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The edition address:
677019, Yakutsk, Sergeljahsky highway, 4,
M&ChHCC NCM, unit S1-01,
tel.(4112) 32-15-26; 39-55-52
The telefax (4112) 32-19-81
E-mail: ysc_tech@sakha.ru
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II. MATERIALS OF CURRENT NUMBER OF " THE YAKUT MEDICAL JOURNAL"

Markovsky V.D., Sorokina I.V., Kaluzhina O.V.

Morphological features of endothelial cover of pulmonary artery and aorta at fetuses and newborns after chronic intrauterine hypoxia (an experimental study)

ABSTRACT

Morphological features of pulmonary artery and aorta in fetuses and newborns have been studied in order to determine morphological changes in endothelial cover of the pulmonary artery and the aorta in fetuses and newborns affected by chronic fetal hypoxia (CFH) as a result of the experiment on laboratory WAG rats on modeling fetal hypoxia of newborns with asphyxia in delivery. According to results of the research in the artery and the aorta of fetuses and newborns affected by CFH deterioration of trophic processes in the endotheliocyte has been found due to thickening of basal membranes. It causes cells flattening, decrease of adhesive properties which is evidenced by decreased expression of receptors CD 34 by endothelium, and their desquamation amplification. Also sclerotic changes in the basal membranes of both vessels have been also determined due to interstitial collagen type III appearance against type IV collagen deficiency.

Keywords: endothelium, pulmonary artery, aorta, chronic intrauterine hypoxia, experiment.

INTRODUCTION

Chronic intrauterine hypoxia is severe stressor that implements its negative influence on not only mother's but the child' body [2]. It determines development of many diseases of organs and systems in such offspring with leading position of cardiovascular diseases [6]. There is data about chronic oxygen insufficiency influence on morphological status of fetal venosus ductus [7], aorta of chick [11] and rats embryos [10], development of pulmonary hypertension with pulmonary vascular remodeling in the experiment [13] in modern literature. However, in our opinion these data are at times contradictory and does not describe comparison of morphological changes in the pulmonary artery and the aorta in fetuses and infants under the influence of chronic intrauterine hypoxia.

The aim of research is to compare morphological changes of endothelial cover of pulmonary artery and aorta in fetuses and neonates undergoing chronic intrauterine hypoxia.

MATERIALS AND METHODS

The experiment modeling intrauterine hypoxia of newborn with asphyxia in labor was

made on laboratory WAG rats. Pregnant female rats have been affected high-altitude hypoxic influence, which corresponded to 7500 m for 20 minutes each day at the same time, since pregnancy registration to delivery. Rats were divided into two groups: control group 1 – fetuses and newborns from mothers who were not exposed to high- altitude hypoxia (18 cases); *experimental* group 2 – fetuses and newborns from mothers who suffered from high-altitude hypoxic exposure (16 cases). Female and offspring were euthanized. The autopsies of animals were made, the tissue pieces of pulmonary artery and aorta were cut for morphological investigation. They were fixed in 10% neutral formalin solution, then subjected to standard paraffin preparation through increasing alcohol concentration, Nikiforov solution (96% alcohol and diethyl ether 1:1), chloroform, then followed paraffin filling. Prepared blocks were sliced on microtome Microm HM-340 into serial sections $4–5\times10^{-6}$ m. The complex of histological, histochemical, immunohistochemical, morphometric methods was morphological processing. Morphological and morphometric studies were performed on microscope Olympus BX-41 (Japan) using Olympus DP-Soft (Version 3:1) and Microsoft Excel 2010 programs, and fluorescent microscope «Axioskor 40» (Carl Zeiss, Germany). The slides were stained with hematoxylin and eosin, van Gieson's Picric Acid Fuchsin, and according to Mallory. Immunohistochemical study was done on paraffin sections ($5–6\times10^{-6}$ m thickness) with direct Koons' method by Brosman's methodology [9]. Collagens III, IV type were defined by monoclonal antibodies (mAbs) to the respective collagens (Novocastra Laboratories Ltd.). Adhesive properties of the cells were defined by mAbs to CD34 (Novocastra Laboratories Ltd.). Optical density of endothelial and collagen immunofluorescence was measured by method of Gubina-Vaculik G.I. and others [5] with a microscope "Axioskor 40" and software Biostat.exe and was represented in conditional units of luminescence (cond. un. lum.). The findings were worked up statistically with the license application package «Statistica 6.0» («Statsoft, Inc») on the PC. The methods of variation statistics have been used, veracity was determined by the Student t-test [3]. All manipulations with animals were carried out according to the rules of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 18.03.1986), Directive Council of the European Society for Protection of Vertebrate Animals (Strasbourg, 24.11.1986).

RESULTS AND DISCUSSION

Macroscopic examination with a magnifying glass (×3, 8 diopters) showed that the intima of the pulmonary artery and the aorta was smooth and shiny without noticeable differences in

both groups. Some microscopicall differences were revealed in blood vessels of the control group, though in the literature they described identical structure [8]. Endothelial cover both in the pulmonary artery and the aorta was represented by mononuclear cells layer, which are located on the basal membrane closely to each other. The average height of the cells in the first vessel was $3,20\pm0,04\times10^{-6}$ m, and in the second – $3,44\pm0,06\times10^{-6}$ m, which was reliably to each other ($p<0,05$). The average width of endotheliocytes reached $7,12\pm0,15\times10^{-6}$ m for the pulmonary artery and $7,25\pm0,18\times10^{-6}$ m for the aorta, that had no significant difference between these values. The nucleus was stained evenly with hematoxylin and was located centrally, where there was a slight protrusion of cells into the vessel lumen. The cytoplasm was uniformly stained with eosin. There were determined 2-3 desquamated cells (2,40±0,09) in one field of view (×1000) in the pulmonary artery, and 1-3 cells (2,15±0,15) – in aorta, it had no significant difference between each other. Optical fluorescence density of endothelial cells was shown by CD 34 marker, which values were 0,495±0,01 cond. un. lum. for pulmonary artery and 0,476±0,01 cond. un. lum. for the aorta, which were not significant comparatively with each other. Well-defined basal membranes, on which the endothelial cells were localized, were stained evenly with eosin and accumulated evenly type IV collagen in the form of immunofluorescence moderate intensity (in the pulmonary artery – 0,526±0,02 cond. un. lum., in the aorta – 0,531±0,02 cond. un. lum.).

Thus, above-mentioned state of the vessels corresponded to the universally recognized notion of the norm and could be used as a control [1].

Microscopic examination of the same name vessels of *research* groups revealed the following differences. Endothelial cells were located tightly to each other on the basement membrane. Change of cells' width and height in both vessels indicated their flattening (Table 1).

The elongated oval nucleus of endothelial cells was located centrally in both vessels. The cytoplasm was evenly stained with eosin.

In one field of view (×1000) both in the pulmonary artery and in the aorta was detected size increase of desquamation fields at the analysis. Thus, this index is slightly higher (4,87±0,15 cells) in the first vessel than in the second (4,60±0,16 cells) one. That is significantly different from control values of corresponding vessels ($p<0,001$) and are not reliable between each other.

Endothelial cells of both vessels in groups with hypoxia accumulated marker CD 34 worse, which is proved by optical density decreasing. Thus, it was 0,397±0,02 cond. un. lum. in the pulmonary artery, and 0,379±0,02 cond. un. lum. in the aorta, which was significantly

different from the control group values ($p<0,001$ and $p<0,01$, respectively).

Comparative analysis of data of optical fluorescence density CD 34 between the pulmonary artery and the aorta in groups with chronic intrauterine hypoxia were not found significantly different.

Basement membranes were slightly thickened in groups with oxygen deficiency in both vessels. A downward tendency of type IV collagen volume in the study group compared with the control one has been established. The relative fluorescence density of the emission of collagen for the pulmonary artery was 0,497±0,02 cond. un. lum., and for the aorta – 0,495±0,03 cond. un. lum. Significant differences between the values of the optical density of the emission of type IV collagen in the vessels of the groups with chronic intrauterine hypoxia has not been established. Interstitial collagen type III was also determined in the structure of the vessels basement membranes, where as it is known, collagen type IV must be present. It may indicate presence of sclerotic changes [4]. These features can cause violations of metabolic processes in endothelial cover with development of degenerative changes with subsequent desquamation of cells [12]. This is confirmed by the significant increase in the area of desquamation fields in the vessels of the study group.

CONCLUSIONS:

1. Chronic intrauterine hypoxia causes the formation of significant morphological changes in endothelial cover of the pulmonary artery and the aorta in fetuses and newborns. It is manifested by trophic processes deterioration in endotheliocytes due to endothelial basement membrane thickening, which in turn results in cells flattening, reduction of adhesive properties, as evidenced by decreased expression of receptor CD 34 of endothelium, and increased desquamation. These changes are more significant in the pulmonary artery.

2. Basal membranes of the pulmonary artery and aorta have sclerotic changes as a result of interstitial collagen type III presence against type IV collagen deficiency.

3. The above-mentioned morphological changes reflect negative influence of chronic intrauterine hypoxia on the morphological status of the pulmonary artery and the aorta, which may be regarded as substrate for endothelial dysfunction formation in these people.

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Markovsky Vladimir Dmitrievich – MD, professor, professor of Pathological Anatomy department, vice-rector for research and education, pathomorphology@ukr.net; **Sorokina Irina Viktorovna** – MD, professor, professor of Pathological Anatomy department, acting head of Pathological Anatomy department, soririna1@mail.ru; **Kaluzhina Oksana Vladimirovna** – graduate student of Pathological Anatomy department, kaluzhina24@ukr.net Kharkov National Medical University, Department of Pathological Anatomy, 4, Lenina avenue, 61022, Kharkov, Ukraine

Table 1

Sizes of pulmonary artery and aortic endothelial cells of fetuses and newborns (M±m)

	Width (10^{-6}м)	Height (10^{-6}м)	Width (10^{-6}м)	Height (10^{-6}м)
Control	7,12±0,15	3,20±0,04	7,25±0,18	3,44±0,06#
Chronic intrauterine hypoxia	7,94±0,11*##	2,96±0,03*	5,95±0,10*	3,06±0,06*

* – P<0,001 – the probability of the difference of two medium is reliable between the control and study groups;

– P≤0,05 – the probability of the difference of two medium is reliable between the corresponding values of the pulmonary artery and the aorta;

– P<0,001 – the probability of the difference of two medium is reliable between the corresponding values of the pulmonary artery and the aorta.

Budnik A.F., Musukaeva A.B., Pshukova E.M., Zakhokhov R.M.

Tumors of Thyroid in Kabardino-Balkarian Republic

ABSTRACT

184 biopsy materials have been investigated since 2013 (16 men and 168 women aged from 89 to 18). Among men middle age of patients amounted to 38,1±12,2, among women 49,2±13,1. Different types of tumors were found out in 67 cases, that was 36,4% of all thyroid pathology. The most frequent kind is a follicle adenoma - 42 supervisions (64,6% of all tumors), 13 from these cases combine with nodular goiter (20% of all tumors) and 4 cases (6,2%) with autoimmune thyroids. Malignant tumors were found out in 25 cases, the most frequent kind is a papillary cancer (19 cases - 28,4%). Other kinds: medullar cancer and undifferentiated cancer for 2 cases (3%), follicle cancer and combination of follicle chasse with papillary for to a 1 case (1,5%).

Keywords: thyroid, cancer, tumors, endocrine system.

Kabardino-Balkarian republic refers to the regions with a low content of iodine in water and soil. The morbidity of thyroid cancer depends on action duration of iodic deficit and his severity [4].

