 1.37	165.22± 4.57	58.32± 7.55*#	21.28± 1.90*#	10.99± 1.81#	6.39± 1.54*#	51.92± 7.08*	40.80± 4.15*#

Values are mean±SD, *p<0.05, #p<0.05 when compared to control, # When compared to boxer and swimmer.

Table 2. Values of correlation coefficient of VJT scores with different anthropometric parameters and body composition in boxers, swimmers and sedentary group

		Age (Yrs)	Body weight (kg)	Body height (cm)	BMI ($kg \cdot m^{-2}$)	Sum of Skinfolds (mm)	%Fat (%)	TF (kg)	LBM (kg)	VJT (cm)
Age	C		0.001	-0.095	0.039	-0.106	-0.060	-0.054	0.033	-0.11
	B		-0.23	0.015	-0.22	-0.20	-0.14	-0.15	-0.25	0.20
	S		0.16	0.09	0.21	0.03	0.08	0.18	0.13	-0.15
BW	C			0.38*	0.95***	0.69***	0.70***	0.86***	0.96***	-0.62***
	B			0.19	0.76***	0.80***	0.79***	0.85***	0.97***	-0.42*
	S			0.84***	0.95***	-0.22	-0.20	0.47**	0.98***	0.31*
BH	C				0.07	0.064	0.062	0.19	0.45*	-0.18
	B				-0.49	0.11	0.11	0.13	0.20	-0.26
	S				0.63***	-0.03	-0.02	0.48*	0.82***	0.35*
BMI	C					0.72***	0.73***	0.86***	0.88***	-0.60***
	B					0.64***	0.63***	0.68***	0.73***	-0.18
	S					-0.26	-0.24	0.43*	0.94***	0.21
Sum of Skinfolds	C						0.99***	0.95***	0.46**	-0.66***
	B						0.97***	0.99**	0.63**	-0.41*
	S						0.99***	0.74***	-0.35*	-0.43*
%Fat	C							0.96***	0.47**	-0.66***
	B							0.99***	0.62***	-0.40*
	S							0.75***	-0.34*	-0.45*
TF	C								0.68***	-0.70***
	B								0.70***	-0.42*
	S								0.34*	-0.20
LBM	C									-0.50**
	B									-0.38*
	S									0.36*
VJT	B									
	S									

***p<0.001, **p<0.01,*p<0.05, B = boxer group, S = swimmer group, C= sedentary group.

Table 3. Simple regression norms for the prediction of VJT score in Indian boxers and swimmers

Group	Simple Regression Equation	SEE (cm)
Sedentary	VJT=44.99–0.78 %fat	2.06
Boxer	VJT=44.54–1.16 %fat	3.92
Swimmer	VJT=52.06–1.02 %fat	3.71
Sedentary	VJT=45.26–0.23 S	2.09
Boxer	VJT=46.23–0.37 S	3.90
Swimmer	VJT=52.59–0.30 S	3.75
Sedentary	VJT=50.58–0.26 LBM	2.34
Boxer	VJT=76.32–0.78 LBM	3.96
Swimmer	VJT=29.69+0.21 LBM	3.87

S = sum of skinfolds, LBM = lean body mass

In the present study VJT score of the swimmers was significantly (P<0.001) higher than the boxers, and sedentary group. Papadopoulos et al. [12] reported lower values of vertical jumps performance in much younger (14.5±1.3 years old) swimmers, compared to an average of 22 years old swimmers in present study. Similarly, the VJT score in boxers in present study (age of an average of 22) was higher, compared to the jumping performance of the younger subjects (male kick boxers with the age 18.5±1.85 years), reported by Ouergui [13]. The variances of the results in jumping performance can be explained by the age of subjects in different studies. It is worth mentioning that in the given study VJT score of the swimmers was lower, compared to data reported for the vertical jump performance in American football players of similar age [17]. The variances of the results in jumping performance in these two studies can be attributed possibly to the special type of movements with higher specific requirements for power performance of the American football players, compared to swimmers.

In the given investigation VJT score was significantly (P<0.001) higher in boxers and swimmers than their sedentary counterparts. This finding was similar to the earlier report in male elite volleyball players who also demonstrated significant positive correlation between height and VJT score [5]. Malaysian male taekwondo players had significantly higher VJT score than their female counterparts [18].

Table 4. Multiple regression norms for the prediction of VJT score in Indian boxers and swimmers

Group	Regression Equation	R	R^2	SEE (cm)
Sedentary	VJT=49.96–0.62 %fat–0.12 LBM	0.70 (P<0.001)	0.4901	1.97
Boxer	VJT=66.91–1.22 %fat–0.44 LBM	0.43 (P<0.02)	0.1849	3.86
Swimmer	VJT=37.90–0.87 %fat–0.24 LBM	0.51 (P<0.01)	0.2601	3.57

LBM = lean body mass

Earlier studies reported significant positive correlation of height with VJT score in male elite volleyball players [5]. In the given study significant positive correlation was found between height and VJT score in swimmers. This finding corroborated with the earlier study in Malaysian martial art players [19].

Vertical jump score of athletes could be predicted by %Fat which is related to the work performed during vertical jump [20]. Previous research suggests that a reduction of body fat by proper dietary planning would help to develop leg power [21]. In the present study the VJT score had significant (P<0.001) negative correlation with the individual skinfold, sum of skinfolds, %Fat and TF. However, LBM showed significant (P<0.05) negative correlation with VJT score in boxers only. Analogous findings were also reported in Malaysian martial art players [19]. Similarly, poor VJT score in professional rugby league players was attributed to the higher value of %Fat [22].

Generally, sedentary individuals have a larger body %Fat. Hence, athletes with lower body %Fat have advantages in vertical jumps. Body %Fat is the best predictor of vertical jump for recreational male athletes. In the present study, sum of skinfold thicknesses has significant negative correlation with VJT score [1,20]. Therefore, an individual who has lower body fat percentage would be able to make more jumping height [18,23].

Simple regression equations have been calculated to find out norms for the prediction of VJT score from %Fat, sum of skin folds and total fat (TF). Multiple regression equations were also calculated to predict VJT score in both the athlete groups from sum of skinfolds and total fat (TF). In the multiple regression equations %Fat was not considered as a predictor variable since it was calculated from the sum of skinfolds. Standard errors of estimate (SEE) of the computed multiple regression equations were smaller than the simple regression equations and the values of these SEE were substantially small enough to recommend the multiple regression norms for practical use in epidemiological studies and also in clinical settings.

Conclusion

One way ANOVA showed significant (p<0.001) difference in body mass, %Fat, total body fat (TF), lean body mass (LBM) and VJT score in boxers, swimmers and sedentary groups. The VJT scores, obtained in the athlete groups, were below average, compared to data from other studies. Higher value of body %Fat imposed the unfavourable effect on achieving higher jumping height mainly in sedentary group. Significant positive correlation of LBM with VJT score was associated with a greater jumping height in swimmers. On the other hand, VJT had significant negative (p<0.05) correlation with sum of all the skinfolds, individual skinfold and total fat in both athlete group as well as in the sedentary group.

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NITRIC OXIDE AND ALLYL ALCOHOL INDUCED HEPATOTOXICITY

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Background. *Nitric oxide (NO) is an important mediator of hepatotoxicity. NO in liver can be derived from two sources: (1) constitutive NO synthase (eNOS) in endothelial cells, and (2) inducible NO synthase (iNOS) in hepatocytes and Kupffer cells.*

Objectives. *The present study was aimed to examine the effect of nonselective NOS inhibitor (L-NAME) and selective iNOS inhibitor (1400W) on the development of allyl alcohol (AA) induced hepatitis in rats.*

Methods. *Male Wistar rats were treated with intraperitoneal injection of saline or AA and L-NAME or 1400W. NO in liver was measured by electrochemical method after eNOS stimulation by calcium ionophore. Total NOS activity and nitrite/nitrate content were measured in liver and blood serum. The activity of free radical oxidation in liver was measured by chemiluminescent method. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed in blood serum*

Results. *AA increased the activity of free radical processes in liver and markers of cytolysis in serum, as well as decreased eNOS and increased iNOS activities. L-NAME considerably inhibited eNOS and augmented the necrosogenic properties of AA, whereas 1400W partially prevented liver damage.*

Conclusion. *It has been concluded that in AA intoxication NO produced from eNOS is beneficial to the liver, but NO derived from the upregulated iNOS has deleterious effect.*

KEY WORDS: **nitric oxide, toxic hepatitis, NOS inhibitors.**

Introduction

Allyl alcohol (AA) is a well known hepatotoxin widely used in animal experiments to induce liver necrosis. AA in hepatocytes is oxidized to acrolein by alcohol dehydrogenase [1]. Acrolein is a potent electrophile being able to attack nucleophilic molecules, e.g. sulfhydryl groups of reduced glutathione. The depletion of reduced glutathione affects the glutathione peroxidase antioxidant mechanisms and results in the activation of free radical oxidation. It has been suggested that the oxidative stress is the key event leading to liver necrosis in AA intoxication [2]. We used the AA model of hepatocyte cell damage in order to investigate the role of nitric oxide (NO) in the pathogenesis of necrotic liver injury. Nitric oxide (NO) is an important mediator of hepatotoxicity, and changes in its generation are paradoxically implicated in both cytoprotection and cytotoxicity [3]. NO in liver can be derived from two sources. Hepatocytes and Kupffer cells contain inducible NO synthase (iNOS), activity of which is markedly increased in inflammation. Endothelial cells contain constitutive NO synthase (eNOS). NO produced by eNOS plays important role in liver microcirculation. It has been shown that aminoguanidine, a relatively selective inhibitor of iNOS, protected against acetaminophen-induced hepatotoxicity [4], as well as thioacetamide-induced hepatotoxicity [5]. Another study concluded that iNOS plays an important role in liver cirrhosis following CCl_4 intoxication to rats [6]. Recently, we have demonstrated that *N*-(3-(Aminomethyl)benzyl)acetamidine (1400W), a strongly selective inhibitor of iNOS, prevented the depletion of reduced glutathione level in liver injured by allyl alcohol (AA) [7]. At the same time, in acute hepatitis caused by thioacetamide, methyl ester of N-nitro-L-arginine (L-NAME), the nonselective inhibitor of NOS, increased liver damage [8].

The present work was carried out to study the effects of nonselective (L-NAME) and selective iNOS (1400W) inhibitors on the formation of NO in liver in AA intoxication, as well as to investigate the correlation between NO production and severity of the liver damage.

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Methods

Animals and treatment

Male Wistar rats weighing 250-300 g were used in experiment. The animals were maintained under control conditions (23 °C, 12-hour light-dark cycle) with water and standard laboratory chow being available *ad libitum*. All rats were randomly assigned to four groups of ten animals each. Rats from the first group were treated with intraperitoneal (i.p.) injection of saline and served as a control. AA (30 mg/kg) was diluted in saline and administered one time intraperitoneally into rats of the second, third and

fourth groups. Third and fourth groups animals received correspondingly L-NAME (10 mg/kg, i.p.) and 1400W (1.5 mg/kg, i.p.) 30 min prior to AA injection. On the next day of experiment rats were anesthetized with a mixture of 100 mg/kg ketamine and 10 mg/kg xylazine and liver was dissected to carry out the studies described below. Blood was also taken for the analysis.

All procedures were in compliance with national and international laws and guidelines for the use of animals in biomedical research [9].

Measurement of NO in liver

The measurement of NO was performed with highly sensitive electrochemical nanosensor which was prepared as previously described [10]. Briefly, a carbon fiber (tip diameter about 500 nm) was covered with polymeric film of nickel (II) tetrakis (3-methoxy-4-hydroxy-phenyl) porphyrin. Measurements were performed using a three-electrode system that consisted of a nanosensor as the working electrode, a platinum counter electrode, and a silver-silver chloride reference electrode.

Liver tissue was cut into thin pieces of 5–6 mm in length using a razor blade and slices were placed on the bottom of thermostated organ chamber filled with Hank's solution. Counter and reference electrodes were positioned in the chamber near the liver slice and the active tip of the nanosensor was lowered near (2–4 μm) the surface of liver tissue with the aid of micromanipulator.

To stimulate the release of NO, 10 μl of eNOS receptor independent agonist calcium ionophore A23187 was injected with a nanoinjector onto the surface of liver slice to reach a final concentration in the medium of 1 μmol/L.

The changes in NO concentration from the background level were monitored with time (amperometry) with a computer-based Gamry VFP600 multichannel potentiostat (detection limit of 1 nmol/L and resolution time <50 ms). NO concentration was calculated by means of a calibration curve.

Measurement of the total NOS activity in liver and nitrite/nitrate content in liver and blood serum

The activity of NO generating system was assayed in 40 mM Tris HCl buffer, pH 8, containing liver homogenate (1 mg of protein/ml), 4 mM FAD, 4 mM H_4biopterin, 3 mM dithiothreitol, and 1 mM L-arginine. The reaction was initiated by adding NADPH (2 mM) and run for 3 h at 37 °C [11]. The combined concentration of nitrite and nitrate, which accumulate quantitatively as the stable oxidation products of NO, was determined as described below.