Thyroid cancer is the most widespread tumor of the endocrine system [2, 6]. In Russia 1,8% are noted in the structure of general morbidity of thyroid tumor, for men - 0,5%, for women - 2,9%. The morbidity of thyroid malignances is characterized by evident positive dynamics [6]. For determination of a role of endemic goiter further researches are needed in epidemiology of thyroid tumor [4].

Thyroid tumors are a very heterogeneous group both on a morphological structure and on a clinical flow. The preoperated diagnostics of these tumors in spite of modern achievements of thyroidology remains at a level from 30 to 70% [1, 2, 3, 7].

Research aim: to conduct the analysis of biopsy material for research of the age-related and sexual features of thyroid tumors in KBR and their connection with other diseases of organ. To confront the results of preoperated and morphological diagnostics for the development of methods of improving surgical help to a patient with this pathology.

MATERIALS AND METHODS

The analysis of operationally-biopsy material of GKUZ "Pathoanatomical bureau" MH of KBR was carried out for 2013. Macroscopic description of objects and histological

conclusions are analysed in all cases. All histological preparations were made on standard methodology, painted by a hematoxylin and eosin, investigational by means of light microscopy.

RESULTS AND DISCUSSION

A common amount of cases was 182 for a year. Distribution of material on sexual character is the following: 16 men and 168 women. Age of patients from 89 to 18. Among men the middle age of patients amounted to 38,1±12,2, among women 49,2±13,1.

On results research the next groups of pathological processes were distinguished: goiter (111 cases - 60,3%), thyrioditis (6 cases - 3,3%), of high quality tumors (25 cases - 13,6%), malignant tumors (18 случаев - 9,8%,), combine defeats (24 cases - 13 %).

Goiter is diagnosed in 5 cases for men and in 106 cases for women, a prevailing form is a diffusely-colloid goiter.

The inflammatory diseases of thyroid were presented by autoimmune thyrioditis of Hasimoto, for men not a single case is marked, for women - 6 (3,3% from a general amount).

The high quality of thyroid tumors are presented by follicle adenoma, 25 of them being revealed on our material. For men this diagnosis meets in 3 cases, middle age of patients 43,3±6,5; women have 22 cases of disease, middle age 38,7±15,2. Age of patients from 76 to 18, most cases were on 30-40 (7 cases - 31,2%) and 50-60 (5 cases - 22,7 %). Correlation of men and women was 1x7, 3. Unlike men follicle adenoma for women is diagnosed in younger and more mature age. These data coincide with data of other researchers [5]. In all presented cases the single incapsulated tumor knot was sized from 2 to 5 cm, surrounding fabric norm follicle structure. In most cases in the tumor there were circulatory disorders expressed in a different degree, forming of cystoid cavity in some cases. In one of investigational biopsies (a woman aged 68) in the cellulose a parathyroid gland of normal structure was found. Clinically adenomas were recognized in 18 cases, making 72%. Those researches in that the diagnosis of adenoma was proposed morphologically, in a clinical conclusion had a "nodular goiter".

Malignant tumors among all diseases of thyroid made 9,8% (18 cases) for a year. For men there were 2 cases of papillary cancer, age of patients being 24, 26 and 43, no metastases are present. One of the cases is diagnosed clinically, in others a diagnosis was proposed "nodular goiter". For the women of malignant tumors of thyroid found out 15. Among them a prevailing form is a papillary cancer - 11 patients from 48 to 89. Middle age of patients in this group 65,1±12,9. Clinically the diagnosis of malignant tumor is proposed in 8 cases (72,7 %), in the cases of divergence the preoperated diagnosis was: key goiter (1 case), cystoadenom (2 cases). Metastases in lymphonoduss found out in one case (9 %), germination of capsule of gland in

three cases (36,4 %). In one case from investigational the knots of papillary chasse were found out in both stakes of thyroid - for the woman of 64.

In addition, from malignant tumors for women were discovered: follicle cancer (2 cases) medullar and undifferentiated on one case. One of cases (woman 45) of follicle chasse interesting that there were knots of papillary chasse in a contralateral stake and isthmus of gland, in lymphonoduss are his metastases. A tumor is recognized clinically. Medullar carcinom found out for the woman of 52 with a clinical diagnosis key goiter. An undifferentiated cancer met for a patient 48, accompanied by metastases in lymphonoduss, a clinical diagnosis is an adenoma.

The defeats of thyroid on our material are presented by next variants:

1) 13 cases (7,1% from the incurrence of supervisions) of follicle adenoma on a background a diffuse colloid goiter. From them there is one man age of that 64, an adenoma clinically is not recognized. Age of women 74 from 37 to, from 12 investigational cases in 6 an adenoma was educed clinically. Goiter in surrounding the tumor of fabric mainly macro- and microfallicular (mixed), the morphological signs of hyperfunction found out in three cases from 12 (25 %).

2) 4 cases (2,2%) of follicle adenoma on a background autoimmune thyroiditis for women 49 from 26 to. In one of these supervisions "adenoma" in a clinical diagnosis was absent.

3) 6 cases (3,3% from an incurrence) of combination of malignant tumors with a key colloid goiter. In this group there are 3 patients of sex of men are 2 cases of papillary carcinom on a background goiters(35 and 51 years) and one case of medullar carcinom with metastases in regional lymphonoduss (34). Among women in this group one case of low differentiated adenocarcinoma (77) and two cases of papillary microcarcinom (31 and 56), not recognized clinically.

4) 1 case (0,5% from an incurrence) of combination of follicle adenoma in the left stake with papillary carcinom in the right stake of thyroid for a man 38. The diagnosis of adenoma was clinically proposed. In operating material were also found out metastases in lymphonoduss.

Malignant tumors for men met in age 24-51, middle age of patients 34,7±10,7, 3 cases from 6 were on the short age-related interval 34-38. For women the age-related range is wider: 89 from 31 to, middle age 60,8±16,2, peak of morbidity 60-70.

Thus, tumors of thyroid in investigational material all ephithelial nature, prevail among them of high quality. On our material goiter for development of follicle adenoma served a background in 10,5 % cases. These data comport with data of other researchers [5]. The bulk of malignant tumors is diagnosed without preceding pathology (66,7 %), a key colloid goiter served in other supervisions a background. While researches of other authors demonstrate another

picture: without preceding changes in fabric of thyroid a cancer is diagnosed at 11,3 %[8]. Interestingly, that a papillary cancer for women is marked on the average in age 65,1±12,9, that goes away with data of other researchers that mark this form of chasse of thyroid in more young age [2].

Adenomas are clinically diagnosed in 22 cases from 42 (52,4%). From all cases, when there was a malignant tumor in a remote thyroid, and it is 25 supervisions, clinically a diagnosis was proposed only in 13 cases, that made 52%. It is necessary to mark that mainly it is related to objective reasons: microcarcinom on a background a key goiter; multidullar height of tumor; difficulties of cytologic verification of follicle and rare forms of chasse of thyroid; a presence of secondary circulator changes is in tumor knots. Intraoperative cytologic and histological research it would be allowed more exactly to put diagnosis and to determine a volume and character of operative interventions.

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AUTHORS:

1) Budnik Antonina Frantsevna - candidate of medical sciences, associate professor of the Department of normal and pathological anatomy, Medical faculty, KBSU. Ph.: +7 (906) 4858622, e-mail: budnik74@mail.ru Address: 360000, KBR, Nalchik, 3, I. Armande Str.

2) Musukayeva Anjelica Batalovna - assistant to the Department of normal and pathological anatomy, Medical faculty, KBSU. Ph. +7 (928) 7058767 e-mail: llaaa@mail.ru Address: 360000, KBR, Nalchik, 3, I. Armande Str.

3) Pshukova Elena Mukhadinovna, Candidate of Medical Sciences, assistant to the Department of normal and pathological anatomy, Medical faculty, KBSU. Ph. +7 (928) 0776361, e-mail: pshukova .71@mail.ru Address: 360000, KBR, Nalchik, 3, I. Armande Str.

4) Zakhokhov Ruslan Maksidovich, Candidate of Medical Sciences, associate professor, chair of the Department of general surgery, dean of medical faculty of KBSU. Ph.: +7 (903) 4254994, e-mail: zakh-rus@mail.ru Address: 360000, KBR, Nalchik, 3, I. Armande Str.

Kipriyanova N.S., Loskutova K.S.

Results of Application of Cryogenic Methods in Treating Various Precancerous and Malignant Tumors of Skin and Mucous Membranes in the Republic Sakha (Yakutia)

ABSTRACT

The authors analyzed the results of cryoablation of benign and malignant tumors of the skin and mucosa in 7927 patients from 1994 to 2014.

The results of the study indicate high efficiency of cryosurgical treatment in outpatient settings.

Keywords: premalignant disease of skin and mucous membranes, cryogenic treatment, prevention, microscopic examinatio.

INTRODUCTION

Cryoablation is a method of local impact with curative intent on tissue specific area by freezing with nitrogen. Pathological tissue undergoes degradation by ultra-low temperatures, and the tumor necrosis process controlled by doctor. The fabric in the impact zone becomes white, cold, dense, not sensitive. As the result, tumor cells die due to the formation of extra- and intracellular ice crystals, blood stasis, resulting in anoxia and tissue necrosis, as well as humoral and cell-mediated immune response of the whole organism to the action of freezing.

However, the cryogenic impact regardless of the type of device for cryosurgery is not able to destroy a large amount of tissue. Therefore the cryosurgical method is used mainly for the treatment of superficial lesions of skin and mucous membranes, where the amount of damage is small, the area available for visual observation and does not require complex control methods [7].

Compared with other surgical interventions cryosurgery has a number of advantages in terms of ease of operation, the possibility of complete destruction of tumor tissue with the activation of protective factors which inhibit further tumor development, painless, and the hemostatic effect of freezing completely satisfactory cosmetic effect to the absence of rough scars, fewer complications arising after the operation, such as the formation of adhesions, gross and keloid seams fester. In addition, during the cryo-therapeutic procedures identified immunomodulatory, adaptogenic, antistress action, increase the body's resistance to the action of stress factors of physical, chemical, psychogenic nature and prevention of allergic and autoimmune diseases. In that case the cryo-therapeutic action is achieved by sensitization of all safety and regulatory systems of the body, including the pro-oxidant system, apoptosis of

immunocompetent system with recovery of T-helper and T-killer subsystems of the immune system. This pathogenetic mechanism is particularly important, because it is known that increasing malignancy and immune system are complex multi-level cross-relationships with immunosuppressive effect of tumor [1,3,4,8,9,10,11].

Great capabilities of the local cryogenic therapy have determined its progressive, pathogenetic development. Currently, the treatment form has become a standard method of cancer therapy in outpatient practice, especially in the treatment of benign and malignant tumors of the skin and mucous membranes of different localization. Indications for cryosurgery are localization of epithelial tumors of the skin in the periorbital area, the nose, trunk, extremities, genitals, and tumor recurrence after radiation therapy, the development of tumors in the scars, etc.

Despite the abovemenntioned information, there are still few data about the morphology and nature of the initial tissue damage during the cryoablation. Destruction of pathological formations followed by the replacement tissue in the overwhelming number of cases is considered to be the main direction of cryogenic treatment, as well as other local treatments. A wide variety of forms of cellular death may be caused by identical phenomena, i.e. many of the features of pathological changes under the influence of physical and chemical agents are more dependent on the duration of exposure than on its nature. Cell survival and death depend essentially on the cooling and freezing of biological objects and thawing.

It is now known that the process of tissue cryoablation includes two phases: initial damage associated with direct tissue necrosis of surface layers under the influence of low temperature, and secondary damage caused by the death of diseased tissue as a result of hemodynamic instability caused by thrombosis and destruction of the walls of the microvasculature in entire volume of the freezing and during the aseptic inflammation. This causes a secondary ischemic tissue necrosis. After cryoablation the superficial tissue damage can be noted. Direct tissue death occurs at a shallow depth relatively to the cryoapplicator, and the tissue bulk is exposed to ischemic necrosis, that is well vascularized or water-rich tissues are sensitive to the cryotherapy.

The repair of skin after the low-temperature degradation is characterized by simultaneous processes of resorption detritus using fibroblastic cells and macrophage populations, as well as the lesion. The result is the rapid formation of regenerate that has organotypic structure [2,5,6].

The purpose of the study. Evaluate the effectiveness of cryosurgical method in oncology practice in the outpatient setting by analyzing the results of treatment of tumors of the skin and

mucous membrane and detection of structural changes occurring in the tissues of distance education immediately after cryoablation for the period from 1994. by 2014.

Materials and methods. Cryogenic treatment in oncology practice has been applied in the Republic of Sakha (Yakutia) from 1994 to the present. During the analyzed period of time (1994 - 2014 gg.) Were treated 7927 patients with benign and malignant tumors of the skin and mucous membranes.

Cryogenic treatment was conducted by cryoablation apparatus KA-02 by 3-4 cycles with two or more fields, depending on the size and location of the tumor. Depth cryotherapy does not exceed 10 mm. Cryotherapy was performed with probes of different diameters (Figure 1). All patients were treated on an outpatient basis. Exposure time depended on the nature of the tumor and is between 30c for superficial skin lesions of small size (superficial basal cell carcinoma, solar keratosis, seborrheic keratosis) and up to 5 minutes at ulcer, cystic tumors with lesions of the underlying tissue (basal cell carcinoma with ulceration, squamous cell carcinoma of the skin, keratoacanthoma, tumors of the skin appendages, angiomatous nevus). In benign processes and tumors of the skin and mucous membranes cryosurgery was performed with the capture of healthy skin 0.5 cm; with superficial basal cell carcinoma - the Taking of 1-1.5 cm: with squamous cell carcinoma of the skin, ulcers, cystic forms of basal cell carcinoma - the Taking of 2-2.5 cm apparently intact skin. Depending on the nature of education krivozdeystvie held from 1 to 3 times. After cryotherapy prescribe anti-inflammatory treatment of the lesion with a solution of potassium permanganate, geliomitsiny ointment, fastin et al., and the development of secondary infection - antibiotics.

All patients underwent preoperative morphological (histological and cytological) study to confirm the diagnosis, determine the histological type of the tumor and the subsequent determination of treatment strategies and select the method of cryosurgery. Full cryosurgery education carried out in 32.8% of cases, 67.2% histological study. All tissue samples for histological studies were fixed in a 10% neutral formalin solution. After washing in running water of its dehydrated in alcohols of increasing concentrations, embedded in paraffin according to standard technique and sectioned at a thickness of 4-5 microns luge microtome Leica. Sectioned, dewaxed with xylene, and stained with hematoxylin and eosin, as well as methods of Van Gieson and Masson, according to conventional techniques.

Results and discussion. Malignancy vast majority of patients were in the age over 60 years, with benign tumors of the skin and mucous membranes - from 30 to 59 years. Hemangiomas and warts have been diagnosed mainly in children.

Localization malignancies were distributed as follows: basal cell skin cancer - 323, squamous cell carcinoma of the skin - 13, cancer of the lower lip - 7. With the benign tumors were treated: intradermal skin papillomas – 722, nevi – 422, hemangiomas - 571, keratopapillomy – 1117, seborrheic keratosis - 595, atheroma – 107, flat warts – 1401, meybomitis eyelid- 54, papillomas and cysts of the oral cavity (uvula, tongue, tonsils, palate, arches) - 143, granulomas - 348, condylomas of vulva – 431

Cryogenic treatment for cervical pathology was used in women of childbearing age and nulliparous women. 1673 treated women of reproductive age with the following pathologies: erosion and pseudo erosion (ectopia, ectropion et al.) of cervix - 1490 (89.1%), cervix leukoplakia - 17 (1.0%), cysts of cervix - 163 (9 , 7%), polyps and a papillomas - 3 (0.2%).

Of 343 patients with malignant tumors aged 41 to 74 years, were treated by this method, the following steps: T1N0M0 - 183, T2N0M0 - 129, T3N0M0 - 29, T4N0 M0 - 2 patients. Neoplasms had the following localization: basal cell carcinoma corner of the eye - 45 patients, nose - 37 patients, pinna – 101, region behind the ear - 23, facial skin -86, body skin - 31; squamous cell carcinoma of skin – 6, bodies - 7; cancer of the lower lip - 7. Basal cell carcinoma was diagnosed in 177 men and 146 women, squamous cell carcinoma of the skin in 8 men and 5 women, cancer of the lower lip in 3 men and 4 women.

Analyzing the results of the treatment are the following epidemiological parameters of the cryogenic treatment.

With basal skin cancer often treated by men 57 years and older, living in the city and visitors, with the localization process behind the ear and nose, the indigenous men in the pinna, usually on the ear lobe. In urban women immigrants often observed localization in the corner of the eye (Fig. 2).

Immediate and long-term results with benign tumors satisfactory (Figure 3). Of 329 patients with basal cancer T1N0M0 100% alive without evidence of recurrence and metastasis. In 5 patients with T2N0M0 process recurrences were observed in 2-3 years, it was made repeated cryotherapy with complete cure.

In 6 patients with advanced cancer process conducted repeated cryotherapy, then 2 - radiation treatment for recurrence. Died from progression of the three patients who initially had locally advanced squamous cell carcinoma of the process of the skin at the site kriolechenie relapses were observed in 2-3 years. Repeated cryoablation were not carried out. Two are held twofold surgery, then radiation therapy, one conducted courses of palliative chemotherapy for lung metastases (which are identified in 4 years at follow-up).

Histological examination of surgical specimens after one cryotherapy revealed that all the structural elements of education completely preserved (Figure 4). This allowed not only morphological verification, but also to assess the state of resection margins.

With twice the cryotherapy tissue in the operating material does not undergo significant changes. We have detected areas of so-called effect of swelling, which is to increase the solubility of gases in the blood at cooling the tissue and then release them during thawing (like decompression sickness) [5] (Figure 5).

In triplicate to the action of freezing nip with krioapplikator identified and destruction of tumor cells adjacent tissue necrosis vessels supplying the tumor cells with blood. Deep tissue collagen fibers were pulping, swollen, fragmented. Vessels determined with difficulty. Perivascular hemorrhage were seen extensive. Collagen fibers in the walls of blood vessels located fragmented, with symptoms of pulping fibrils. endothelial cells with signs of vacuolar degeneration and necrosis. Determined vessels were with focal destruction of the walls and a violation of the microcirculation in the form of thrombosis. Walls were swollen, with foci of tears, bleeding. Lumens of enlarged veins were occlusive with fibrin, red and mixed thrombi characteristic effects of coagulation and rapid blood clotting. Were filled with plasma foam bubbles of gas that may be associated with the effect of heave.

Thus, when the primary cryoablation arise: 1) structural elements of the mechanical damage of tissues located beneath krioapplikator; 2) damage to the vessel walls, and breach of the rheological properties of blood. In this case, it is the vascular tissue is exposed to the maximum destruction. This is due to the fact that the greatest amount of water, which is a target for cryoablation, is concentrated in the blood vessels. For cryoablation effects characterized by the appearance of stasis, thrombosis, loss of extensive deposits of fibrin. In addition, revealed venous vasodilatation due, apparently, to a reflex reaction to the cycle of cryotherapy on a "freeze-thaw". Destruction of vessels and the violation rheological properties of blood lead to the blockade of the blood flow in the hearth of cryotherapy, the formation of ischemic focus.

Conclusions.

Analysis of the results and experience of 20 years of using cryogenic method in treatment of benign and malignant tumors of the skin and mucous membranes revealed a high therapeutic efficacy of the treatment of benign and malignant tumors (96%) in the early stages of the disease. Results of the study indicate a high efficiency of cryosurgical treatment in an outpatient setting, so it does not cause the overall reaction on the part of the body, do not require frequent visits to a specialist, non-invasive, simple enough to learn, well tolerated by patients and is simple to

perform. Strictly dosed cryotherapy allows to distinguish the tumor and screening of blood flow removes the tumor by blocking blood flow in the hearth of cryotherapy. State of tissue removed depends on the duration and frequency of cryotherapy. Single and double freezing tissue saves material for high-grade histology.

Authors:

Kipriyanova Nadejda Sidorovna – Doct. med. sciences, Professor, Medical Institute M.K. Ammosov North Eastern Federal University, high-level certificate physician – oncologist CBI RS(Y) Polyclinic, kiprinad2@mail.ru

Loskutova Kunney Savvichna – Cand. med. sciences, head of Pathologic Department FSBSI YSC CMP, chief non-staff pathologist MH RS(Y) Republican Hospital №1 – National Centre of Medicine, loskutovavs@mail.ru

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Smirnov A.V., Snigur G.L., Perlin D.V., Gurov D.U.

Algorithm optimization of pathoanatomical diagnosis of prostate cancer with application of an immunohistochemical method

ABSTRACT

Pathological differential diagnosis of primary high-grade prostatic adenocarcinoma (Gleason sum 6) and benign prostatic hyperplasia in transrectal prostate biopsy has been conducted with using an optimized panel of antibodies to cytokeratin 34βE12, cytokeratins 5 and 6, protein P63, AMACR, PSA and/or PSAP, which in 100% of cases conduced to establish the appropriate diagnosis. This immunohistochemical analysis can be recommended for carrying out life-time pathotogical diagnostics of prostate cancer besides the traditional research of a material from 12-24 points of biopsy with coloring of micropreparations with hematoxylin-eozin and determining a degree grade by Gleeson's scale.

Keywords: pathologic anatomy diagnosis, biopsy, prostate cancer, immunohistochemical study.

INTRODUCTION

Prostate cancer (PCa) is the most common solid tumors in men in the United States and in the European Union [1]. In Russia and other countries of UIS in men older than 60 years, prostate cancer is the most common malignant neoplasm (MN) [2]. In the period from 1999 to 2009 in our country, the incidence of prostate cancer has increased by 2.8 times, showing the highest values among all MN [3]. Due to the widespread use of prostate-specific antigen (PSA) screening number of men with prostate cancer detection at early stage increases in developed countries, including Russia [4, 5]. Under the new conditions the share of the biopsy increases, in which the differential diagnosis between high-grade adenocarcinoma of prostate, tumor-like "mimic" atrophic process, prostatic intraepithelial neoplasia (PIN), atypical small acinar proliferation (ASAP), sclerosing adenosis, which necessitates not only the use common differential diagnostic morphological criteria, but also to include in the currently under development standards of prostate biopsy reliable immunohistochemical methods in certain quantities. Among manuals, differential diagnostic immunohistochemical criteria of prostate well-differentiated adenocarcinoma occupy an important place [6]. If in one diagnostic case even 12 specimens (the amount of biopsies depends on the volume of prostate and can be increased up to 24) present, it is necessary to select a sufficient diagnostic panel, and question of the economic

feasibility sharply raises to use all possible number of biomarkers that increases the cost of biopsy.

The aim of our study was the optimization of prostate biopsy algorithm with using immunohistochemical biomarkers.

MATERIALS AND METHODS

We carried pathologic diagnosis of prostatic adenocarcinoma (n = 32) and benign prostatic hyperplasia (BPH) (n = 32) in combination with a PIN, chronic prostatitis, focal atrophy, atypical small acinar proliferation (ASAP) on biopsies of patients GBUZ "Volgograd Regional uronefrologichesky center".