Measurement of the nitrite/nitrate concentration

The combined nitrite/nitrate concentration in the NO generating system as well as in liver homogenate and blood serum was assayed after the reduction of nitrite to nitrate by cadmium using improved Griess method with the help of commercially available QuantiChrom™ Nitric Oxide Assay Kit (BioAssay Systems).

Free Radical Oxidation Assay

The activity of free radical oxidation in liver homogenate was measured by chemiluminescent method [12].

Evaluation of Hepatotoxicity

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed as the markers of hepatocellular death using commercially available kits (Sigma-Aldrich).

Statistical analysis

Values are expressed as mean±SEM from 4 to 6 experiments. Values $P<0.05$ are considered as statistically significant. Statistical analysis was performed using ANOVA followed by the Student *t* test.

Results

The release of NO was measured ex-vivo using electrochemical nanosensors placed on the surface of the liver tissue. We stimulated NO release by a calcium ionophore, as it causes a large increase in intracellular calcium, rapid eNOS activation and maximal receptor-independent NO production. So far as, iNOS is not sensitive to Ca^{2+}, we can assume that the amount of NO measured by this method directly reflects the functional state of the eNOS located in the hepatic sinusoidal endothelial cells. The maximum or peak concentration of released NO from the liver of control rats was 160.0±12.2 nmol/L (Figure 1). By contrast, stimulation of liver from the AA injured animals resulted in an approximately two-fold reduction in the amount of NO release. Administration of the nonselective NOS inhibitor

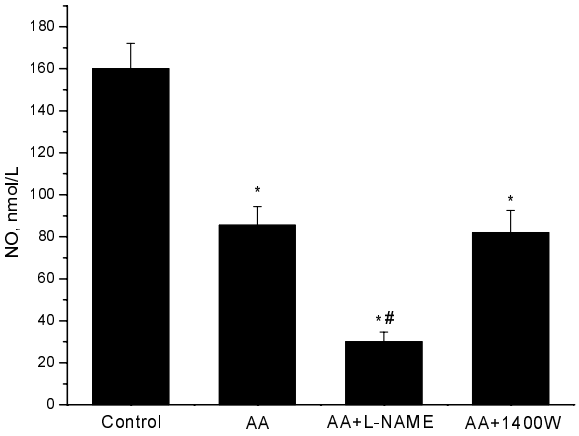

Fig. 1. The effect of L-NAME and 1400W on calcium ionophore (A23187, 1 mmol/L) stimulated nitric oxide release from liver of AA-treated rats. Rats were given AA (30 mg/kg, i.p.) with or without the administration of L-NAME (10 mg/kg, i.p.) or 1400W (1.5 mg/kg, i.p.) 30 min before AA. * — significantly different from control, $p<0.05$; # — significantly different from AA alone, $p<0.05$. Values are mean±SEM of 5–6 rats.

L-NAME markedly decreased the peak NO release (more than in five times, as compared to control), whereas iNOS inhibitor 1400W did not change the NO production significantly.

In contrast to the calcium ionophore stimulated NO yield, the activity of NO generating system was about 1.5-fold increased after intoxication, thereby suggesting that AA liver damage is accompanied by the considerable iNOS induction (Figure 2). Both L-NAME and 1400W significantly diminished the total NOS activity.

Serum and liver nitrite/nitrate concentrations correlated well with total NOS activity and were significantly increased 24 hours after AA administration (Figure 3). Both inhibitors effectively prevented the increase in nitrite/nitrate level.

The activity of free radical oxidation evaluated by the intensity of spontaneous chemiluminescence of the liver homogenate was almost three-fold higher in the poisoned animals in comparison with control (Figure 4). It is worth noting that 1400W significantly inhibited the free radical processes in hepatocytes, whereas L-NAME administration resulted in the increase of the chemiluminescence intensity.

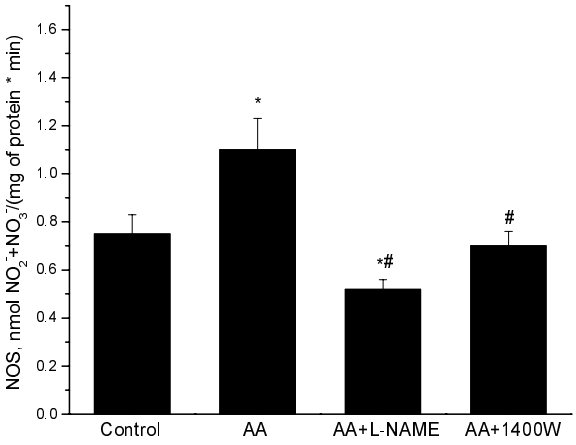

Fig. 2. The effect of L-NAME and 1400W on the total NOS activity in liver of AA-treated rats. Rats were given AA (30 mg/kg, i.p.) with or without the administration of L-NAME (10 mg/kg, i.p.) or 1400W (1.5 mg/kg, i.p.) 30 min before AA. * — significantly different from control, $p<0.05$; # — significantly different from AA alone, $p<0.05$. Values are mean±SEM of 6–8 rats.

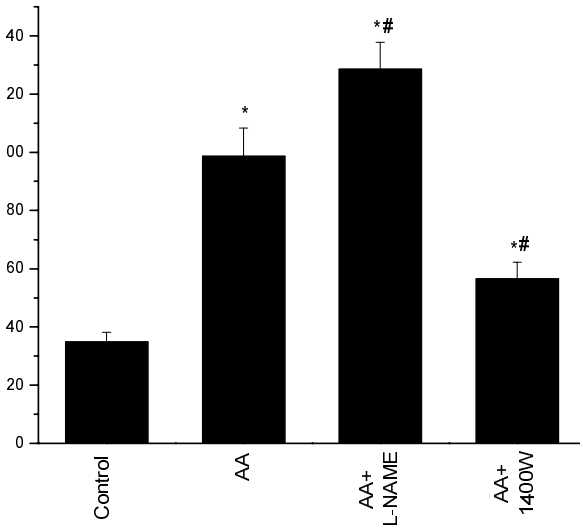

Fig. 4. The effect of L-NAME and 1400W on the chemiluminescence intensity of AA-treated rats liver homogenate. Rats were given AA (30 mg/kg, i.p.) with or without the administration of L-NAME (10 mg/kg, i.p.) or 1400W (1.5 mg/kg, i.p.) 30 min before AA. * — significantly different from control, $p<0.05$; # — significantly different from AA alone, $p<0.05$. Values are mean±SEM of 6–8 rats.

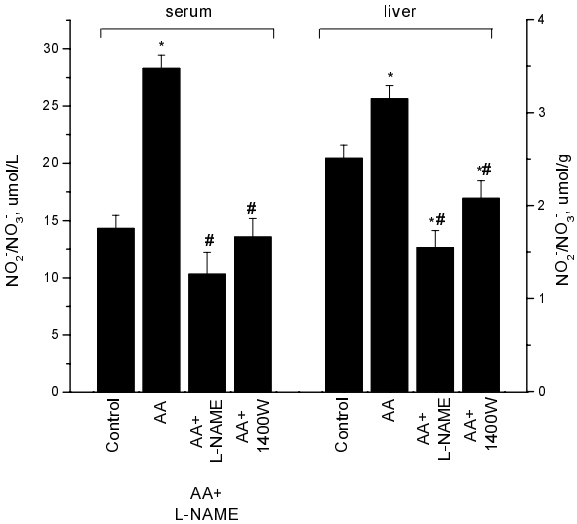

Fig. 3. The effect of L-NAME and 1400W on the combined nitrite/nitrate content in blood serum and liver of AA-treated rats. Rats were given AA (30 mg/kg, i.p.) with or without the administration of L-NAME (10 mg/kg, i.p.) or 1400W (1.5 mg/kg, i.p.) 30 min before AA. * — significantly different from control, $p<0.05$; # — significantly different from AA alone, $p<0.05$. Values are mean±SEM of 6–8 rats.

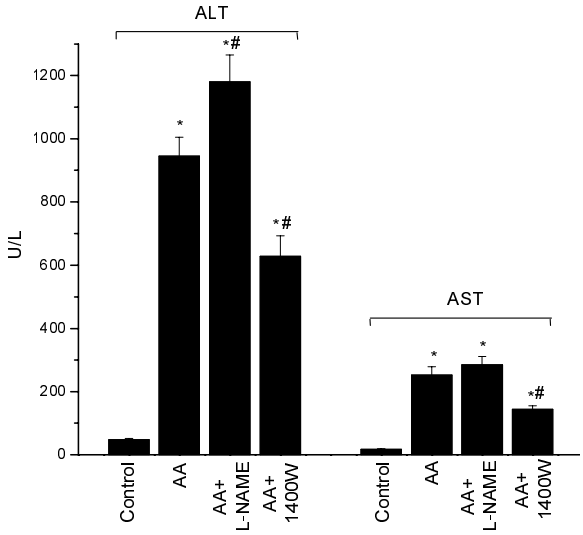

Fig. 5. The effect of L-NAME and 1400W on ALT and AST activities in blood serum of AA-treated rats. Rats were given AA (30 mg/kg, i.p.) with or without the administration of L-NAME (10 mg/kg, i.p.) or 1400W (1.5 mg/kg, i.p.) 30 min before AA. * — significantly different from control, $p<0.05$; # — significantly different from AA alone, $p<0.05$. Values are mean±SEM of 6–8 rats.

The activities of serum ALT and AST indicate the presence of hepatocyte cytolysis in animals treated with AA. These markers were especially elevated in the group administered with AA and L-NAME, which suggests significant damage of hepatocyte membranes in these rats (Figure 5). iNOS inhibitor partially decreased ALT and AST activity, as compared to the animals treated with AA alone.

Discussion

It is generally accepted that NO is an important regulator in the hepatotoxicity of many chemicals [4, 5, 6]. However, the role of NO in the development of liver necrosis is still not fully known and demands further studies. NO is reported to be produced in liver by both eNOS (endothelial cells) and iNOS (parenchymal and Kupffer cells). Previously we have shown that necrosogenic poison AA sharply decreases the reduced glutathione content in hepatocytes but inhibition of iNOS partially prevents this effect [7]. In this study we demonstrate that AA rapidly upregulates iNOS in liver. Depletion of the glutathione may weaken cellular antioxidant defense to such a point that the NO produced by iNOS may cause tissue injury. This suggestion is confirmed by our observation. The activity of iNOS as well as the intensity of free radical processes in liver tissue and the markers of cytolysis in serum (ALT and AST), which were markedly increased after AA injection, went down significantly in animals treated with the strongly selective iNOS inhibitor 1400W. These results distinctly show that the upregulation of iNOS in macrophages and hepatocytes following by the activation of free radical reactions in liver cells may be at least one of the AA necrosogenic effect mechanisms.

We also demonstrate here that in contrast to iNOS, hepatic eNOS is significantly inhibited in liver necrosis. This effect of AA, like the activation of iNOS, can also be the reason of the necrotic liver damage. After administration of the nonselective NOS inhibitor L-NAME we observed the deep depression of eNOS activity simultaneously with the considerable raise of the chemiluminescence intensity in liver and cytolytic markers activity in serum. This phenomenon can be be explained by the role of NO derived from eNOS in maintaining perfusion, and preventing hypoxia, platelet adhesion and thrombosis in liver capillaries.

Conclusion

In the AA intoxication NO produced from eNOS is clearly beneficial to the liver, whereas the inducible NO production has an opposite effects. Further research should provide a solid basis for therapeutic approaches to either supplement NO to the liver for its protective effect or suppress iNOS to prevent liver damage.

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L-ARGININE, BUT NOT L-NAME PROTECTS AGAINST LIVER INJURY INDUCED BY EXPERIMENTAL ISCHEMIA-REPERFUSION

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Background. *Hepatic ischemia-reperfusion (I/R) injury occurs upon restoration of hepatic blood flow after a period of ischemia.*

Objective. *The study establishes that stimulation or blockade of nitric oxide synthesis has a protective effect during ischemia-reperfusion.*

Methods. *Male albino rats which were divided into four equal groups: sham-operated control, ischemia and reperfusion group (0.9 % saline i.p.) for 3 days, group pre-treated with L-arginine (25 mg/kg i.p.), group pre-treated with L-NAME (10 mg/kg i.p.) for 3 days before ischemia-reperfusion maneuver. Complete ischemia of the median and left hepatic lobes was induced by clamping the left branches of the portal vein and the hepatic artery for 45 min. Rats were sacrificed after 3-h reperfusion. Nitric oxide synthase 3 (endothelial) and nitric oxide synthase 2 (inducible) expression, nitric oxide stabile metabolites (NO_2, NO_3) content, AST and ALT activities were determined. Histological examination of liver tissue was performed.*

Conclusions. *Relative NO deficiency, due to eNOS inhibition, is central in the pathogenesis of hepatic ischemia reperfusion injury. Replacing NO content with either precursors or via donor drugs represents novel methods in ameliorating ischemia-reperfusion injury.*

KEY WORDS: **hepatic ischemia-reperfusion, nitric oxide, NOS isoforms, L-arginine, L-NAME.**

Introduction

Ischemia-reperfusion (I/R) of the liver is a complex of pathophysiological processes that occurs during surgery, trauma, liver transplantation and can lead to serious liver injury. Basic mechanisms of liver IR are not completely understood at present time, but we claim that reactive oxygen and nitrogen are important in this process. Overproduction of free radicals by activated Kupffer cells and neutrophils causes a number of toxic effects, including changes in lipid and protein oxidation, excretion of proinflammatory mediators and microvascular dysfunction, which together lead to cell and tissue damage [1]. According to Bahde H. (2010), the hepatic microcirculation violations and endothelial dysfunction also play main role in the pathophysiology of I/R at the time of reperfusion [2]. The biologically active molecule of nitric oxide (NO) may play a pivotal role in the process of free radicals formation.