Prostate biopsies were fixed in 10% buffered formalin solution for 24 hours at room temperature and worked up in the standard manner. Paraffin sections 3-5 μm were prepared, stained with hematoxylin and eosin. After micropreparations investigation pathological anatomical diagnosis (conclusion) was established.

For immunohistochemical investigation (IHC) next biomarkers (mouse and rabbit monoclonal antibody) were used for basal epithelial cells: cytokeratin 34βE12 (1:100, Thermo Scintific), cytokeratins 5 and 6 (1:100, Thermo Scintific), protein P63 (1:50, Santa Cruz); oncomarkers: alpha-methylacyl coenzyme A racemase, AMACR (1:100, Thermo Scintific), ERG 1,2,3 (1:50, Santa Cruz); and marker of low molecular weight calcium-binding protein - protein S-100 (1:00, Thermo Scintific) and prostate biomarkers: prostate specific antigen, PSA (1:200, Thermo Scintific) and prostatic acid phosphatase, PSAP (1:3000, Thermo Scintific). Procedures of dewaxing, unmasking antigens, hematoxylin staining were performed in accordance with the recommended protocol with further immunophenotype analysis. Pathological anatomical diagnosis (conclusion) based on results of immunohistochemical investigation of micropreparations.

RESULTS AND DISCUSSION

In each case of transrectal prostate biopsy at first we have examined micropreparations from 12 points of biopsies (768 micropreparations) stained by hematoxylin and eosin. After that, we have selected suspected to adenocarcinoma 2-4 paraffin blocks from each patient and made sections for IHC examination (571 slides). Thus, 16,7-33,3% of biopsy material often from the most questionable tissue samples examined by IHC method.

Immunophenotype of prostatic adenocarcinoma (Gleason 6) in 100% of cases was characterized by cytokeratin 34βE12 (-), cytokeratins 5 and 6 (-), protein P63 (-), AMACR (+),

PSA (+), PSAP (+). Biomarker ERG 1,2,3 demonstrated less informative diagnostic significance and was positive in 6 cases (18.8%). Virtually in all cases, prostate cancer occurred on a background of BPH. In 18 cases (43.8%) adenocarcinoma of the prostate was accompanied by PIN 2-3 degrees.

In contrast, 100% of BPH immunophenotype characterized by cytokeratin 34βE12 (+), cytokeratins 5 and 6 (+), protein P63 (+), AMACR (-), ERG 1,2,3 (-) at the PSA (+), PSAP (+). It should be noted, that the cytoplasmic expression of cytokeratins 5 and 6 in 9.4% of cases has been hailed as "questionable." In 6 cases (18.8%) BPH was accompanied by PIN. In addition, in 25 cases (78.1%) of BPH focal atrophy of prostatic glands was detected, and in 29 cases (90.6%) BPH accompanied by varying severity of lymphoid infiltration.

In 2 cases (6.3%) of BPH foci of atypical small acinar proliferation were detected and characterized by the presence of small glands with moderate cytological atypia and immunophenotype: cytokeratin 34βE12 (+), cytokeratins 5 and 6 (+), P63 protein (+), AMACR (-).

In one case (3.1%) of BPH thickened hyalinized periacinar basement membrane detected with atrophy of some prostatic glands and sclerosing adenosis suspected, however, expression of protein S-100 was negative.

Transrectal biopsy of the prostate is considered as "gold standard" for prostate cancer diagnosis [7], the implementation of IHC for differential diagnosis of prostate adenocarcinoma, PIN, focal atrophy, atypical small acinar proliferation is an essential element of diagnostic search and included in various national manuals [5, 6, 8].

To reduce the cost of the biopsy examination PIN-cocktail can be used for IHC, composed of antibodies against AMACR - tumor markers (cytoplasmic expression) and anti-p63 - a marker of basal cells (nuclear expression) that contributes to the proper establishment of diagnosis in 92-97% of cases [9,10]. Furthermore, it is noted that the use of the multiblock system reduces the antibody expense per specimen of tissue without loss of information about the object labeling [6, 7].

Thus, in the differential diagnosis of primary high-grade prostatic adenocarcinoma with Gleason score = 6 and BPH during transrectal prostate biopsy an optimized panel of antibodies to cytokeratin 34βE12, cytokeratin 5 and 6, protein P63, AMACR, PSA, PSAP presented, which allowed 100% of cases to establish correct diagnosis.

The results of this study allow to recommend to include immunohistochemical examination with using the following biomarkers: 34βE12, cytokeratins 5 and 6, protein P63,

AMACR, PSA and/or PSAP in standard method of biopsy to diagnose prostate cancer in addition to traditional analysis of material from 12-24 points of biopsy stained by hematoxylin and eosin with Gleason grades estimation.

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K.S. Loskutova, M.P. Kirillina, A.S. Innokentyeva, T.N. Zharnikova

Male Breast Cancer in the Republic Sakha (Yakutia)

ABSTRACT

Data of pathomorphological and immunohistochemical research of breast cancer at male population of Yakutia are presented in this article. At all male patients BC is revealed in the setting of gynecomastia before metastases in lymph nodes, presented in a structure of infiltrative ductal BC with an immunophenotype of luminal A subtype.

Keywords: male breast cancer, pathomorphology, immunohistochemistry, Yakutia.

INTRODUCTION

The male breast cancer (BC) is the rare disease diagnosed approximately in 1% of all cases of BC. In 2008 in Russia on 52469 new cases of a breast cancer about 500 diseases at men are revealed [2].

BC rarity at men, insufficient acquaintance of doctors to early symptoms of an illness, small awareness of the man's population on possibility of development of a tumor in a mammary gland result in low vigilance of doctors, lack of complex inspection at a gynecomastia and, as a result, to identification of BC at men in later stages in comparison with women [6,8]. Men get sick, on average, for ten years after women, is the most frequent in the 6-7th decades of life though this pathology meets aged from 9 till 90 and more years [8]. Thanks to an anatomic structure of a mammary gland, at men the minimum manifestations of an illness can be revealed even at a usual palpation, however early stages of a cancer are diagnosed only for 34% of patients. Approximately in 20% of supervision the diagnosis of a breast cancer at men at primary address isn't established. In 46,8% of supervision at primary address local distribution of process, from them in 40% of cases - with metastatic defeat more than 3 lymph nodes is noted [4,8]. Most often the tumor is localized in the central departments of gland, more than 2 cm in size, is frequent with existence of metastases in axillary lymph nodes (at 50% of patients). Therefore the increase and consolidation of axillary knots can be the first symptoms of a disease. In 21% of cases patients for the first time address to the doctor apropos is long not healing ulcer in a mammary gland [6]. At 1/3 patients the tumor rather quickly sprouts skin, is connected with people around to fabrics, with the advent of symptoms of "umbilikation", "platform", "a lemon crust", etc. Approximately the areola fold thickening (Krause's symptom), a symptom of "retraction of a nipple" is found in a half of patients at the time of the address. In rare instances

(approximately at every 7th patient) fixing of a tumor to a big pectoral muscle meets [3]. Allocations from a nipple (from serous to bloody) meet rather seldom.

Risk factors of BC at men can be divided into two groups: 1) existence of androgenic insufficiency, for example at a cryptorchidism, a secondary testicular atrophy, at violation of an exchange of androgens and estrogen at chronic hepatopathy, such as cirrhosis, and also at the liver failure caused by chronic intoxication (medicamentous, professional, etc.); 2) excess estrogen stimulation at long reception of preparations of the estrogen (at adenomas, a prostate cancer, at hyperfunction of bark of adrenal glands with obesity, an arterial hypertension and diabetes, etc.) [5]. In particular, explain these frequencies of bilateral changes in chest glands. The synchronous cancer is noted at 2% of patients and more than at a half of them during 1 year — 20 years before detection of a malignant tumor existed the centers of consolidations in chest glands. There are data on such risk factors as racial accessory [15], the ionizing radiation and genetic predisposition to this disease. In pathogenesis of a cancer of chest gland at men many note a role of repeated injuries.

It is considered that at men develops in 30 — 70% of cases of BC against a gynecomastia, in particular its nodal form, thus the risk of transition of a nodal form to BC makes 9,3-12,2% [4]. At 26,3% with the focal gynecomastia forms elements of intraductal proliferation of an epithelium, in 7,9% - atypical ductal proliferation of an epithelium in a varying degree came to light [7]. Are available sported about existence of BRCA1 and BRCA2 at BC at men [9,15]. 7-27% of sick BC of men in the anamnesis had an instruction on existence of a malignant tumor of a mammary gland for direct relatives of the first and second generation.

Because of small number of supervision BC morphology at men is studied absolutely insufficiently, there are no large-scale researches on the analysis of dependence of the forecast of a disease on degree of a histologic malignant index of BC at men. There are ambiguous and inconsistent data on existence for men of lobular and noninvasive forms of a pro-current cancer [8,12].

In literature a number of features of the hormonal status of BC at men is noted: existence of higher level of receptors of an estrogen men, than at women, lack of growth have a receptor - positive tumors with age. Clinically significant levels of receptors of an estrogen are present at a tumor approximately at 75% of patients, and at 43% of patients – receptors of progesterone [4].

MATERIALS AND METHODS

As a material of this research macro - and microscopic preparations of mammary glands of 5 men with BC were used. Histologic processing of the material was carried out by the

standard techniques. Immunohistochemical (IGH) research was conducted with reinstatement of anti-genes under pressure in the Epitop Retrieval Solution pH 6,0 solution with use of monoclonal antibodies of Novocastra (Great Britain), "HercepTest" (Dako A/c, Glostrup, Denmark), Diagnostic Biosystem according to instructions of manufacturing firms. Antigen positive cells and cellular structures were identified on their brown coloring at the light-optical level.

RESULTS AND DISCUSSION

During 2009-13 BC was diagnosed at 5 men. Thus, despite such a small quantity, we note a tendency to growth of the BC incidence rate at men. So, if 1 case was diagnosed in 2009, in 2010-11 – any, in 2012 and 2013 – on 2 cases. Men were aged from 50 till 64 years, from them 4 (80%) – at the age of 50-54 years (54,6±3,76). It is a decade earlier, than is noted by other researchers (in 6-7 decades of life). 4 (80%) BC are revealed at representatives of a indigenous nationality (yakuts). All men had no excess body weight, didn't abuse alcohol.

In total BC had a histologic structure of an infiltrative ductal cancer, with existence out of a tumor of changes, characteristic for a gynecomastia. At 4 (80%) the sizes of a tumor were 2,0 cm and less, with localization in an around the nipple zone. At 1 man at the age of 64 years the big size of a tumor – 6,5 cm is noted.

One of the microscopic criteria characterizing biological behavior of a carcinoma of a mammary gland is their histologic malignant degree. In our research in 4 cases (80%) GI malignant degree, in 1 case (20%) – GII degree is established. Thus, at 80% of patients the favorable factor of 5-year survival is established. In a number of researches direct link with the forecast of a disease is established: the tumor malignant degree is higher, the survival period is shorter. Patients with a tumor of the I malignant degree survive 5 years and more after operation in 75% of supervision, more than 10 years — in 45%; at tumors II and III malignant degrees — in 53 and 27% and in 31 and 18% of supervision respectively [4].

In the regional lymph nodes metastases aren't found in all men (100%). It corresponds to results of a number of researches in which distinct direct dependence between histologic degree of a malignant of BC at men and identification frequency the regional metastases is established [1]. And existence of metastases in lymph nodes has the most significant predictive value [13].

All studied BC had infiltrative growth. At IGH with anti-genes to a muscular aktin of SMA total absence of a layer the mioepithelial cells is revealed, at their existence out of a zone of tumoral growth, by places with a gynecomastia picture. Studying E-kadkherin - protein the epithelial intercellular adhesive contacts, the transmembran glycoprotein providing homotypic

adhesion in the epithelial fabrics is of interest. This gene is responsible for safety of cellular communications in the epithelial tissues of adults, and also acts as a supressor of a fabric invasion. There are data that decrease in quantity E-kadkherin in a number of carcinomas, is an adverse predictive factor and happens mainly at the transkriptsion level [11]. In our research in 3 of 5 BC (60%) lack of expression E-kadkherin is revealed. Also there was no reaction with a bazal keratin of CK5/6. Thus, 100% of the studied tumors expression a pan-cytokeratin of PCK and granular an epithelial membrane anti-gene of EMA (figure 1).

HER2/neu is the most important independent factor of the forecast at BC, including at men. According to Bloom et al. [16] HER2 hyperexpression at IGH research was revealed only in 1 case from 58 supervision (1,7%). According to the European institute of Oncology (European Institute of Oncology, IEO), the frequency of an expression of a gene of Her2/neu makes 15% [12]. In our research the hyper expression of HER2 is established in 1 case from 5 supervision (20%) (figure 2). .

All BC investigated by us were receptor - positive. The average total point of the assessment of positive cells and intensity of IGH-reaction rated 5,6 (ER estrogen receptors) (figure 3) and 6,6 (PGR progesterone receptors) (figure 4). Thus we revealed the growing tendency of receptor - positive tumor cells at older ages, as ER (diagram 1), and PGR (diagram 2).

The criterion of an indicator of proliferative activity of Ki-67 has already been applied at breast cancer of women, however its role in the forecast of male cancer hasn't precisely been found out yet and demands further studying. In total BC in our research had very low proliferative activity (the Ki-67 index less than 14%), and also low indicators of existence of a mutant gene-supressor p53.

CONCLUSIONS:

1. BC at men, despite increase in frequency of occurrence is rare tumor.

2. Under the timely complex inspection of doctors, BC at men can be revealed in early stages before metastases of lymph nodes.

3. The infiltrative pro-current cancer is the BC main histologic form at men, in the presence of gynecomastia as a background process.

4. 80% of cancer of mammary gland have low histologic malignant degree G1.

5. According to molecular and genetic classification BC immunophenotype at men corresponds luminal A (80%) and B (20%) subtypes of BC that allows to use the complex

treatment with inclusion of hormonal therapy with anti-estrogen, and also aromatase inhibitors at 80% of patients.

6. In studying of this disease at men it is necessary to consider challenges demanding further studying and accumulation of supervision, large-scale researches for studying of risk factors, predictive importance of biological markers, ethnic and genetic features.

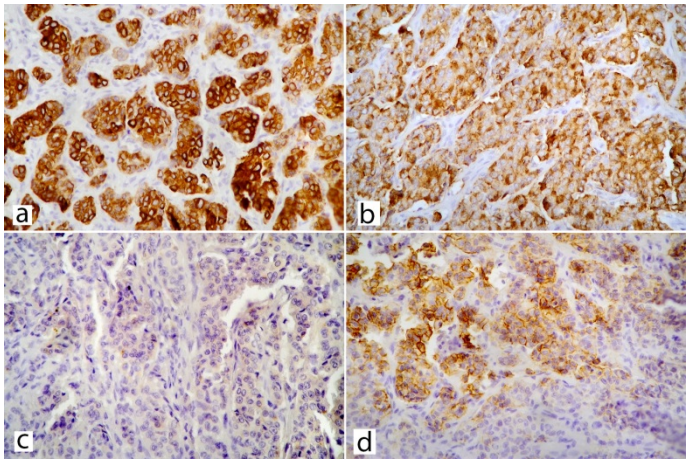

Figure1. The patient Zh., 54. Infiltrative ductal cancer, malignance degree G2: a - bright membranous and cytoplasmatic expression (PanCK, clone 5D3 fnd LP34); b - moderated granular cytoplasmatic expression of epithelial-membranous antigen (EMA, clone GP1.4 Ber-EP4); c - absence of expression of basal keratin (Cytokeratin 5/6, clone D5/16B4); d - loss E-cadherin by tumor cells: groups of tumor cells with remained membranous antigen expression in a zone of "thawing" membranes with transition in fields of total absence of expression (E-cadherin, clone NCH-38) (x200).

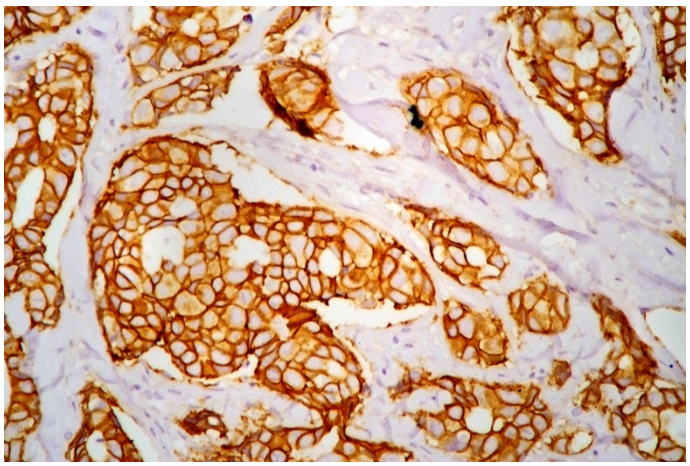

Figure 2. The patient S., 52. Hyperexpression of oncoprotein Her/2-neu (+3) in tumor cells of infiltrative ductal cancer (x200)

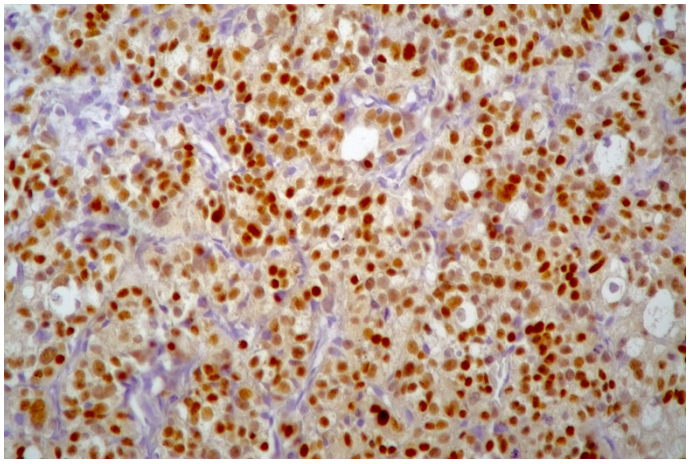

Figure 3. The patient Ya., 64. Estrogen-positive infiltrative ductal mammary gland cancer (ER, clone1D5): a share of stained cells in scores =5 (from 2/3 to 100 % of cages), intensity of expression in scores =2 (strong nuclear stain), total score =7 (X200).

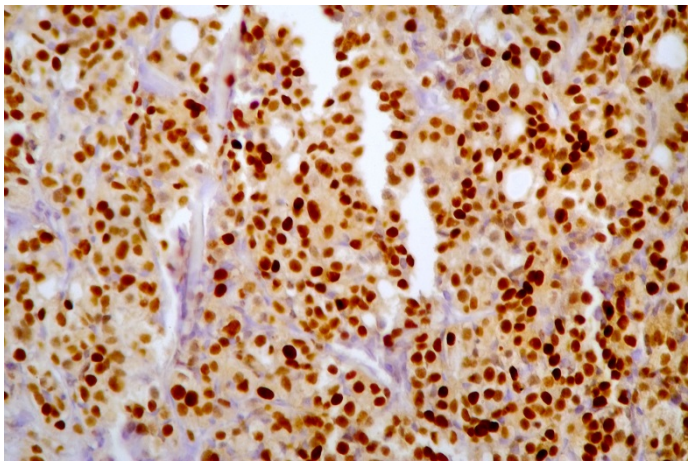

Figure 4. The patient B., 64. Progesterone-positive infiltrative ductal mammary gland cancer (PGR, clone PgR636): a share of stained cells in scores =5 (from 2/3 to 100 % of cages), intensity of expression in scores =2 (strong nuclear stain), total score =8 (X200).

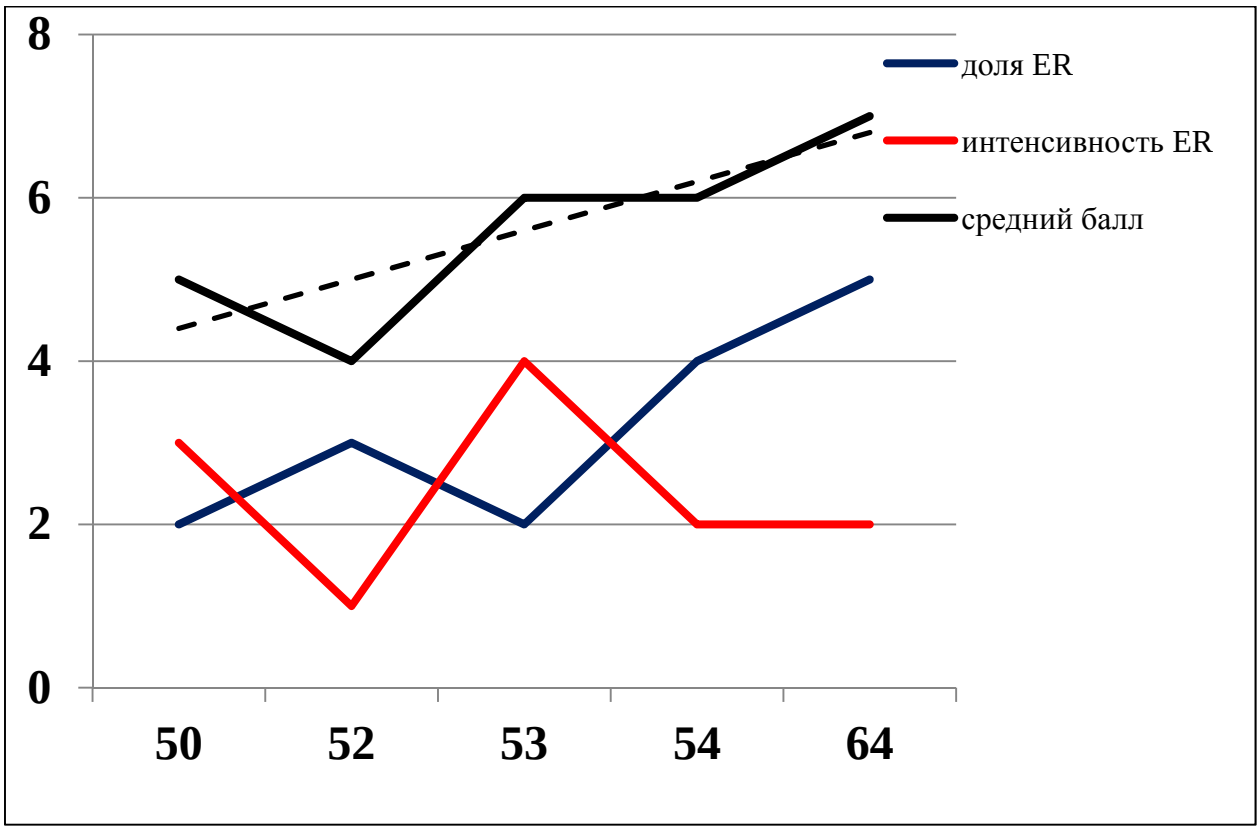

Diagram 1. The level of expression ER with aging in male breast cancer.