However, the data about the protective or damaging role of NO during I/R are controversial. According to some studies the application of NO precursors minimizes the adverse effects of reperfusion and improves hepatic microcirculation [3,4]. On the other hand, studies of some researchers suggest that inhibitors of NO synthesis can prevent liver damage during I/R [5].

This study shows that stimulation or blockade of nitric oxide synthesis has a protective effect during ischemia-reperfusion.

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Methods

NO precursor – L-arginine and non-selective NOS blocker – L-NAME (N-nito-L-arginine methyl ester) were procured from Sigma; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were procured from "Filisit–diagnostic", while TNF-α, IL-1β, IL-6, eNOS, and iNOS ELISA kits were procured USCN Limited Inc.

Animal model

Male white rats (Ternopil State Medical University vivarium, Ukraine) 8 to 10 weeks old, were used for these experiments. All animals were fasted 12 h before experimentation and allowed water *ad libitum*. All animals received care in compliance with the "Guide for the Care and Use of Laboratory Animals" (National Institute of Health Publication № 85-23, revised 1985). The studies were carried out according to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the local animal protection committee.

Induction of ischemic and reperfusion injury

The hepatic ischemia-reperfusion (I/R) protocols were performed as described in a previous study by Oleshchuk, 2012 [6]. There was no mortality

observed associated with this model. The liver of each rat was exposed through a midline laparotomy after the induction of anesthesia (thiopental sodium 20 mg/kg i.p.). Complete ischemia of the median and left hepatic lobes was induced by clamping the left branches of the portal vein and the hepatic artery for 45 min. The right hepatic lobe was perfused to prevent intestinal congestion. After the time frame sufficient for ischemia development, the ligatures around the left branches of the portal vein and hepatic artery were removed. In order to accurately evaluate the blood flow in the median and left hepatic lobes after ischemia, the right branches of the portal vein and the hepatic artery were ligated to prevent shunting to the right lobe after reperfusion and perfused for 2 h. The wound was stitched with 3.0 silk suture. Sham-operated animals were similarly prepared except that no ligature was placed to obstruct the blood flow to the left and median hepatic lobes. Instead, it the blood flow to the right lobe of the liver was occluded. Rats were sacrificed in all groups after 1-h ischemia followed by 3-h reperfusion. Twenty four white rats were divided into four equal groups (n=6 each group). Group 1 (sham-operated control group) and group 2 (ischemia and reperfusion group) were given 0.9 % saline (1 mL/kg, i.p.) for 3 days. Group 3 was pre-treated with L-arginine (25 mg/kg i.p.), group 4 – with L-NAME (10 mg/kg i.p.) for 3 days before, (last time – 10 min before) induced ischemia-reperfusion maneuver.

Peripheral blood and hepatic tissue procurement

Blood sampling for determination of liver enzymes. Blood samples were obtained from the right ventricle via left anterior thoracotomy at the time of sacrifice. Blood was collected in a sterile syringe without anticoagulant and centrifuged at 2000 g to separate the serum. The serum samples were stored at -20 °C until use for AST and ALT assays.

Blood sampling for cytokine and NOS determination. Serum was removed from blood samples by clotting for 2 hours on ice; serum was centrifuged at 2,500×g (4 °C), filtered, aliquoted, and frozen at -20 °C until assayed for eNOS and iNOS. Small liver samples were collected from each rat, then frozen immediately and stored in liquid nitrogen until used for eNOS and iNOS assays.

Determination of liver enzymes function

Determination of AST (EC 2.6.1.1) and ALT (EC 2.6.1.2) in plasma was performed by Raytman-Frenkel method, using a standard kit "Filisit-diagnostic" (Ukraine) according to the manufacturer's instructions. The activity of AST and ALT in serum were expressed in mmol/(L×h).

NO_2 and NO_3 assays.

NO_2 and NO_3 in serum of blood and liver homogenates (1:10) were determined as described by several authors [7].

NOS assays

Determination of eNOS activity was performed by ELISA method using "Enzyme-linked Immunosorbent Assay Kit for Rat Nitric Oxide Synthase 3, Endothelial (NOS3)", USCN, Life Science Inc, E90868Ra.

Determination of iNOS activity was done by ELISA method using "Enzyme-linked Immunosorbent Assay Kit for Rat Nitric Oxide Synthase 2, Inducible (NOS2)", USCN, Life Science Inc, E90837Ra.

eNOS and iNOS expression was investigated in blood plasma and liver tissue. Blood was collected using EDTA as an anticoagulant. Samples were centrifuged for 15 min at 1000 g/min at 2–8 °C within 30 min after collection. Determination was carried out immediately or frozen at -20 °C.

The procedure of liver cells lysis was performed as follows:

1. Preparing liver homogenates in isotonic NaCl solution at a ratio of 1:10.

2. Liver cells were centrifuged for 5 min at 300 g, and then the supernatant was removed.

3. Cells were washed twice with isotonic NaCl solution, after each wash the suspension was centrifuged at 300 g for 5 min.

4. Buffer (PBS) was added to the normal liver tissue lysate (1 ml of buffer at 1×10^6 liver cells). It was centrifuged for 5 min at 300 g.

5. The supernatant was collected. Determination of enzyme activity was carried out immediately or the supernatant was frozen at -20 °C.

Determination of eNOS and iNOS concentration were performed by ELISA method and according to the manufacturer's instruction. eNOS activity in serum and in hepatocytes (1 ml – 1×10^6 cells) was expressed as U/ml, and iNOS activity was expressed as ng/ml.

Histopathology study

A portion of the tissue from the ischemic liver lobe was fixed in 10 % neutral-buffered formalin solution for 5 days, embedded in paraffin, and sectioned. The sections were stained with hematoxylin and eosin.

Statistical analysis

Statistical analysis was carried out by OriginPro Program. Results are expressed as mean±standard deviation. Differences between experimental groups were analyzed with an unpaired 2-tailed Student *t* test. All differences were considered statistically significant at a $p<0.05$.

Results

The results indicate that after 45 min post-ischemia followed by 2 h reperfusion the activity of ALT in blood increased from (0.44±0.10) in the sham control group compared to (2.35±0.09) in I/R group

animals, which is 5.3 times increase. Accordingly, AST activity increased from (1.63±0.12) to (4.27± 0.37), which is 2.6 times increase. The described dynamics indicates that cytolysis process of hepatocytes is evolving under simulated IR injury (Table 1).

The significant increase in ALT and AST activities, that occurred in I/R group, was significantly suppressed by pre-treatment with 25 mg/kg L-arginine (Table 1). Thus, the transaminases activity was significantly lower: ALT (53.2 %) and AST (33.6 %). The activity of ALT and AST increased by 38 % and 84 % with the introduction of L-NAME compared to those of animals with the I/R alone. The activity of these enzymes was different from the control values by 7.4 and 4.8 times, respectively. This highlights a further progression of cytolysis and output of enzymes from the damaged hepatic tissue as a result of L-NAME pre-treatment.

Therefore, it was found that stimulation of NO synthesis in the animals with I/R improved function of the liver and blockage of NO synthesis by nonselective NOS inhibitor L-NAME aggravates liver injury.

Stimulated iNOS activity was registered in I/R treatment, content of which in the liver increased by 57.6 %, while in the serum – by 185.0 %. Whereas, the concentration of eNOS in the liver was reduced by 38.5 % and in the blood by 18.2 % compared with the group 1 (Table 2).

These changes took place on the background of a decline in serum levels of stable metabolite of nitric oxide NO_2^- by 51.7 %. This value has not changed significantly in the liver relative to the control group of similar animals. The level of NO_3^- in the blood did not change significantly compared to the liver where it was reduced by 65.5 % (Table 3). Quantitative indicators can be evaluated as a manifestation of a decrease in the total content of final metabolites of NO in both blood and liver during I/R trial.

The intensity of metabolism of nitric oxide under conditions of prophylactic administration of its precursor was evaluated by the concentration of its stable metabolites (Table 3) and the activity of NO-synthase isoforms (Table 2).

The level of nitric oxide metabolites in the blood increased: NO_2^- – by 3.7 times, and NO_3^- – by 31.7 %, respectively, after administration of L-arginine, and in liver homogenates the content of nitrite anion was not changed (p>0.05), and the nitrate anion increased respectively by 3.1 times.

We observed one-way influence of the substance on the expression of NO-synthase isoforms, both in blood and in the liver. Enzyme immunoassay results showed that the use of L-arginine in I/R treatment leads to increased eNOS content in hepatocytes (43.5 %) and the reduction of iNOS (19.2 %) (Table 2). A similar pattern was observed

Table 1. Effect of L-arginine on ALT and AST activity in the liver of sham and experimental groups of rats

Data	Sham (group 1)	I/R injury (group 2)	L-arginine +I/R (group 3)	L-NAME+I/R (group 4)
ALT	0.44±0.10	2.35±0.09 p<0.001	1.10±0.09 p<0.005 p_1<0.001	3.25±0.12 p<0.001 p_1<0.001
AST	1.63±0.12	4.27±0.37 p<0.001	2.83±0.20 p<0.01 p_1<0.05	7.87±0.15 p<0.001 p_1<0.001

Results are expressed as mean±SD (n=6); significantly different: p – from sham, p_1 – from I/R injury group; activity of ALT and AST are expressed in mmol/(l´h)

Table 2. eNOS and iNOS content in liver and blood of sham and experimental groups

Groups	Blood		Liver	
	eNOS U/ml	iNOS ng/ml	eNOS U/ml (1 ml – 1×10^6 cells)	iNOS ng/ml (1 ml – 1×10^6 cells)
Sham (group 1)	3.18±0.17	24.95±0.97	7.95±0.60	2.68±0.16
I/R (group 2)	2.60±0.14 p<0.05	71.22±4.01 p<0.001	4.86±0.24 p<0.001	4.22±0.16 p<0.001
L–arginine+I/R (group 3)	3.50±0.24 p>0.1 p_1<0.05	53.54±1.74 p<0.001 p_1<0.001	6.98±0.17 p>0.1 p_1<0.001	3.41±0.14 p<0.05 p_1<0.001
L-NAME+I/R (group 4)	1.14±0.12 p<0.001 p_1<0.001	31.40±1.61 p<0.001 p_1<0.001	2.99±0.20 p<0.001 p_1<0.001	2.56±0.06 p>0.1 p_1<0.001

For this and for the following table the results are expressed as mean±SD (n=6); significantly different: p – from sham, p_1 – from I/R injury group

Table 3. NO_2^- and NO_3^- content in liver and blood of sham and experimental groups

Groups	Blood		Liver	
	NO_2^-, micromol/l	NO_3^-, micromol/l	NO_2^-, micromol /kg	NO_3^-, micromol /kg
Sham (group 1)	1.62±0.08	10.18±0.41	1.70±0.11	9.29±0.59
I/R (group 2)	0.78±0.05 p<0.001	9.90±0.46 p>0.05	1.67±0.16 p>0.05	3.20±0.77 p<0.001
L–arginine+I/R (group 3)	2.84±0.07 p<0.001 p_1<0.001	13.05±0.63 p<0.01 p_1<0.01	2.07±0.10 p>0.05 p_1>0.05	9.94±0.60 p>0.05 p_1<0.001
L-NAME+I/R (group 4)	0.43±0.04 p<0.001 p_1<0.01	8.65±0.12 p<0.05 p_1<0.05	0.98±0.03 p<0.001 p_1<0.05	1.73±0.07 p<0.05 p_1>0.05

in blood. Inducible NO-synthase was reduced by 24.8 % after the use of L-arginine, but remained higher by more than 2.1 times, compared with sham group of animals. Endothelial isoform of the enzyme increased by 34.5 % compared to its value in group 1. To summarize, we can say that prophylactic administration of NO precursor L-arginine before I/R was followed by the activation of a constitutive NO-synthase, with a simultaneous decrease of inducible NOS activity and increase in nitric oxide metabolites level.

It is logical that the expression of both isoforms of nitric oxide synthase, on the background of inhibition of L-NAME, decreased eNOS content was even lower than in the group of animals without correction by 56.5 and 38.5%, and the expression of iNOS decreased by 55.9 and 39.3 % in blood and hepatocytes.

Therefore, L-NAME reduced expression of all NOS forms (Table 2). It was also shown that the level of nitrite and nitrate anions after administration of non-selective blocker of NO synthesis decreased by 45.0 and 12.6% in the serum and by 41.1 and 46.0 % in liver homogenates compared to IR. The levels were also significantly lower in the affected organ (by 1.7 and 1.8 times) compared to the group 1 of animals. We can hypothesize that reduction of end metabolites of nitric oxide synthesis correlates with inhibition of NO synthase expression in both serum and liver (Table 3).