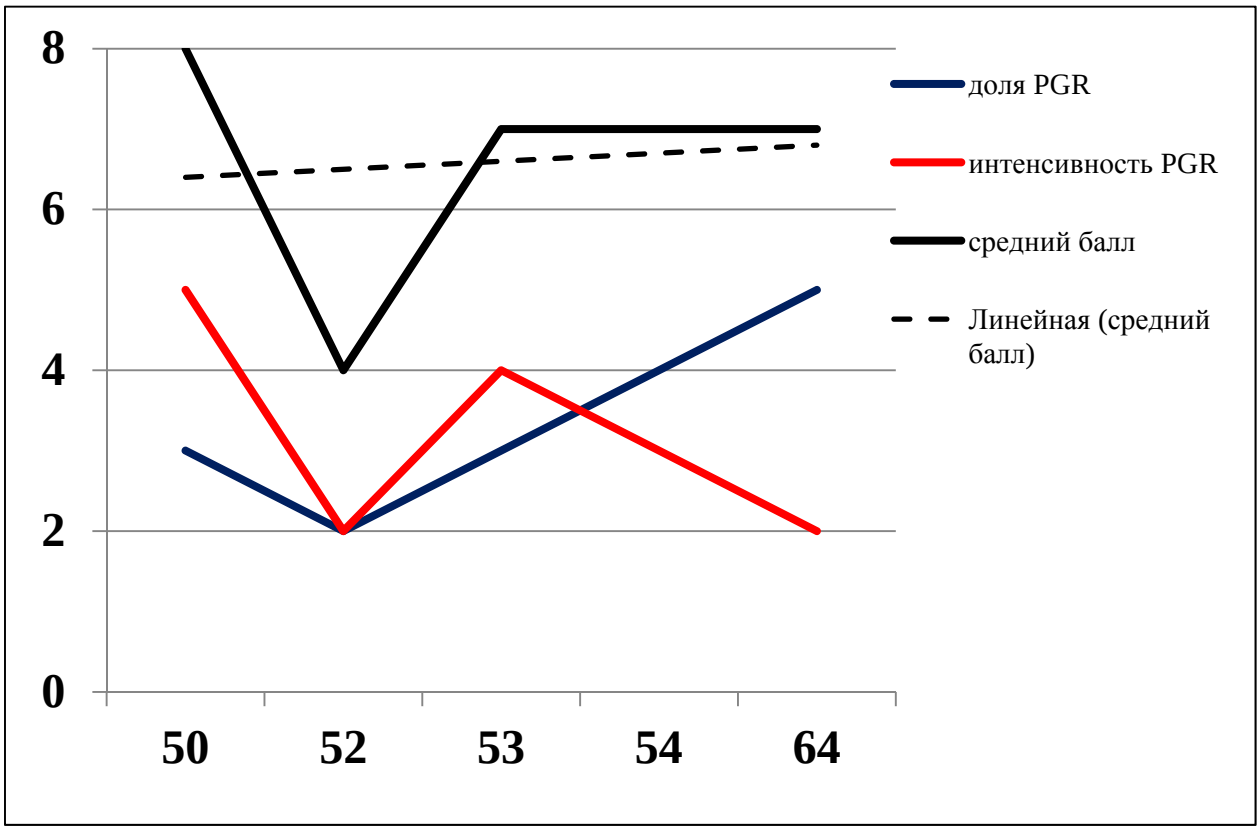

Diagram 2. The level of expression PGR with aging in male breast cancer.

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Authors:

ЛОСКУТОВА Кюнняй Саввична – к.м.н. гл. внештат. патологоанатом МЗ РС(Я), зав.ПАО ГБУ РС (Я) «РБ №1-НЦМ», зав. лаб. ФГБНУ «ЯНЦ КМП», lockutovaks@mail.ru; **КИРИЛЛИНА Мария Петровна** – к.б.н., зав. учеб.-науч. лаб. Клиники МИ ФГАОУ ВПО СВФУ им. М.К. Аммосова, kirillinamp@ mail.ru; **ИННОКЕНТЬЕВА Александра Степановна** – зав. отделением биопсий РБ№1-НЦМ; **ЖАРНИКОВА Татьяна Николаевна** – к.м.н., зав. отд. ГБУ РС (Я) «ЯРОД», н.с. ФГБНУ «ЯНЦ КМП», zharnikova_tn@mail.ru.

LOSKUTOVA Kunnjay Savvichna - cand. med. sciences, chief non-staff pathologist of MH RS (Ya), head of the pathologic department SBI RS (Ya) «RH №1-NCm», head of the laboratory FSBSI «YSC CMP», lockutovaks@mail.ru <mailto:lockutovaks@mail.ru>;
KIRILLINA Maria Petrovna – cand. boil. sciences,head of lab. Clinics of MI FSAEI HPE NEFU of M.K.Ammosov, kirillinamp@ mail.ru;
INNOKENTIEVA Alexandra Stepanovna – head of the department of biopsy RH№1-NCM;
JARNIKOVA Tatyana Nikolaevna – cand. med. sciences, head of the department SBI RS (Ya) «YROD», research ass. FSBSC “YSC CMP”, zharnikova_tn@mail.ru.

Khayrullin R.M., Kometova V.V.

The prognostic significance of a degree of ordering tissue structures at invasive-lobular breast cancer

ABSTRACT

A retrospective analysis of uneven distribution of tissue structures in breast cancer as a criterion for the prognosis is carried out. The standard statistical indicators which reflect variability of morphological parameters were applied. We found out the individual coefficient of variation of cancer structures in some optimal number of analyzed visual fields of the histology microslide to be the most informative and objective parameter statistically conjugated with the prognosis.

Keywords: prognosis of breast cancer, cancer structures, morphometry.

INTRODUCTION

Breast cancer in their clinical, morphological and molecular genetic features is a heterogeneous disease [11, 14]. Breast cancer has many subtypes which are different by its clinical outcomes. Morphological verification of breast cancer is 95.6%, within the same histological types and the same stage of the prognosis can vary significantly. It was suggested the set of morphological parameters for prognosis and risk of recurrence [3]. The interpretation of these parameters is quite complicated. In pathomorphology the best way to increase of the informational content is the unification of results using quantitative research methods [1, 16]. Of particular the prognostic interest represent the degree of ordering of the structure and components of the tumor tissue. The experience of the morphological study of micropreparations of different histological types of breast cancer and of cancer's structures that make up the tumor tissues, not only in different cases of observations, but also in the different fields of view of micropreparation of the one patient [15]. The number of cancer structures from observation to observation can vary by tens of times. A comparative study of breast tissue of patients with breast cancer before and after radiotherapy led to the conclusion that the damaging effect of radiation on the tumor leads to significant disturbances and changes of parenchymal-stromal relationship not only in the tumor tissues, but also in the surrounding tissues [2]. The absence of any strongly regularities of the heterogeneity of the distribution and the ordering of tissue structures lead to the conclusion that the need to find indicators that would adequately reflect this heterogeneity, and at the same time is sufficiently informative. In quantitative morphometric studies this group of indicators is parameters of the variability of variables and includes the

absolute difference between the extremes (maximum and minimum values), a relative measure of magnitude (relative difference of limits), standard deviation (σ), and finally, the coefficient of the variation (CV) [1]. The purpose of this study was to determine the variability of the distribution of the structural elements of the parenchyma and stroma of infiltrating lobular type of breast cancer and its covariation with prognosis.

MATERIALS AND METHODS

The material for the study included 69 biopsies of tumor tissue of patients with breast cancer at the age of 59,3±8,8 years, which were treated in the Ulyanovsk Regional Clinical Oncology Center from 2004 to 2006. All permission on the research was granted by Ethical Committee of Ulyanovsk State University. For morphometric analysis were using methods described previously [8-9, 13]. For the estimate of the total score of malignancy used appropriate criteria [4-5, 10, 12]. The obtained data were processed using the software package Microsoft Excel 2003 and Statistica for Windows 8.0. (StatSoft Inc., USA).

RESULTS AND DISCUSSION

Preliminary univariate ANOVA analysis of variance by the criterion of five-year survival showed no significant differences in the group of patients who died of observations depending on the duration of their lives after moment when was diagnosed the breast cancer. Critical statistically significant differences were obtained only in the separation criterion five-year survival rate in the binary scale: "The patient is alive to the present (default value 0)" - "The patient died after 1-5 years after diagnosis (default value 1)." In accordance with the purpose of the study for identify the prognostic value of quantitative indicators characterizing the degree of ordering (or heterogeneity) structures were identified by their values for the above two groups of patients.

Table 1.

The main morphological features were the heterogeneity and disorder of the location of the cancer complexes in the stroma of tumor's. Their number varied in a wide range, from 38 to 898 structures in one field of view area of 0.152 mm^2. As can be seen from Table 1, from of all of the analyzed indices of the individual variability greatest level of significance of differences were obtained for the mean value of the square deviations of the number of cancer's structures in the field of view. It was 20.22±2.72 for patients with long-term survival over 5 years and 23.8±2.49 for patients who died within 5 years after diagnosis of breast cancer ($p<0.004$). The smallest statistically significant difference was observed for the absolute and relative difference of extremes (see Table. 1). Thus, the most informative and objective from a statistical point of

view, the parameter characterizing the degree of ordering and distribution of tissue elements in the tissue of the lobular type of breast cancer is an individual coefficient of variation of cancer structures (CV) in 30 fields of view. The substantial side of criteria, which were proposed by of different authors, depends on the skill and style of presentation of conclusion of a pathologist, often contains a large amount of special morphological information, and is difficult for understanding and summarizing for the clinician in matters of the medical tactics [3]. In recent years were undertaken intensive efforts to create of optimal pathologic assessment scales to improve diagnosis of breast cancer [6-7]. However, the methodological approaches implemented in them, based on standard statistical mean values of the variables or, more often, of the frequency. The interpretation of these data is of great complexity and is not devoid of abstraction for a particular patient. According to our results, the most objective measure of individual forecasting five-year survival for patients with lobular breast cancer option should be regarded the standard deviation of the number of cancer structures analyzed for the same number of fields of view. Other words, for patients with lobular infiltrative type of breast cancer having individual «σ» of the absolute number of cancer structures no more than "23" for 30 fields of view microscope slide, may be forecast 95% chance to five-year survival. In cases with individual variations of σ more than "32" five-year survival prognosis is unfavorable.

CONCLUSION

Summarizing the results of the study, it must be concluded that the a variant of infiltrative type of lobular breast cancer is characterized by of statistically significant differences in the degree of ordering of structures of cancerous tumor's tissue that can be highly reliable parameters for predicting the five-year survival rate of patients.

Table 1

Mean values and confidence intervals of parameters of the individual variability of the number of cancer structures determined in 30 fields of vision for each patient (the observation case)*

The parameter of the variability	Prognostic group	M±m	95% confidence interval	Level of the significance of differences
Absolute difference of extremes	Survival over 5 years *n*=20	74.25±11.32	50.57-97.93	p<0.021
	Survival less than 5 years *n*=8	123±13.45	91.20-154.79	
Relative difference between the extremes	Survival over 5 years *n*=20	2.47±0.38	1.69-3.26	p<0.021
	Survival less than 5 years *n*=8	4.1±0.45	3.04-5.16	
Standard	Survival over 5	20.22±2,72	14.53-25.92	p<0.004

deviation (σ)	years *n*=20			
	Survival less than 5 years *n*=8	35.41±3.17	27.92-42.90	
Coefficient of variation (CV)	Survival over 5 years *n*=20	8.80±1.13	6.43-11.17	p<0.013
	Survival less than 5 years *n*=8	15.21±2.57	9.13-21.30	

Note: *- the parameter of the statistical variability which was obtained for the characteristic of each patient (for n=30 fields of vision) of the corresponding variable, was considered as the independent individual parameter

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Sosina S.S., Golderova A.S., Loskutova K.S., Efremova S.D., Alekseevay E.A.

A modern method of early diagnosis of preoncologic changes of gastric mucosa

ABSTRACT

In order to detect precancerous forms of chronic gastritis in the conditions of the North with use of modern noninvasive immunoenzymometric methods of diagnostics (the GastroPanel test set) a number of population from the settlements Vitim and Tolon of the Lensky region of RS(Ya) with chronic gastritis are examined. We revealed that the survey method «Gastropanel» allows to receive information on structure and function of gastric mucosa as well as to identify patients with expressed atrophic gastritis referring to a group of oncological risk with high sensitivity and specificity.

Keywords: atrophic gastritis, “Gastropanel”, identification.

Atrophic gastritis often proceeds without symptoms and often remains not diagnosed. In most cases Helicobacter pylori infection is the cause of atrophic gastritis, which is always associated with gastritis. A half of humanity is infected by this infection (about 3,5 billion people). Almost at 50% of the persons infected with Helicobacter pylori atrophic gastritis is diagnosed, which in most cases leads to a cancer of stomach and in 90% of cases it is the reason of development of stomach ulcer. Stomach ulcer is noted at 10% of world’s population.

In 1994 the International Agency for Research on Cancer (IARC) and the research organization under jurisdiction of WHO presented results of the conducted researches that Helicobacter pylori infection is a major risk factor of development of cancer of stomach. The infection of Helicobacter pylori is considered as a causal factor of development of cancer of stomach (carcinogen class 1). According to these provisions Helicobacter pylori infection starts a chain of the reactions that leads to development of atrophic gastritis, which in turn causes a cancer of stomach at some patients. At patients with atrophic gastritis of the stomach the risk of development of the cancer of stomach is more frequent in 5 times, in comparison with other population. If mucosa of all of the stomach is damaged (heavy atrophic gastritis of a body and antral department), the risk increases up to 90 times. If the atrophic process is localized only in antral department, the risk is increased up to 20 times, besides, the risk of development of stomach ulcer is 25 times higher, than in other population.

Ablation of Helicobacter pylori infection leads to the treatment of atrophic gastritis. Atrophic gastritis slowly recovers after the successful ablation. The risk of development of the diseases connected with atrophic gastritis decreases respectively or disappears completely .

Now the "Gastro Panel" or gastroscopy with histologic research of bioptat are the only methods of diagnosis of gastritis and atrophic gastritis, as well as of their severity and localizations.

Creation of the "Gastro Panel" test panel is the result of decades of the basic medical researches conducted in Finland. By means of conducting "Gastro Panel" on blood test, it is possible to define a state and functional activity of all mucous membrane of stomach. This new method is noninvasive, safe and convenient for the patient. In most cases by means of "Gastro Panel" results of the research of a state and functional activity of mucous membrane of the stomach are found out similar to results to endoscopy with biopsy research. However, the endoscopy with bioptat capture is a subjective method of the research in many respects depending on professional skills and experience of an endoscopist and pathologist. It is necessary to notice that "Gastro Panel", in comparison with the gastroscopy and biopsy research is more sensitive method of inspection and allows to diagnose considerably smaller changes of structure and functional activity of mucous membrane of the stomach [5].

According to researches of the Yakut scientific center Russian Academy of Medical Science of RS (Ya) of Loskutova K.S., Argunova V.A. (2007) [4] contamination of Helicobacter pylori of adult population is 76,1% in Yakutia. In the general structure of the examined children and teenagers [3] contamination of Helicobacter pylori was 58,5%, atrophic gastritis at 8,5% of teenagers and 34% of adults from among indigenous people of the North was combined with an intestinal metaplasia, displasia of a mucous membrane of a stomach [6].

According to researches of the scientific research institute of therapy RAMS in Novosibirsk [2] frequency of atrophy in a stomach body at the population of Novosibirsk, urban and country people of Yakutia made respectively 10,1, 16,7 and 25,6%, and in antral department – 10,7, 25,6 and 8,9%. The total atrophy is registered in 1% in all groups. The infection of Helicobacter pylori is revealed at 78 – 88%.

According to researches of employees of clinical hospital of Yakutsk and NEFU [1] with the use of "Gastropanel" the stomach body atrophy (isolated or in combination with an atrophy of antralny department) is revealed among persons aged till 50 years was in only in 6,1% of cases, and in in a group of persons is more senior than 50 years – is diagnosed in 31,2% of cases.

And, the atrophy of antral department of the stomach with low indicators of postprandial level gastrina-17.

Thus, studying and development of issues of the early diagnostics, prevention and treatment of precancer changes mucous of the stomach has the perspective direction in decrease of the incidence of stomach cancer in the population of the North.

Aim of the Research: Identification of the precarcinogen forms of chronic gastritis in the Northen conditions with use of a modern noninvasive immune ferment method of the diagnostics of "Gastropanel" and their comparison with the morphological picture of stomach mucous.

MATERIALS AND METHODS

21 patients with chronic gastritis (10 men and 11 women) aged from 26 till 67 years residents of the settlement of Vitim and settlement of Tolon of the Lensky region of RS (Ya) are examined including 11 Yakuts and 10 Russians. Except 2 patients, who have lived in the north for 6 years, all of them were born in a settlement or moved from other areas of the republic.

For diagnostics of the phenotype of gastritis the GastroPanel test set of Biohit firm (Finland) is applied including the immunofermental analysis of antibodies to Helicobacter pylori (IgG), pepsinogen 1(PG1), pepsinogen 2 (PG2) and gastrin-17 (G-17). Functional heterogeneity of departments of stomach serves as a basis for using "Gastropaneli": in antral department G-17 and PG 2 were produced, and in a stomach body - PG1 and PG2. Respectively, when progressing the atrophy of a body of stomach the decrease in production of PG1 will be observed, and at multifocal gastritis – decrease in PG1, and at multifocal gastritis – the decrease in PG1 at the normal or increased G-17 values.

RESULTS AND DISCUSSION

According to "Gastropanel" at 18 patients (85,7%) gastritis was associated by Helicobacter pylori, the infection with a cytotoxic strain of CagA. Increase of indicators of PG2 (more than 10 mg/l) at 14 patients (66%) corresponds to high activity of inflammatory process in a mucous membrane of stomach. According to G-17 hormone 15 patients (71,4%) had a level of acidity of gastric juice within norm, easy decrease in acidity was noted at 3 patients, the hypochlorhydria and an achlorhydria are noted at 2 patients, the increased acidity of gastric juice at 4 patients. The PG1 level was reduced (less than 25 mcg/l) at 2 patients that testifies to the atrophy of fundal department mucous of the stomach. 4 patients had a low ratio of PG1/PG2 (lower than 3) that testifies to the atrophy of mucous membrane of the body of stomach.

The fibroezophagogastroduodenoscopic study with morphological research of bioptat of antral and fundal mucous departments of the stomach was carried out at the patients. The morphological picture of active gastritis was revealed at 19 patients (90,4%) and atrophic changes mucous of the stomach were noted at 3 patients with the low level of ratio of PG1/PG2 is revealed.

Conclusion: Thus, "Gastropanel" allows to receive information on structure and function mucous of the stomach, to identify with high sensitivity and specificity of patients with the expressed atrophic gastritis, groups of oncological risk.

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Authors:

1. Sosina Svetlana Stepanovna – cand. med. sciences, senior lecturer of the department of propaedeutic and faculty therapy with endocrinology and ART, Medical institute Northeast federal university of M.K.Ammosov, sosinasveta@gmail.com <mailto:sosinasveta@gmail.com>

2. Golderova Ajtalina Semenovna – doctor of med. sciences, head, chief researcher of FSBSI «YSC CMP», hoto68@mail.ru <mailto:hoto68@mail.ru>

3. Loskutova Kjunnjaj Savvichna – cand. med. sciences, chief non-staff pathologist RS (Y), he3ad of pathologic department RH 1- NCM

4. Efremova Svetlana Dmitrievna – junior research associate of FSBSI “YSC CMP”

5. Alekseeva Elizaveta Aleksandrovna - junior research associate of FSBSI “YSC CMP”

Evseyev A.N., Plekhova N.G., Evseyeva A.A.

Morphological changes in gastric mucosa in hemorrhagic fever with renal syndrome

ABSTRACT

Gastric biopsy specimens in acute hemorrhagic fever with renal syndrome cases were examined using indirect immunolabeling of the viral antigen assisted by confocal laser scanning and electron microscopy. Degenerative and necrotic cellular changes combined with signs of focal hyperplasia of gastric focal epithelium and incipient shift of epithelial differentiation towards mucocytes to the profound epithelial proliferation combined with poor differentiation of highly specialized cells (chief and parietal cells) were revealed. Ultrastructural virus-specific inclusions in the cells were found and dependence thereof on the adaptive rearrangement of gastric mucosa was established.

Key words: Hemorrhagic fever with renal syndrome, immunofluorescence, immunoelectron microscopy, gastric mucosa, erosions, acute ulcers, endothelium, macrophages

Generalized microvessel lesion with damage to different tissues and organs and subsequent inflammatory and reparative processes represent a pathogenic pathway of hemorrhagic fever with renal syndrome (HFRS) (1, 2, 3, 4). No morphological study into the mechanisms of gastric mucosa (GM) damage in HFRS have ever been done till the present date. There are almost no data on the condition of different GM regions based on comprehensive morphological assessment using immunoelectron microscopy.

The aim is to provide comprehensive morphological assessment of GM changes at the acute stage of HFRS and establish the relationship between the changes and the antigen availability in different cells.

MATERIALS AND METHODS

Gastric biopsy specimens of ten HFRS patients obtained within 6 to 14 days from the onset of clinical manifestation of HFRS were fixed in 10% neutral formalin and embedded in paraffin. Serial sections were stained by Ehrlich's hematoxylin and eosin; neutral mucopolysaccharides were identified by PAS-reaction, acidic mucopolysaccharides – by Alcian blue staining with additional hematoxylin staining of nuclei. In addition, immunofluorescence by means of confocal laser scanning microscopy and immunoelectron microscopy of biopsy

specimens was used. Gastric biopsy specimens were studied with electron microscope JEM-100S (JEOL, Japan) with an accelerating voltage of 80 kV in a diffraction contrast mode.

RESULTS

Gastroscopy of HFRS patients at different stages of the disease revealed mucosal thickening of gastric body, cardia and antrum due to hyperemia and edemas. Here and there, gastric folds appeared ridged with punctate hemorrhage, acute erosions and ulcers. With light microscopy, gastric changes were found to be diffuse, involving superficial and deep layers. Degenerative changes and necroses of superficial epithelial structures and focal stromal hemorrhage were prevailing (see Fig. 1). Gastric foveolar epithelium was represented by tall columnar cells with pyknotic nuclei located in the basal part. Signs of hypersecretion were found in superficial-foveolar epithelium – a thick layer of PAS-positive mucin that was located on the cell surface (Fig. 2).

Foveolar cell cytoplasm was found to contain alcianophilic granules with glycosaminoglycans. Lamina propria of gastric mucosa presented with stromal edema, microvasculature hyperemia and stases, focal hemorrhage, polymorph cell infiltration.

Microscopic study: erosion base was found to be covered with mucus and fibrin and infiltrated with polymorphonuclear leukocytes. In addition, vascular hyperemia, stases, red blood cell aggregation (erythrocyte sludge) with microthrombi in stromal microvessels were identified.

Acute ulcers looked like oval defects of mucosa. The ulcer base was colored grayish-red; some ulcers presented with arrosion-damaged vessels. Histopathology of ulcer base revealed necrotic matter impregnated with fibrin and infiltrated with polymorphonuclear neutrophils. Areas of fibrinoid necrosis and granulations were found below the necrotic matter (Fig. 3).