During histological examination of liver tissue of animals, which previously were administered L-arginine and subsequently simulated ischemia-reperfusion, we found that the trabecular structure of the liver lobules was preserved. The central veins were dilated and well visualized. Periportal areas had lymphocytic and histiocytic infiltration, particularly around the bile duct (Fig.1).

Histological examination of liver tissue in ischemia-reperfusion model on the background of the previous L-NAME administration showed that the trabecular structure of the liver lobules was significantly changed (Fig. 2).

Sinusoids were greatly expanded and they were free from red blood cells in central lobular areas. Narrowed and saturated cell macrophages in the central and peripheral parts of the liver lobules were not present as well. Portal tracts expanded mainly due to the plethora of vessels and severe perivascular lymphocytic and histiocytic infiltration. Signs of small droplets of fat and protein degeneration were present. Enhanced bile ducts and the presence of a

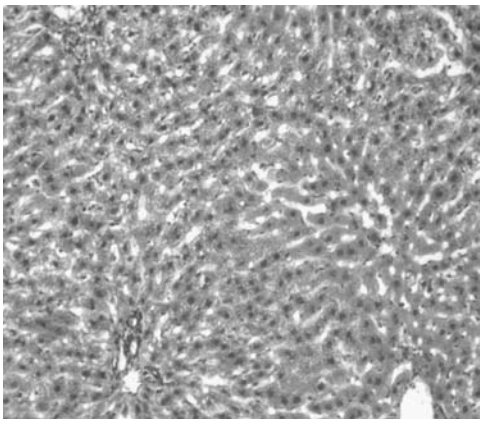

Fig. 1. Histological structure of the liver after administration of L-arginine in ischemia-reperfusion injury. Hematoxylin-eosin. Magnification power 160×.

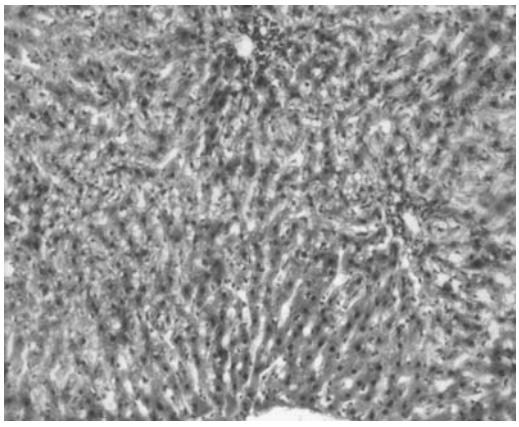

Fig. 2 Histological structure of the liver during ischemia-reperfusion and application of L-NAME. Hematoxylin-eosin. Magnification power 160×.

small amount of bile can be estimated as intrahepatic cholestasis. A large number of cells with pyknotic nuclei and necrotic cells were observed during histological observation in periportal zone of acinus.

Discussion

I/R is a series of multifaceted cellular events which take place on the resumption of oxygen delivery to the affected organ after a period of hypoxia. IR occurs in the liver during procedures that are associated with vascular inflow obstruction followed by restoration of blood flow. I/R may result in major hepatocellular damage [8].

NO plays a significant role in the acute phase of I/R, as this phase is associated with a rapid decrease in available NO. This decrease occurs either by depressed production by eNOS in sinusoidal endothelial cells, increased degradation by reactive oxygen species, or both [9]. In this I/R study expression of liver iNOS increased and eNOS level decreased. It was determined during our previous I/R studies that the levels of IL-1β, IL-6 and TNF-α in I/R were significantly increased [6]. Therefore, cytokines provoked stimulation of iNOS expression. To summarize, there is a lack of synthesis of NO during the early periods of reperfusion, which may be due to inhibition of eNOS [9]. As for this study, we have found that after 2 hours of reperfusion levels of nitrates do not change significantly in the blood with decrease in the liver, and conversely decreased blood nitrite levels.

We have shown that the application of L-arginine during I/R trial causes activation of nitric oxide synthesis. Increased concentrations of NO stable metabolites nitrite and nitrate anions in both blood and liver is consistent with our previous results and other scientific data [10, 11]. We observed one-way impact on the content of NO-synthase isoform in blood and in the liver. The level of endothelial form increased and inducible – declined. Results of our histological studies have shown that the precursor of nitric oxide synthesis has protective influence on the morphological structure of hepatocytes.

Blocking the enzymatic synthesis of NO in liver I/R led to the aggravation of the pathological process, as evidenced by the results of our biochemical and histological studies.

Thus, the introduction of non-selective blocker of NOS L-NAME increased activity of ALT and AST, indicating a further increase cytolysis of hepatocytes and this is consistent with L. M. Wang et al. (2003) [12]. Our results on the liver degradation by complete blocking enzymatic NO formation during I/R suggest NO protective role during early reperfusion.

Conclusion.

Ischemia reperfusion injury is a well-defined threat to the liver during periods of interruption and restoration of oxygen delivery. It occurs in certain procedures such as hepatic resections and orthotopic liver transplantation. Relative NO deficiency, due to eNOS inhibition, is central in the pathogenesis of this injury. Replacing NO per se either precursors or via donor drugs represents a novel method for ameliorating hepatic ischemia-reperfusion injury.

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INFORMATION SUPPORT SYSTEM OF MEDICAL SYSTEM RESEARCH

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Background. *Medical system research requires information support system of implementing data mining algorithms resulting in decision trees or IF-THEN rules. Besides that, this system should be object-oriented and web-integrated.*

Objective. *The aim of this study was to develop information support system based on data mining algorithms applied to system analysis method for medical system research.*

Methods. *System analysis methods are used for qualitative analysis of mathematical models diseases. Algorithms such as decision tree induction and sequential covering algorithm are applied for data mining from learning data set.*

Results. *Taking into consideration the complexity of mathematical equations (nonlinear systems with delays), scientific community requires the appearance of new powerfull methods of exact parameter identification and qualitative analysis. From the point of view of theoretical medicine, uncertainties arising in models of diseases require to develop treatment schemes that are effective, take into account toxicity constraints, enable better life quality, have cost benefit. Multivariate method of qualitative analysis of mathematical models can be used for pathologic process forms of classification.*

Conclusions. *The complex qualitative behavior of diseases models depending on parameters and controllers was observed in our investigation even without considering probabilistic nature of the majority of quantities and parameters of information models.*

KEY WORDS: **data mining, system analysis, medical research, decision making**

Introduction

Here, we would like to present our results in field of application of system analysis methods to problem of clinical medicine. We emphasize that effects of uncertainty should be taken into account in such complex systems. It will be shown that even considering deterministic models of such nonlinear systems, we observe different qualitative behavior closely dealt with parameters values. Let's start from the origin of this problem. Nowadays, a lot of models describing physiological indices of human body at different diseases and treatment schemes are obtained. Primarily, they are based on regression analysis. More complex ones use neural networks and evolutionary programming. The most significant attempts to construct mathematical models at different levels of hierarchy of human organism were made by John Murray [3], Keener and Sneyd [2], G.I.Marchuk [1], Mackey and Glass (they investigated nonlinear phenomena applying dynamic systems and introduced notion of dynamic diseases). Without considering uncertainty all these models can be applied for patients from determined groups (primarily for given age and a lot of other restrictions).

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Methods

As for projects stimulating given research, we would like to note the following. During the last years Medical Informatics Department performs investigations initiated by Healthcare Ministry of Ukraine in order to develop and use general system analysis algorithm to study different diseases [4–9]. Namely, in fields of oncology (melanoma, leukemia), infectious diseases (flu), therapy (bone tissue diseases). Naturally, there arises a problem to develop a general model for disease. It is incorrect to state that we managed to offer unique universal algorithm to construct disease general model. More correctly is to say that this approach can be used for diseases of different nature. We believe this approach can be extended to processes in sociology and demography, as well as for economy and finance. A lot of them have the same nature as human diseases. Let's take into consideration special medical terminology (as little as possible). First of all, the most recognized definition of disease states that disease is a set of

pathologic processes weakening vitality and activity of a human organism. Here, pathologic process is a set of pathologic (that is abnormal) and protectoral reactions within human organism. The most significant is modeling pathologic process.

Results

Based on this reason we offered general model for pathologic process including three counterparts:

(i) the *reason* or cause of disease (it may be some external factor (like bacteria, chemicals) or own modified cells (tumor cells);

(ii) *immune system* supports organism with help of specific antibodies (sort of predators) and plasmatic cells (their ancestors);

(iii) *normal cells*, tissues and organs (it is necessary to consider them to satisfy some constraints of toxicity).

We used our own software for these researches: Software Environment for Medical System Researches (SEMSR). Conceptual model of software environment of system medical investigations support is developed. Model implementation of data structure for medical investigations in terms of XML-technology is offered. Interface which is Web-integrated, user-oriented and adjustable is developed. Mathematical methods of system analysis of pathologic processes in form of Java-classes hierarchy are implemented. Software tools to execute system medical investigations, to prepare results obtained for presentation in Internet and visualization are developed.

Uncertainties in medical system research

Uncertainties in such models may be parametric. Some of the parameters may be unknown functions. As for uncertainty in control, it is necessary to take into account all possible scenarios. Note, the purpose of this article is not to present methods to identify these uncertainties. For these purpose we need to present powerful and deep mathematical apparatus of adjoint systems, sensitivity functions and minimax aposteriorial estimation. Here, we would like to answer two questions:

(i) why is it so important to take into account uncertainties?

(ii) the basic uncertainties in models of diseases.

To answer question № 1, we should say that the mathematical solutions of equations have different qualitative behavior. In practice we can observe different forms of disease (subclinical, acute, chronic, lethal). Search of treatment scheme is dependent on such forms.

In our research we investigated uncertainties in the following issues: maturation time for plasmatic cells τ, influence of antigen on target-organ damage rate σ, relation between target-organ damage rate and immune response $\xi(m)$, therapy scheme (polychemiotherapy, radiotherapy), surgery interventions. Note, the three last ones are non-parametric. They depend on unknown function like controller.

Approach of Compartmental Systems

Problems of population dynamics, pharmacokinetics, mathematical epidemiology, and others are described by compartmental systems with time delay. Even in the linear case, the solution of such equations leads to approximate computation procedures, which makes it impossible to find solutions of the following problems in explicit form:

– determining the time instant at which the number of infected persons does not exceed some level i^* (mathematical epidemiology);

– estimating the time when no more than d^* medical product units (pharmacokinetics) remain in the organism of a patient, etc.

Explicit solutions of such problems can be obtained on the basis of exponential type estimates. A number of works are devoted to the construction of exponential estimates for systems with delay. In [1], an estimate for a linear system is obtained on the basis of the Cauchy formula. An approach based on Lyapunov functions with conditions of the Razumikhin type was developed in [2]. In [3], an estimate is found from the solution of a difference inequality for a Lyapunov–Krasovskii functional. In [4], a differential difference inequality is constructed for a Lyapunov–Krasovskii functional. For compartmental systems, a promising approach is proposed in [5] and the method of construction of a class of exponential estimates is based on the Hale–Lunel inequality.

Software Development Based on Data Mining Technology

The objective is to develop and implement an algorithms of diagnostic classification applying decision tree induction and sequential covering methods and to study problem of their computational complexity.

The solved problem belongs to wide class of differential diagnostics problems. In medicine the notion of “differential diagnostics” means systemic approach based on evidence for determining causes of symptoms observed in case if there are few alternative explanations and also to reduce list of possible diagnoses.

One of approaches expressing natural process of thinking for differential diagnostics is data mining method. We are interested in the problem of computational complexity of the algorithms for real clinical data such as, for a example, for biochemical data in case of polytraumas.

Software implementation of decision tree induction

The methods are implemented within Netbeans developer system in Java language. The database of learning tuples is deployed on MySQL server. At fig.1 the conceptual model of informational system is presented. Class *DecisionTree* implements decision tree induction method. Class *DataManager* is processing calls from *DecisionTree* running queries to *mysql* database retrieving learning data.

Database *mysql* consists of two tables – table *attribute* for storage of information on attributes and table *categorized_data* – for learning tuples. The structure of tables in SQL syntax is shown below:

```
CREATE TABLE mysql.attribute (
id integer not null unique,
        attribute_name varchar(25),
        attribute_field_name varchar(25),
   primary key (id)
 ) ENGINE=InnoDB;
CREATE TABLE mysql.categorised_data (
id integer not null unique,
        A1 varchar(12),
        A2 varchar(8),
        A3 varchar(7),
   ……..
        A21 varchar(7),
        class varchar(28),
   primary key (id)
 ) ENGINE=InnoDB;
```

Classes of this project are included in package *decision_tree.model*. There are beans-classes *Attribute, Attribute_for_list* and *CategorisedData* for processing data of corresponding tables. SQL-queries for retrieving corresponding data including calculations of information indices are implemented in class AttributeListPeer.

Problem of computational complexity of decision tree induction algorithm

As it was shown in the work [11], time of decision tree induction algorithm running is estimated with value

$$O(p \times \#(D) \times log(\#(D))) \quad (1)$$

Our goal was to check this result experimentally. Experiments were executed varying amount of attributes p. Decision trees were constructed for each value of p. At fig. 2 and 3 there are shown estimates of decision tree induction times according to [4].

Computational complexity of sequential covering algorithm

Due to analysis of sequential covering algorithm we conclude that computational complexity is determined by product of amount of possible values of class attribute K (quantity of external cycle iterations) and computational complexity of procedure *Mine_one_rule (D, Att_vals, c)* executed inside each cycle.

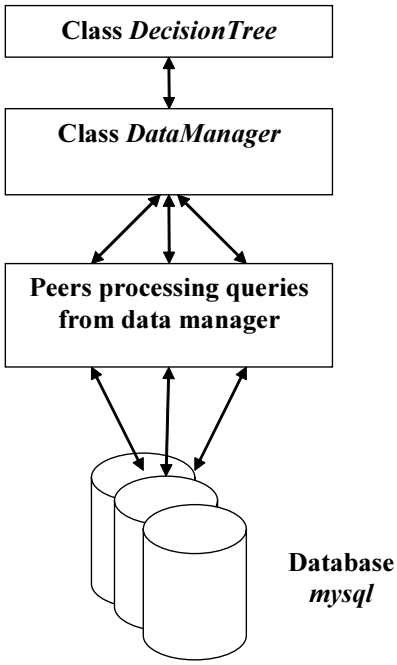

Fig. 1. Conceptual model of informational system of decision tree induction

Procedure *Mine_one_rule (D, Att_vals, c)* includes execution of p iterations. For each iteration for a certain attribute A_i we calculate the measure for each of K_i values of attribute. That is internal body of cycle in procedure *Mine_one_rule (D, Att_vals, c)* is executed $\sum_{i=1}^{p} K_i$ times. The measure is executed as a result of 4 SQL-queries with complexity $O(log(N))$ (according with MySQL 5.0 documentation). That is procedure *Mine_one_rule (D, Att_vals, c)* has computational complexity $O\left(\sum_{i=1}^{p} K_i \times log(N)\right)$.

Summarizing we have sequential covering algorithm complexity of the order

$$O\left(K \times \sum_{i=1}^{p} K_i \times log(N)\right) \quad (2)$$

Conclusions

So, even without considering probabilistic nature of the majority of quantities and parameters, we saw the complex qualitative behavior of diseases models depending on parameters and controllers. At different values of these quantities we observed subclinical, acute, chronic or lethal forms of pathologic processes.

Taking into consideration complexity of mathematical equations (nonlinear systems with delays),

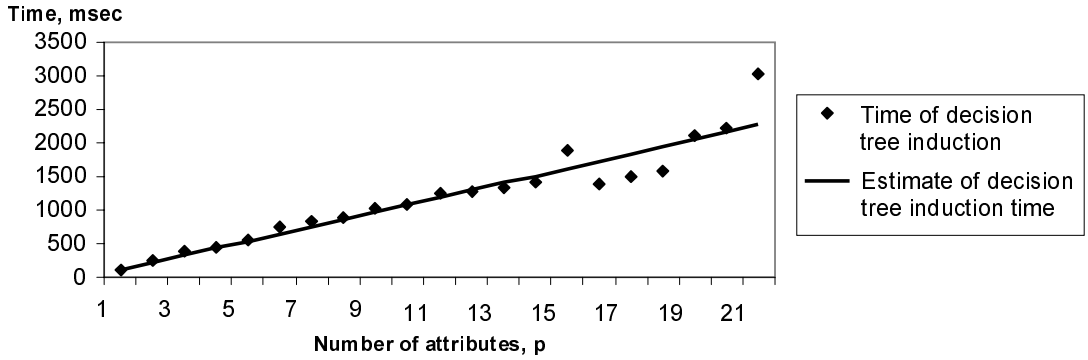

Fig. 2. Estimate of algorithm complexity based on information gain.

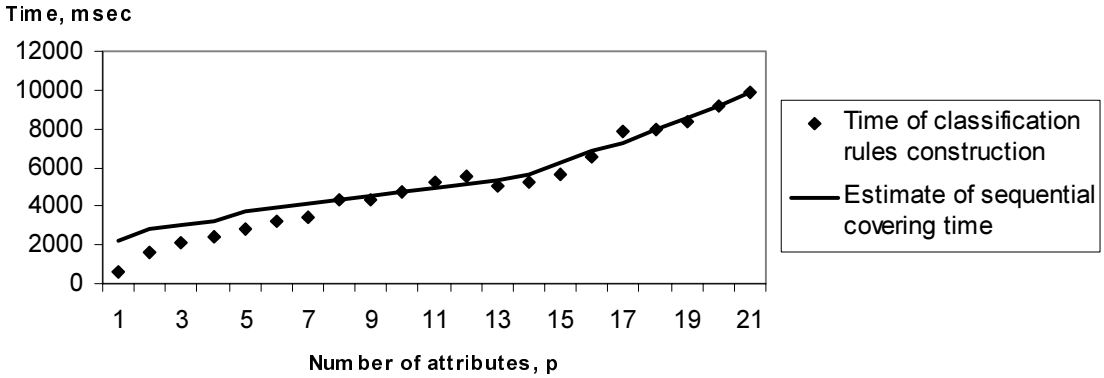

Fig. 3. Estimate of complexity of sequential covering algorithm.

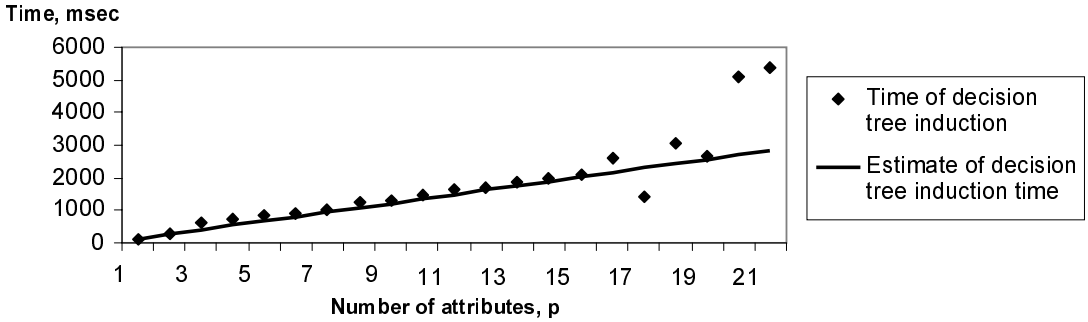

Fig. 4. Estimate of complexity based on information gain ratio.

it requires appearance of new powerfull methods of exact parameter identification and qualitative analysis.

From point of view of theoretical medicine, uncertainties arising in models of diseases require development of treatment schemes that are effective, take into account toxicity constraints, enable better life quality, have cost benefit.

In future works our idea will be to compare behavior of pathologic processes using both deterministic and stochastic models and to extend such models to demographic processes.

In the work here we considered the problem of development and implementation of decision tree induction and sequential covering methods based on information indices for construction of diagnostic classification algorithm.

While investigating this example, the problem of computational complexity of decision tree induction algorithm was observed that:

– decision tree induction time based on information indices is well approximated with estimate (1) at small number of attributes (in this case to 15–16);

– when increasing number of attributes (in this example over 15–16), the time of decision tree induction begins to deviate essentially from estimate (1) independent on search of information measure;

– at small number of attributes decision trees induced constructed based on either information gain or information gain are identical; i.e., information measure determining splitting attribute doesn't affect on decision tree induced;

– computational complexity of sequential algorithm is well approximated by (2). Such estimate was checked changing an amount of attributes as well as number of learning tuples.

The perspective of this investigation is comparative performance analysis depending on volume of set of learning tuples.

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THE INFLUENCE OF ANTIRETROVIRAL AND ANTITUBERCULOSIS AGENTS ON THE BIOCHEMICAL AND HISTOPATHOLOGICAL INDICES OF LIVER FUNCTION IN RATS

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Background. *Standard antituberculosis treatment and highly active antiretroviral therapy are frequently associated with hepatotoxicity leading to drugs discontinuation.*

Objective. *This study aimed to assess the signs of hepatotoxicity in albino rats in case of simultaneous usage of tuberculostatics (TBS) and antiretroviral agents (ART).*

Methods. *Healthy rats were divided in 4 groups: 1st control group; 2nd group was given tuberculostatics (isoniazid – 50 mg/kg, rifampicin – 50 mg/kg and pyrazinamide – 1500 mg/kg); 3rd group was given ART (efavirenz – 150 mg/kg and stavudine – 5 mg/kg); 4th group was given TBS and ART. The animals were sacrificed painlessly on the 29th day; blood and liver samples were obtained. The main biochemical and histopathological indices were determined.*

Conclusions. *Comparing with control group, repeated usage of TBS caused the prominent liver injury with cytolysis and cholestasis signs, decreasing of CYP3A and CYP2E1 isozymes activity and dysfunction of protein synthesis by the liver. ART (efavirenz and stavudine) caused the elevation of transaminases activity with the increase of serum bilirubin level at the background of increase in cytochrome 450 isoforms 3A and 2E1 activities and total serum protein. The antiretroviral agents in case of simultaneous administration with the antituberculosis drugs diminished the hepatotoxic effects of first-line drugs for tuberculosis treatment which was confirmed by the study of liver histopathology. Such results of our experimental study give encouragement for further detailed clinical research of drug-drug interaction of both pharmacological groups due to the rising cases of HIV-associated tuberculosis in the whole world.*

KEY WORDS: **Isoniazid, rifampicin, pyrazinamide, efavirenz, stavudine, liver, cytochrome P450.**

Introduction

The global burden of tuberculosis (TB) is enormous today (Fig. 1) [1]. The overlapping epidemiology of HIV and TB as simultaneous infections has had catastrophic consequences. The interaction between these diseases is bidirectional. HIV infection increases the risk of both primary and reactivated TB [2], and this risk increases markedly with advancing of HIV disease. The case fatality rates of HIV-associated TB are high; the estimated aggregate case fatality rate of HIV-infected TB is about 40 %, and may be over 50 % in many developing countries [3].

When choosing therapy for patients with HIV and TB, drug–drug interactions should be carefully considered. Rifampicin is an upregulator of CYP450 enzymes that catalyzes the metabolism of a number of other drugs, including the NNRTI (Non-nucleoside reverse-transcriptase inhibitors) efavirenz (EFV). Concomitant Highly Active Antiretroviral Therapy (HAART) during TB therapy is complicated by high pill burden, concerns about drug-drug interactions, paradoxical immune reconstitution reactions and the main problem is overlapping drug toxicities (table 1) [4, 5, 6].

The treatment of tuberculosis is complicated by drug-induced hepatotoxicity, with reported rates ranging widely, from approximately 3 to 25 %, depending on the hepatotoxicity definitions, the regimens, the methodologies, and the study populations [7].

Hepatotoxicity is a relatively common adverse drug reaction leading to treatment interruptions in HIV patients, observed with different drug combinations [8]. Currently, the top priority for World Health Organization (WHO) is to increase coverage of ART for HIV-positive TB patients towards the 100 % target [1].

Isonazid (isonicotinylhydrazine, INH) in treatment of all types of TB is associated with mild to moderate elevation of liver enzymes and sometimes with severe hepatotoxicity. Rifampicin (RFP), which is commonly used in combination with INH, was

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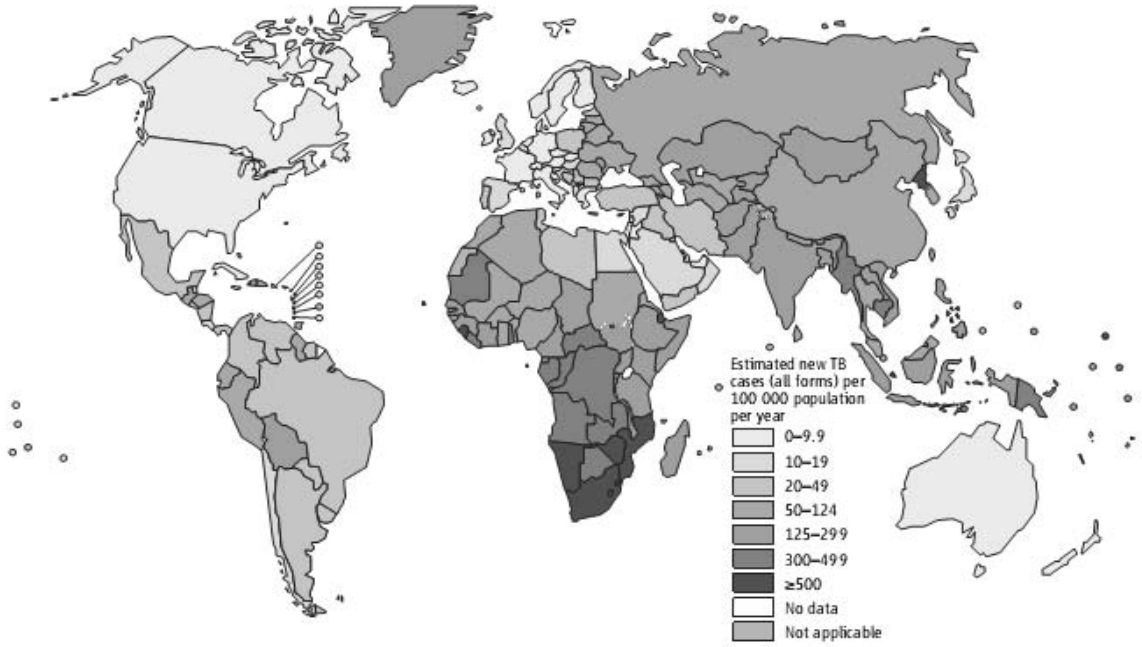

Fig. 1. Estimated TB incidence rates, 2012. *Reproduced from* World Health Organization. Global tuberculosis report 2013.

Table 1. Overlapping or additive adverse drugs effects of antiretroviral and first-line antituberculosis agents

Toxicity	Antiretroviral agents	Antituberculosis agents
Peripheral neuropathy	Stavudine, didanosine	Isoniazid , ethambutol
Gastrointestinal intolerance	All	All
Hepatotoxicity	Nevirapine, efavirenz, all nucleoside reverse transcriptase inhibitors and protease inhibitors	Isoniazid, rifampicin, pyrazinamide
Neurotoxicity (CNS)	Efavirenz	Isoniazid
Skin rash	Abacavir, amrenavir, nevirapine, efavirenz	Isoniazid, rifampicin, pyrazinamide

reported to result in higher rate of liver injury, especially with the simultaneous administration of pyrazinamide (PZA). Thus in this study we investigate the influence of first-line antituberculosis and antiretroviral drugs alone and in combination on main functions of the liver.

Methods

INH, RFP and PZA were secured from "Borshchahivskiy CPP" (Ukraine). Antiretroviral agents – EFV and d4T (Aurobindo Pharma Ltd., India) were kindly provided by State Institution "Ukrainian Center for AIDS Prevention of the Ministry of Health of Ukraine".

Animals and study design

The white inbred rats (200±20 g, n=24) were taken from TSMU vivarium. Animals were randomly distributed into 4 groups (n=6): 1 – intact group; 2 – rats which were treated with combination of antituberculosis drugs isoniazid (50 mg/kg), pyrazinamide (1500 mg/kg) and rifampicin (50 mg/kg); 3 – rats which were treated with the components of HAART: efavirenz (150 mg/kg) and stavudine (d4T, 5 mg/kg); and 4 – rats for which both antiretroviral and antituberculosis drugs in the abovementioned doses were used. A suspension of granulated tablets was introduced via the tube into the rat stomach once a day during 28 days. Rats of intact group were given equivalent quantity of distilled water. The rats were weighted and sacrificed under Ketamine hydrochloride general anesthesia on the 29th day. The blood and liver samples were taken for investigation. All animals' procedures were performed according to the rules and requirements of European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and local Ethic Committee.

Biochemical analysis

Serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase, concentration of total bilirubin, serum total protein, creatinine and urea were determined

using standard test kits «Lachema». Thiobarbituric acid-reactive substances (TBARS), catalase activity were determined as described earlier [9, 10]. Plasma ceruloplasmin level was determined as well [11]. The liver tissue was homogenized by using the homogenizer Silent Crusher S (Heidolph, Germany). The concentrations of lipid hydroperoxides [12], TBARS, superoxide dismutase (SOD) [13], catalase activity and reduced glutathione level (G-SH) [14] were analyzed in liver homogenates. p-hydroxylase activity as a marker of CYP2E1 and N-demethylase activity as a marker for cytochrome P450 – CYP3A activity in rat hepatic microsomes were determined; liver microsomes were prepared by differential ultracentrifugation [15]. We determined the content of the active form of INH in serum of experimental animals also [16].

Histopathological studies

Slices of the liver (from six animals of each group) were fixed in 10 % formalin, and then Lilly-fixator was applied. Samples were embedded in paraffin; 5–6 μm sections were routinely stained with haematoxylin and eosin and observed using microscope LOMO Biolam.

Statistical analysis

Results were presented as the mean ± standard error of the mean (M±m) and analyzed by the Mann-Whitney-test and ANOVA-test using Statsoft STATISTICA 10 (at Systemic Statistical Analysis Department of TSMU). A probability level of less than 0.05 was considered as significant. The distribution of indices was estimated using Shapiro-Wilk normality test.

Results

Combination of the most effective and common antituberculosis agents has the most prominent negative effect on liver function. We observed the rising of liver transaminases levels and cholestatic syndrome: increasing of ALT and AST activity by 163 and 118 %, respectively, increasing of alkaline phosphatase and total bilirubin level – by 77 and 104 %, respectively, in comparison with intact control (table 2). Also, there are some problems with liver functions due to decreasing of total protein serum level by 40 %.

The influence of antiretroviral agent on indices of cytolysis and cholestasis was milder. We noted the increase in ALT and AST activities by 42 and 45 % comparing with the control group rats (table 2).

The main positive findings of our study are that the concomitant administration of EFV and d4T with INH, RMP and PZA decreased the hepatotoxicity of antituberculosis drugs (Figure 2). The group of rats which received the treatment of all five drugs had decreased levels of ALT, AST and alkaline phosphatase activity by 34, 36 and 27 %, respectively, total bilirubin level was lower by 23 %, and total serum protein level was higher by 95 % comparing with rats which were treated with antituberculosis agents alone. However, there was no full restoration of all indices. It was noted that the ALT, AST and alkaline phosphatase activities and total bilirubin levels were still higher in rats treated with antituberculosis agents

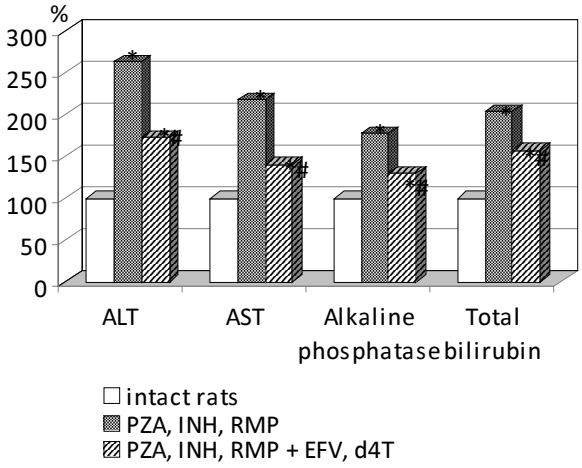

Fig. 2. The cytolysis and cholestasis indices in case of antituberculosis and antiretroviral agents' administration. Notes: p<0.05 comparatively with * – intact rats, # – rats which were treated with combination of antituberculosis drugs isoniazid (INH, 50 mg/kg), pyrazinamide (PZA, 1500 mg/kg) and rifampicin (RMP, 50 mg/kg).

Table 2. The changes of biochemical indices of serum in case of antiretroviral and antituberculosis agents usage (M±m, n=6)

The group of animals	Index				
	ALT, mccat/l	AST, mccat/l	Alkaline phosphatase, mmOl/(l·hour)	Total bilirubin, mcmol/l	Total protein, g/l
Control rats	0.85±0.03	1.15±0.07	1.86±0.08	2.38±0.10	60.82±1.88
INH, RFP, PZA	2.24±0.08*	2.51±0.06*	2.38±0.17*	4.86±0.29*	36.88±0.76*
EFV, d4T	1.21±0.04*	1.43±0.06*	2.07±0.04	3.23±0.05*	74.01±0.12*
INH, RFP, PZA+EFV, d4T	1.48±0.04*#·	1.61±0.06*#	2.40±0.20*#	3.74±0.14*#·	71.87±4.54*

Notes. Statistical significance level is p<0.05 for this and for the following tables comparing with:
1. * – intact rats;
2. # – group of rats which received antituberculosis agents INH, RFP, PZA;
3. · – group of rats which received antiretroviral agents EFV, d4T.

alone than in control rats by 73, 40, 29 and 57 %, respectively.

The biochemical markers of intensive oxidative stress were detected after administration of combination of antituberculosis agents (Table 3). It was observed that the levels of HPL and TBARS in liver tissue increased by 86 and 144 % respectively; TBARS in blood serum – by 99 % in comparison with intact group on the 29th day. The decrease in SOD activity (by 39 %) and catalase activity in liver homogenates and blood serum – by 55% and 32.5%, respectively, was observed. Simultaneously, the levels of G-SH and CP were lower by 29 %, the level of ceruloplasmin was higher by 19 %, respectively, compared to control group.

Antiretroviral agents caused some lipoperoxidation of cells membranes: HPL level in the liver increased by 21 %, TBARS in the liver tissue and serum – by 50 and 23 %, respectively, comparing with control rats (table 3). As for indices of endogenic antioxidant defense, SOD and catalase activity in the liver were lower by 46 and 40 %, in the serum – by 31 and 32 %, respectively, comparing with control group. The G–SH was lower by 22 %, ceruloplasmin level increased by 16 %.

The levels of primary and secondary products of lipids membranes peroxidation were lower in the group of rats which received combination of antiretroviral and antituberculosis agents comparing with the rats which were treated with antituberculosis combination INH, RMP, PZA alone. For example, HPL level was lower by 24 %, TBARS in liver tissue and serum – by 32 and 30 %. As for activity of SOD and catalase in the liver, they were higher by 20.8 and 21 %, respectively.

The antiretroviral agents EFV and d4T caused the increase of N-demethylase and p-hydroxylase activity by 34 and 26 %, respectively. Similarly, antituberculosis drugs INH, RMP and PZA caused N-demethylase and p-hydroxylase activity decrease by 54 and 33 %, respectively, all results comparing with intact rats (Figure 3).

The level of serum free INH was lower by 18 % in rats which received combined treatment of EFV, d4T and INH, RMP, PZA, comparing with the group of animals which were treated with antituberculosis agents alone (table 4).

Histologic findings were done as described below. We observed the lobular disarray, dilation of central veins, and signs of fatty and hyaline-drops protein dystrophy in case of treatment with antituberculosis drugs. All cells had different structure and sizes. We found increased number of cells with kariopicnosis, fragmentation and karyolysis (Fig. 4 (1)). ART had no the influence on lobular structure of the liver (Fig. 4 (2)). However, we noticed the hyperplasia of hepatocytes due to the enlargement of homogenous cytoplasm and hyperplasia of granulated nuclei. Cholestasis signs were not

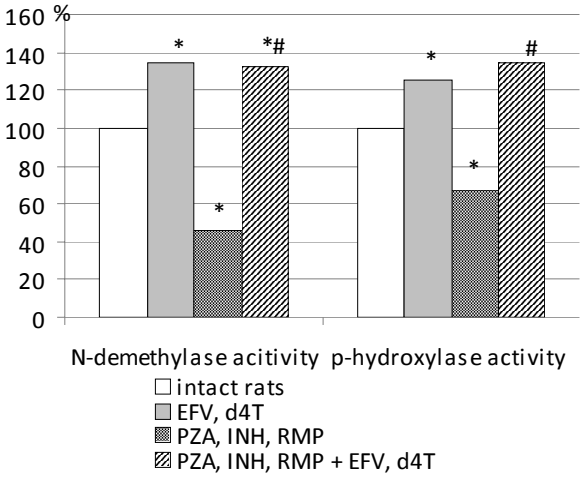

Fig. 3. The influence of ART and antituberculosis agents on N-demethylase and p-hydroxylase activity of liver microsomes.

Table 3. The influence of antiretroviral and antituberculosis agents on prooxidant and antioxidant homeostasis (M±m, n=6)

Index	The group of animals			
	Control rats	INH, RFP, PZA	EFV, d4T	INH, RMP, PZA+EFV, d4T
HPL, U/kg	5.1±0.26	9.47±0.22*˙	6.19±0.11*#	7.20±0.21*#˙
TBARS (liver), mcmol/kg	6.73±0.23	16.45±0.36*	10.08±0.28*#	11.22±0.61*#
TBARS (serum), mcmol/l	0.81±0.04	1.62±0.02*˙	1.01±0.03*#	1.14±0.01*#˙
SOD (liver), U/kg	5.07±0.09	3.09±0.09*˙	2.76±0.08 *#	3.73±0.13*#
SOD (serum), U/l	4.43±0.07	3.23±0.15*	3.05±0.12 *#	3.38±0.10*˙
Catalase (liver), cat/kg	7.99±0.07	3.59±0.23*	4.82±0.17*#	4.35±0.14*#
Catalase (serum), cat/l	8.61±0.25	5.81±0.32*	5.82±0.35*	6.44±0.47
G-SH, mmol/kg	2.37±0.04	1.67±0.03*	1.85±0.08*	1.7±0.06
Ceruloplasmin, mg/l	329.6±10.0	390.8±13.5*	383.5±6.9	371.9±10.8

Notes. In this and all the following tables: statistical significance $p<0.05$ comparatively with:
1. * – control rats;
2. # – group of rats which received antituberculosis agents INH, RFP, PZA;
3. ˙ – group of rats which received antiretroviral agents EFV, d4T.

Table 4. The influence of antiretroviral and antituberculosis agents on serum isoniazid level in rats (M±m)

Indices	Animals groups	
	Antituberculosis agents	Antituberculosis +antiretroviral agents
The content of free INH, mg/l	11.8±0.6	9.7±0.4*

Notes. * – the significance level p<0.05 comparing with the rats which received antituberculosis drugs.

observed. In case of drug administration from both pharmacological groups, we noticed the saved lobular structure of the liver (Fig. 4 (3)). The structure of hepatic cells was normal; cytoplasm was saturated and homogeneous in all cells of the liver lobule. The histological structure of the liver was improved comparing to the liver structure of the rats which were treated with combination of antituberculosis drugs.

Discussion

At least one-third of the 34 million people living with HIV worldwide are infected with latent TB. Persons co-infected with TB and HIV are 21–34 times more likely to develop active TB disease than persons without HIV. Coinfection with HIV leads to difficulties in both the diagnosis and treatment of tuberculosis. The problem of simultaneous administration of antituberculosis and antiretroviral drugs in patients with HIV-associated tuberculosis is ambiguous due to overlapping side effect profiles of drugs both pharmacological groups, the adherence challenges of polypharmacy and their drug-drug interaction especially on the stage of metabolism by liver microsome enzymes. Today state-of-art in combination of such drugs is described below. The treatment of patients with TB and HIV should include the first line drugs for TB treatment (common combination of isoniazid, rifampicin, and pyrazinamide) and NNRTI based antiretroviral therapy regimen, if possible; efavirenz and rifampin are the preferred components [18]. It is known that EVR is metabolized by isoenzyme of CYP450 – CYP3A preferably and by CYP2B and 2A also [6]. This NNRTI causes the induction of CYP3A in the liver, but not in the intestine [19]. Nucleoside reverse-transcriptase inhibitors (NRTIs), like d4T, are metabolized by glucuronidation pathway and don't have any influence on CYP450 [20]. Concerning the antituberculosis drugs, RMP is a potent inducer of CYP3A isoenzyme, as well as of other P450 isoforms, more potent than rifapentine and rifabutin [21]. PZA can induce CYP2E1 [22]. We also know that hepatotoxicity of INH depends on activity of this isoenzyme [23].

The SAPiT trial – an open-label randomized controlled trial in Durban, South Africa to determine optimal timing of ART initiation in relation to TB treatment – showed that initiation of ART during tuberculosis treatment in patients with sputum smear positive for tuberculosis and HIV-infection, reduced mortality by 56 % and was associated with improved outcomes [17]. Our study reasserts the results of this trial.

We can see the improving of all liver functions due to the repeated administration of components of ART, especially total serum protein level. Such results are explained because of induction of enzymes of the liver endoplasmic reticulum by EFV [19]. The influence of antiretroviral agent on indices of cytolysis and cholestasis was mild. The increase in total bilirubin level was noted. We can explain this by the fact that one of the mechanisms of EFV-induced cholestasis is inhibition of bile acids transport [24]. Our results are supported by the clinical data of the similar studies which showed the absence of risk of isoniazid–associated hepatitis in case of concomitant HAART [25].

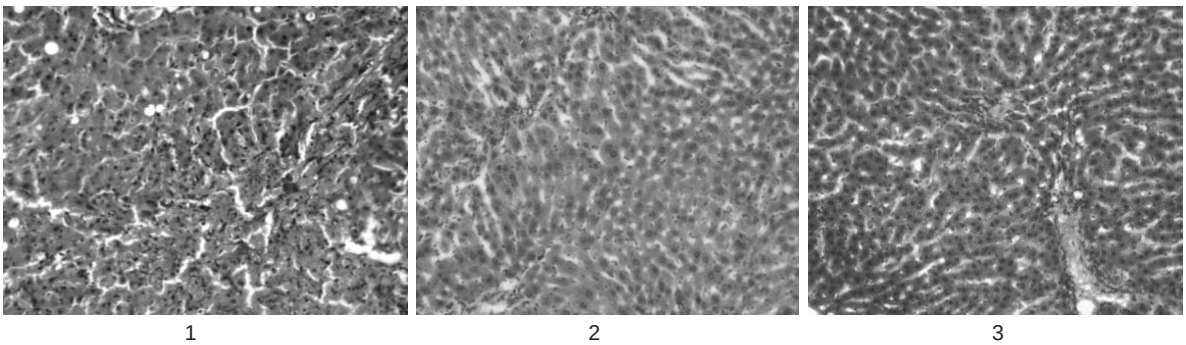

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Fig. 4. Histopathological structure of rat liver in case of administration of antiretroviral and antituberculosis agents alone and in combination: 1. Liver structure of rats which were treated with combination of PZA, INH and RMP. Haematoxylin and eosin stain. ×160; 2. Liver structure of rats which were treated with antiretroviral agents for 28 days. Haematoxylin and eosin stain. ×160; 3. Liver structure of rats which received combination of antituberculosis and antiretroviral agents. Haematoxylin and eosin stain. ×160.

Liver biopsy is the traditional gold standard to ascertain the degree of liver injury. Our biochemical results are confirmed by histological observations. Functionally, the liver can be divided into three zones, based upon oxygen supply. Zone 1 encircles the portal tracts where the oxygenated blood from hepatic arteries enters. Zone 3 is located around central veins, where oxygenation is poor. Zone 2 is located between the first two zones. The main mass of CYP450 enzymes is located in zone 3 of hepatic acinus [29]. Some studies showed increased activity of CYP3A and CYP2E1 in case of isoniazid and rifampicin administration [30]. That's why the decreasing of activity of these isozymes can be explained by higher hepatotoxicity of such dose of PZA which was added to the combination of INH and RMP. We can observe the necrosis of hepatic cells in centrolobular part of liver lobule which is the main cause of depression of such liver function as detoxification. We can suggest that the decrease in concentration of toxic metabolite of INH due to the combination of RMP and EVF is the key factor in decreasing of isoniazid hepatotoxicity, and the absence of cases of liver toxicity during preventive INH therapy with the HAART background [25, 31].

Possible mechanisms in decreasing the negative influence of antituberculosis agents in case of their combination with antiretroviral agents can be explained by the fact that inducers of cytochrome P450 enzymes stimulate synthetic and antitoxic liver functions and can decrease the quantity of necrotic hepatocytes [26]. Due to this we can see improved metabolism and detoxification processes due to the stimulation of cytochrome P450 isozymes in case of simultaneous usage of antituberculosis and antiretroviral agents. Hepatotoxicity of INH is linked to its metabolites - monoacetyl-hydrazine, hydrazine, acetyl-isoniazid and others [27]. Isoniazid is metabolized by genetically polymorphic arylamine N-acetyltransferase type 2 (NAT2), and recent studies showed that isoniazid concentrations may be more toxic for slow acetylators after a standard therapeutic dosing [28], because of relative overdosing and higher concentration of INH toxic metabolites in the blood. That's why the increase in isonicotinyl hydrazine metabolism can accompany the mitigation of its hepatotoxic effects.

Conclusion

The present study showed that the combination of first-line antituberculosis drugs (isoniazid, rifampicin and pyrazinamide) in case of their intragastric administration during 28 days caused the prominent liver injury with cytolysis and cholestasis signs, decreasing of CYP3A and CYP2E1 isozymes activity and dysfunction of protein synthesis by the liver.

Antiretroviral agents (efavirenz and stavudine), after their administration into the stomach for 28 days, caused the elevation of transaminases activity with the increase of serum bilirubin level at the background of cytochrome 450 isoforms 3A and 2E1 activity rising and the increase of total serum protein.

The antiretroviral agents in case of simultaneous administration with the antituberculosis drugs diminished the hepatotoxic effects of the first-line drugs for tuberculosis treatment. Such results of our experimental study may facilitate the detailed clinical research of drug-drug interaction from both pharmacological groups due to the rising cases of HIV-associated tuberculosis in the whole world.

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ТУБЕРКУЛЬОЗ У ПРАКТИЧНІЙ ДІЯЛЬНОСТІ СІМЕЙНОГО ЛІКАРЯ

І. Т. П'ятночка, С. І. Корнага, Т. В. Корнага
ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО

Вступ. *У статті дано характеристику сучасному сімейному лікарю як універсалу, який стоїть на сторожі збереження здоров'я людини, і показано його роль у боротьбі з туберкульозом. Надзвичайної гостроти набула проблема хіміорезистентного туберкульозу, зокрема мультирезистентного, від якого в Україні страждають 23,4 % хворих на вперше діагностований туберкульоз та 58,6 % із повторними випадками захворювання.*

Мета роботи *– дати характеристику сімейному лікарю як універсалу, а також показати його роль у боротьбі з туберкульозом на сучасному етапі.*

Результати. *У Тернопільській області в останні роки спостерігають позитивну динаміку епідеміологічного процесу, зменшення захворюваності й смертності від туберкульозу. В 2014 р. ефективність лікування хворих на вперше діагностований туберкульоз легень з бактеріовиділенням становила 87,6 %. Однак кількість хворих, які померли від туберкульозу протягом одного року спостереження, за останнє десятиріччя зросла з 15,6 до 22,2 % (p<0,05). Це свідчить про несвоєчасне виявлення туберкульозу на різних рівнях медичної служби, зокрема і первинної ланки. Летальність серед хворих на мультирезистентний туберкульоз становила 17,2 %, з них до одного місяця – 1/3 осіб.*

Висновки. *Сімейний лікар надає першу медичну допомогу та ліквідовує вогнища інфекційних захворювань, проводить серед населення пропаганду здорового способу життя, відповідального ставлення до свого здоров'я як найвищої особистої і суспільної цінності.*

КЛЮЧОВІ СЛОВА: **туберкульоз, сімейний лікар.**

Вступ

Туберкульоз відомий у світі, відколи існує людство. Це одна з найдавніших хвороб, які можуть уражати всі органи людини. Періоди епідемії туберкульозу змінювалися періодами стабілізації. Останній спалах туберкульозу розпочався з 90-х років минулого століття. У 1995 р. в Україні проголосили епідемію туберкульозу, захворюваність перевищила епідемічний поріг – 50 випадків на 100 тис. населення. У 2005 р. цей показник був найвищим – 84,1 на 100 тис. населення. Упродовж 2006–2015 рр. спостерігали покращення епідеміологічної ситуації, і вже у 2014 р. захворюваність на туберкульоз, поширеність і смертність від нього становили, відповідно 53,2, 90,2 та 12,2 випадку на 100 тис. населення. Водночас зросла захворюваність на ко-інфекцію туберкульоз/ВІЛ/СНІД – 10,4 на 100 тис. населення. Надзвичайної гостроти набула проблема хіміорезистентного туберкульозу, зокрема мультирезистентного (стійкість до найефективніших протитуберкульозних препаратів – ізоніазиду і рифампіцину), від якого в Україні страждають 23,4 % хворих на вперше діагностований туберкульоз та 58,6 % із повторними випадками захворювання.

У результаті проведення основного курсу хіміотерапії вилікування нових випадків бацилярного туберкульозу легень спостерігають у 55 % пацієнтів від тих, які захворіли [1]. Такий низький відсоток видужання зумовлений несвоєчасним виявленням хворих, помилками діагностики, низькою прихильністю пацієнтів до тривалої хіміотерапії, як і самими вадами її проведення. Усе це зобов'язує невідкладно спрямувати значні зусилля на покращення профілактики і, передусім, своєчасну діагностику туберкульозу на рівні первинної ланки (сімейної медицини), де помилки сягають 80,2–96,4 % [2]. Причини помилок щодо діагностики туберкульозу, в кінцевому результаті, зводяться і до зниженої фтизі-

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атричної настороженості, що неприпустимо в період значного поширення мультирезистентного туберкульозу. Лікування такого туберкульозу надзвичайно складне, тривале (≥2 роки), дороге (іноді в 100 разів дорожче, ніж терапія чутливого до ліків туберкульозу), малоефективне і часто супроводжується побічними токсичними реакціями [3].

Мультирезистентний туберкульоз – надзвичайно небезпечна форма туберкульозу, яка нещадно карає людей за їх байдужість до свого здоров'я, слабку обізнаність у питаннях боротьби із цією недугою, що є наслідком вкрай недостатнього проведення санітарно-просвітницької роботи серед населення і хворих на різних рівнях медичної служби та прорахунків в організації боротьби з туберкульозом взагалі.

Виникла нагальна потреба перебудови санітарно-просвітницької діяльності на зразок економічно розвинутих європейських країн. Суть її полягає у виробленні навичок ведення здорового способу життя в дитячому та юнацькому віці; пропагування здорового способу життя в соціальних популяціях людей, об'єднаних спільним устроєм життя чи специфікою праці; зміні пасивного ставлення людини до свого здоров'я шляхом підтримування її матеріальної зацікавленості у збереженні здоров'я в доброму стані, тобто людині не вигідно буде хворіти; розробці для кожної людини індивідуальної програми профілактики захворювань з усуненням впливу вже добре відомих негативних чинників (алкоголь, куріння, гіподинамія тощо), а також наданні людині рекомендацій щодо вибору професії з урахуванням показників психофізіологічних і професійних тестів [4]. Втілення в життя такої індивідуальної програми профілактики захворювань є можливим лише за присутності сімейного лікаря, який найбільш повно ознайомлений із характерологічними особливостями конкретного пацієнта і станом його здоров'я. Великий досвід у цьому напрямку мають деякі країни Європи [5]. Адже і поняття "лікар загальної практики" склалося еволюційним шляхом у XIII–XIX ст. в окремих європейських країнах, коли лікарську допомогу надавали в індивідуальному комерційному порядку. В результаті цього з'явився сталий контингент пацієнтів, які вважали доцільним протягом усього життя звертатися виключно до "свого" лікаря, якому довіряли завдяки його професіоналізмові та індивідуальним якостям. За таких умов лікар був одночасно сімейним і лікарем загальної практики.

Мета роботи – дати характеристику сімейному лікарю як універсалу, а також показати його роль у боротьбі з туберкульозом на сучасному етапі.

Матеріали і методи

Використано літературні дані про сімейного лікаря як універсала, наведено основні епідеміологічні показники щодо туберкульозу в Україні й по Тернопільській області за останні 10 років і сформульовано головні завдання, які стоять перед сімейним лікарем відповідно до сучасного Уніфікованого клінічного протоколу первинної, вторинної (спеціалізованої) та третинної (високоспеціалізованої) медичної допомоги дорослим "Туберкульоз" (2014) [6].

Результати та їх обговорення

Сімейний лікар – якісно нова форма лікарської діяльності, що володіє великим об'ємом знань і вмінь. Це лікар-універсал, який має ґрунтовні знання з головних розділів медицини – профілактики та діагностики. Він надає першу медичну допомогу, попереджує та ліквідовує вогнища інфекційних захворювань, проводить серед населення пропаганду здорового способу життя. За даними світової статистики, кваліфікований сімейний лікар може самостійно вирішити до 80 % проблемних завдань на етапі первинної допомоги, включаючи і лікування в домашньому або денному стаціонарі.

Поряд із цим, однією з основних вимог щодо високої якості роботи сімейного лікаря є доброзичливе ставлення до пацієнта і високий професіоналізм та, безперечно, відсутність грубості й байдужості до хворої людини. Ще у XII ст. англійський лікар Sydenham сказав, що лікар повинен так поводитися із хворим, як би він хотів, щоб поводилися з ним – увічливо і з турботою. До того ж, сімейний лікар зобов'язаний очищувати свою мову від нетактовних і брутальних слів. Не слід думати, що слово – дрібниця, не в словах справа. Адже слово виражає думку, а думка породжує дії, які можуть закінчуватися фатально.

Слід уникати і такої крайності, коли діагноз має таємничий характер. Це особливо лякає хворого, який не повинен знати про своє захворювання. У противному разі, залежно від психіки пацієнта і стану його здоров'я, рідні повинні бути інформовані про діагноз, методи лікування, прогноз. Інформацію про захворювання слід надати хворому в спокійній, простій, доступній формі з надією на видужання чи, хоча б, на покращення його стану.

Дуже важливими в діяльності сімейного лікаря є його індивідуально-психологічні особливості. Надто важливо встановити розумний, щиросердечний, безкорисливий контакт із хворим та завоювати його довіру. Отож, якщо люди шукають у тебе допомоги – ти хороший лікар. Цього можна досягнути, поряд із високою

професійною майстерністю, індивідуальним підходом сімейного лікаря до хворого, манерою звертання та, зрештою, зовнішнім виглядом. До того ж, незайвим є нагадати слова Гіппократа: "Лікар повинен бути скромним і стриманим, добрим та ввічливим, постійно збагачувати свої знання і прислухатися до думки колег, бачити свою мету не у здобуванні слави та грошей, а в полегшенні страждань і зціленні хворих, у самовідданому служінні людям, які звертаються до нього за допомогою та за порадою".

Отже, сімейна медицина – це медицина сьогодення і майбутнього у вітчизняній охороні здоров'я. Особливий, довірливий характер стосунків з родиною, тривале спостереження за кожним її членом дають змогу постійно і ретельно займатися лікувально-профілактичною роботою, спрямованою на відновлення та збереження здоров'я людини.

Дуже важлива роль сімейних лікарів у масових заходах, спрямованих на раннє виявлення і профілактику туберкульозу. До того ж, більшість пацієнтів із симптомами респіраторних захворювань звертається в лікувальні заклади загальної медичної мережі, де і виявляють туберкульоз та навіть завершують лікування хворого. Отже, ефективне проведення протитуберкульозних заходів не можливе без широкої участі в цій роботі лікарів загальної практики – сімейної медицини. Переваги сімейної медицини полягають у тому, що сімейному лікарю відомі конкретні умови праці, побуту, бюджет і харчування сім'ї, наявність хворих, зокрема і на туберкульоз, а також осіб з підвищеним ризиком виникнення цього захворювання, яких щороку направляють на профілактичні обстеження. До слова, в останні роки ризик інфікування продовжує зростати, оскільки сучасні заходи щодо розриву ланцюга передачі туберкульозної інфекції від хворої людини до здорової недостатньо ефективні: несвоєчасне виявлення хворих, незадовільна їх ізоляція та малоефективне лікування, зокрема мультирезистентного туберкульозу, сприяють поширенню туберкульозної інфекції. Отож пацієнтів із такими симптомами, як кашель, що триває понад 2–3 тижні, нічне потовиділення, загальна слабість, безпричинне схуднення, тривале, понад тиждень, підвищення температури тіла більше 37 °С, необхідно направляти на рентгенологічне обстеження, дворазове дослідження мокротиння на мікобактерії туберкульозу. Сімейний лікар проводить лікування хворих на туберкульоз в амбулаторних умовах, здійснює контрольовану хіміопрофілактику в групах підвищеного ризику за рекомендацією фтизіатра.

Туберкульоз у групах ризику активно виявляють шляхом призначення скринінгового флюорографічного обстеження 1 раз на рік. Крім цього, в лабораторних умовах і вдома сімейний лікар діагностує та надає екстрену допомогу при таких невідкладних станах, як спонтанний пневмоторакс, гостра дихальна недостатність, кровохаркання і легеневі кровотечі, гострі алергічні реакції на протитуберкульозні препарати. Випадки туберкульозу та латентної туберкульозної інфекції за умов епідемії туберкульозу виявляють шляхом щорічної туберкулінодіагностики (проба Манту з 2 ТО), яку проводять здоровим дітям від 4 до 14 років. Дітям до 4 років та дітям підліткового віку туберкулінодіагностику проводять за бажанням батьків, у групах ризику – за епідпоказаннями, зокрема в туберкульозних вогнищах. Профілактичну променеву діагностику призначають дітям віком 15 і 17 років. У разі виявлення на рентгенограмі порожнин розпаду, дрібновогнищевої дисемінації пацієнта направляють до фтизіатра на дообстеження з результатами дворазової бактеріоскопії мазка мокротиння. У випадку негативних мазків мокротиння і за наявності вогнищевих або інфільтративних змін на рентгенограмі легень хворому призначають антибіотики широкого спектра дії (за винятком рифампіцину, аміноглікозидів та фторхінолонів) до 2 тижнів, після чого повторюють рентгенограму легень. За відсутності позитивної динаміки пацієнта негайно скеровують до фтизіатра. До фтизіатра направляють осіб при підозрі на позалегеневі форми туберкульозу (кістково-суглобового, периферичних лімфатичних вузлів, сечостатевих органів, очей, нервової системи та інших локалізацій).

До дитячого фтизіатра на консультацію направляють дітей: які контактували з хворим на туберкульоз; із гіперергічними реакціями на пробу Манту з 2 ТО (інфільтрат 17 мм і більше або реакція з наявністю везикул, некрозу чи лімфангоїту незалежно від розміру інфільтрату); при зростанні розміру інфільтрату на пробу Манту на 6 мм і більше; за наявності ускладнень вакцинації БЦЖ (холодні підшкірні абсцеси, периферичні лімфаденіти, виразки розміром понад 10 мм, келоїдні рубці, персистувальна та дисемінована БЦЖ-інфекція з ураженням різних органів); при інших ситуаціях, що пов'язані з ризиком захворювання на туберкульоз. Сімейний лікар організовує щеплення вакциною БЦЖ дітей, які не були вакциновані в пологовому будинку, контролює розвиток післявакцинних реакцій.

Лікування хворих на туберкульоз проводять за стандартними режимами під безпосереднім наглядом медперсоналу відповідно до Уніфікованого клінічного протоколу первинної, вторин-

ної (спеціалізованої) та третинної (високоспеціалізованої) медичної допомоги дорослим "Туберкульоз" (2014) [6]. Хіміопрофілактику дітям та дорослим проводять на основі призначених фтизіатром препаратів. Найпоширенішою схемою хіміопрофілактики є шестимісячне приймання ізоніазиду (5–8 мг/кг). Кількість курсів превентивної хіміотерапії призначають індивідуально.

Сімейний лікар повинен проводиьти санітарно-просвітницьку роботу в сім'ї, з хворим, як і з населенням загалом. Санітарно-просвітницька робота – це безперервний процес, який полягає у висвітленні властивостей збудника туберкульозу, шляхів його передачі, проявів захворювання, лікування та профілактики. Без чіткого розуміння хворим, що таке туберкульоз, без належних гігієнічних навичок, правильної поведінки в сім'ї, колективі, розумної прихильності до лікування боротьба з туберкульозом, зокрема хіміорезистентним, приречена на невдачу. Однак не варто впадати в "закодований" песимізм, потрібно нівелювати негативні явища стосовно навіть такої небезпечної форми, як мультирезистентний туберкульоз. У зв'язку з цим, сімейний лікар повинен бути проінформований про позитивні зрушення в подоланні туберкульозу, недоліки в роботі як загальномедичної, фтизіатричної служби, так і первинної ланки, тобто сімейного лікаря.

У Тернопільській області в останні роки спостерігають позитивну динаміку епідеміологічного процесу, зменшення захворюваності й смертності від туберкульозу. В 2014 р. ефективність лікування хворих на вперше діагностований туберкульоз легень з бактеріовиділенням становила 87,6 %. Однак, поряд з цим, спостерігали низку недоліків. Зокрема, кількість хворих, які померли від туберкульозу протягом одного року спостереження за останнє десятиріччя зросла з 15,6 до 22,2 % ($p<0,05$). Це свідчить про несвоєчасне виявлення туберкульозу на різних рівнях медичної служби, зокрема і первинної ланки. Виявлено недоліки і при роботі у вогнищах туберкульозної інфекції, оскільки за останні 10 років захворюваність контактних осіб у вогнищах туберкульозної інфекції (на 1000 контактних) зросла з 5,2 до 9,9 ($p<0,05$). Це вже явний недолік у роботі фтизіатрів і сімейних лікарів. За 2010–2014 рр. на тлі зниження захворюваності на туберкульоз легень (з 506 до 444 осіб) значно збільшилась кількість рецидивів туберкульозу – з 23,8 до 61,2 % відповідно ($p<0,05$). До слова, із загальної кількості хворих, які перебували на стаціонарному лікуванні в обласному протитуберкульозному диспансері, мультирезистентність констатовано у 8,5 % осіб, зокрема з 2005 до 2014 р. вона зросла з 4,2 до 17,4 % ($p<0,05$). Летальність серед хворих на мультирезистентний туберкульоз становила 17,2 %, з них до одного місяця – 1/3 осіб. Найбільшу поширеність мультирезистентного туберкульозу спостерігають у м. Тернопіль, Тернопільському, Теребовлянському та Підволочиському районах (53,7 %). Це надзвичайно тривожна ситуація в роботі медичної служби, передусім фтизіатричної, у цих районах.

Висновки

На завершення, акцентуємо увагу на тому, що сімейна медицина – це медицина сьогодення і майбутнього, а сімейний лікар – універсал, який маючи ґрунтовні знання з основних розділів медицини, зокрема і фтизіатрії, надає первинну профілактичну допомогу прикріпленому за сімейно-територіальним принципом населенню в умовах амбулаторно-поліклінічного закладу чи вдома. Сімейний лікар надає першу медичну допомогу та ліквідовує вогнища інфекційних захворювань, проводить серед населення пропаганду здорового способу життя, відповідального ставлення до свого здоров'я як найвищої особистої і суспільної цінності. Адже "Здоров'я – це не все, але все без здоров'я – ніщо" (Сократ).

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TUBERCULOSIS IN FAMILY PRACTICE

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Background. *In this article we describe a modern family doctor as a comprehensive paediatrician, who preserves human health, and their importance in fight with tuberculosis. Drug-resistant tuberculosis has become an urgent matter, multidrug-resistant TB in particular. In Ukraine 23.4% of patients suffer from TB diagnosed for a first time and 58.6% from recurrent cases of the disease.*

Objective. *The aim of our research is to present a family doctor as a comprehensive paediatrician and demonstrate their importance in fight with tuberculosis in modern times.*

Results. *In recent years in Ternopil region the positive dynamics of epidemiological process, decrease in incidence and mortality from tuberculosis is evidenced. In 2014, the efficiency of treatment of patients who were diagnosed with bacterial pulmonary tuberculosis for a first time, was 87.6%. However, the number of patients, who died of TB within one year of observation over the last decade, increased from 15.6% to 22.2%, ($p < 0.05$). This proves an untimely tuberculosis diagnosis at different levels of medical services, at primary one in particular. Mortality rate in patients with multidrug-resistant tuberculosis is 17.2%, 1/3 persons died up to one month.*

Conclusions. *Family doctor provides first medical aid, eliminates foci of infectious diseases, promotes healthy lifestyle among population, and is responsible for their health as the highest personal and social value.*

KEY WORDS: **tuberculosis, family doctor, healthcare.**

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