Confocal microscopy revealed specific coarse-granular luminescence in GM capillary epithelium and endothelium, which was indicative of Hantavirus antigen (Fig. 4).

Electron microscopy study of gastric biopsy specimens revealed degenerative changes in GM superficial epithelial cells. Mucocytes were found to have lost their microvilli; the entire apical surface was occupied by vacuoles that pushed mucoid granules and the nucleus to the periphery of the cell; the cells were overloaded with mature mucoid showing degenerative changes (Fig. 5).

An ultrastructural study of gastric biopsy specimens revealed positive response to Hantavirus antigen in macrophage type cells. Hantavirus inclusion particles were found in cytoplasm areas of higher ribosome content and a great number of endoplasmic reticulum

cisterns (Fig. 6). Mucocyte and macrophage cytoplasm presented with solid viroplasts, double-layer membrane structures as well as laminar structures.

Clasmatotic outgrowths on the surface of the cells, expansion of endoplasmic reticulum, mitochondrial vacuolation with cytolemma rupture were observed. Vacuolated cells were observed on the top of the folds, the sides of the foveolae, and in the depth of the cristae. A part of the cells was found to lose microvilli and desmosomes and separate from the adjacent cells of the epithelial layer. Chromatin was often condensed into large dense lumps. Cellular nuclei were often subject to condensation and looked like apoptotic corpuscles. Intercellular spaces appeared extended with polynuclear leukocytes, lymphocytes and macrophages contained therein.

The cytoplasm of the principal cells presented with sites of both higher and lower electronic density with a great number of vacuoles varying in size. The number of rough-surface endoplasmic reticular structures appeared somewhat decreased, the nuclei acquired an irregular shape with a plicate surface and clusters of chromatin near the karyolemma. The cytoplasm also presented with solid viroplasts, double-layer membrane structures as well as laminar and tubular structures.

Ultrastructurally, accessory cells presented with reduction in the apical part of the cytoplasm, loss of microvilli, invagination in the intracellular tubules, virus-induced structures available, increased number of lysosomes and autophagosomes, rarefied matrix and reduced mitochondrial cristae, and extended areas of intercellular contact.

The proper lamina of gastric mucosa presented with cellular infiltrations of plasmacytes, lymphocytes, neutrophilic polynuclear leukocytes and erythrocytes. Endothelial cells of blood vessels appeared swollen, bearing signs of villous transformation, with extension of the granular ergastic reticulum, mitochondrial destruction, clusters of myelin-like structures, and virus-like particle bearing erythrocytes and macrophages in the vascular lumens.

Our investigation has shown that GM at the acute stage of HFRS presents with the prevalence of alterative ultrastructural changes in the cells combined with signs of focal hyperplasia of gastric focal epithelium, incipient shift of epithelial differentiation towards mucocytes secondary to the profound epithelial proliferation combined with poor differentiation of highly specialized cells (chief and parietal cells) were revealed. Ultrastructural virus-specific inclusions in the cells were found and dependence thereof on the adaptive rearrangement of gastric mucosa was established.

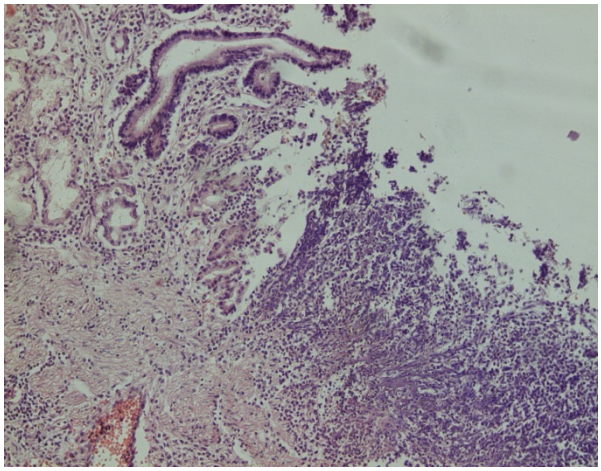

Fig. 1.General view of a biopsied fragmen to gastric mucosa: multiple superficial hemorrhage, degenerative and necrotic changes of foveolar epithelium, stromal edema. Disease duration: 7 days. H&E staining, x 80.

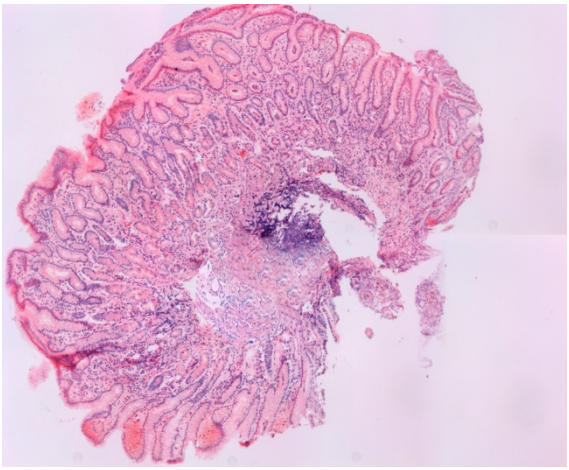

Fig. 2.Mucin hyperproduction in gastric mucosa. Biopsy. Disease duration: 9 days. PAS-reaction, x100

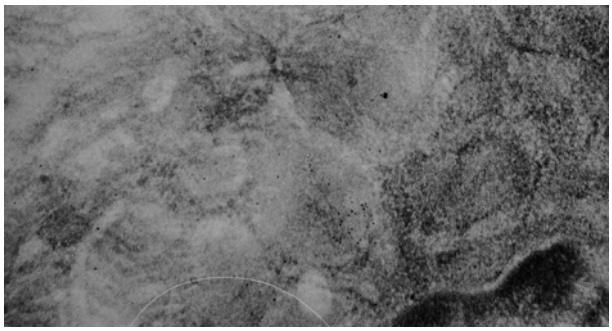

Fig. 3.Acute gastric ulcer: the area of fibrinoid necrosis extends to muscular coat, abundant infiltration with polymorphonuclear leukocytes, stromal edema, hyperemia and stases in the vessel. Biopsy. Disease duration: 10 days. H&E staining, x 100.

Fig. 4.Granular specific luminescence of Hanta virus antigen in GM epithelial and endothelial cells. Gastric biopsy specimen. Disease duration: 7 days. Laser scanning confocal microscopy. Indirect IFA, x 1000

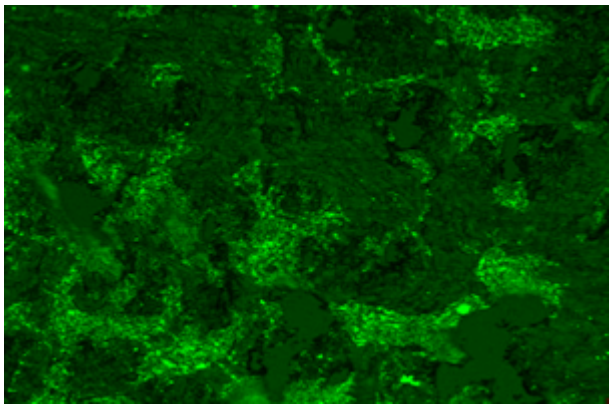

Fig. 5.Gastricmucosa: free surface of mucocytes contains single microvilli, close fitting secretion granules located in the apical part with destructive changes. Biopsy. Disease duration: 7 days. Electronogram, x 3000.

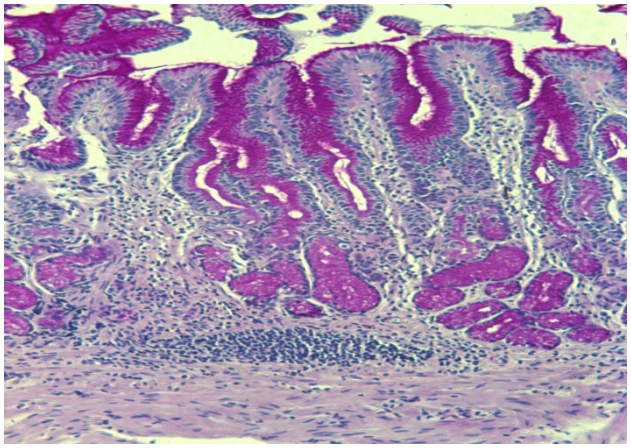

Fig. 6.Macrophage type cell in the gastric biopsy specimen of a HFRS patient. A good view of well-developed endoplasmic reticulum, chromatin condensation at the karyolemma, and virus-specific structures with a positive response to Hantavirus antigen (x50000)

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Authors:

1. **Evseev AlexeyN.** «Far Eastern state Medical University» of Ministry of healthcare of the Russian Federation, Khabarovsk str. Muravyov-Amursky 35

candidate of medical Sciences , head of the course of pathological anatomy Department of pathological anatomy and forensic medicine

Contact phone 89141580564 slave. 8(4212)409564

E-mail alexevseyev@yandex.ru

2. **Plekhova NatalyaG.**

Head of Central research laboratory of «Pacific medical University» of Ministry of healthcare of the Russian Federation

690002, Vladivostok, ProspektOstryakova, 2

D. SC.N. Plekhova Natalya hundred 89623396391

e-mail: pl_nat@hotmail.com

3. **Evseeva Alena A.** senior laboratory of the Department of pathological anatomy and forensic medicine of the medical faculty «Far Eastern state Medical University» of Ministry of healthcare of the Russian Federation Khabarovsk street Muraveva-Amur 35
G. Khabarovsk St. Brick 36A D. 23 sq servant.8(4212)409564
e-mail: alenaevseeva.93@mail.ru

L.S. Bykhalov , A. V. Smirnov

The Characteristic of Expression of BCL-2 and BAX Apoptosis Regulatory Proteins in Lungs with the Development of HIV/TB Co-infection

ABSTRACT

In this study increase of expression Bcl-2 in zones of granulomatous inflammation and in lung parenchyma is revealed as well as an increasing rate of Bax-positive cells with change in the ratio of expression Bcl-2/Bax (coefficient of apoptosis). The balance pro– and antiapoptotic proteins of Bcl-2 family defines sensitivity of the cell to proapoptotic incentives that indicates activation of anti-apoptotic mechanisms in infected cells at VICh/TB coinfecton.

INTRODUCTION

Global medico-social problems related to HIV infection and tuberculosis remain unresolved and are considered as a threat to all humanity. The incidence of tuberculosis among HIV-infected individuals has increased significantly in the last few years, suggesting a number of common social and pathogenetic patterns and mechanisms of development co-infection HIV/TB, as well as mutual influence of infectious agents on each other [2,8]. The main characteristic of the clinical and morphological patterns of combined co-infection HIV/TB can be called the generalization of tuberculosis with multiple hematogenic dissemination of Mycobacterium tuberculosis (MBT) with the formation of caseous necrosis and infiltrates in various organs, these changes in lungs being observed in 100% of cases [1]. One of the major mechanisms of cell death in combined TB and HIV infection is apoptosis [5]. Despite numerous studies on the regulation of apoptosis [5,6,7], the role of protein BC1-2 and Bax in co-infection HIV/TB remains understudied.

The aim: to assess a level of expression of BC1-2 and Bax in the foci of granulomatorus inflammation in lungs at patients who died of generalized tuberculosis in co-infection HIV/TB.

MATERIALS AND METHODS

Autopsy material from patients (n=30) with generalized co-infection HIV/TB, stage 4 - group 1 was investigated. In the comparison group – the group 2 - autopsy cases (n=30) with mono-infection, with acute progressive pulmonary tuberculosis were investigated. Lung samples were extracted from corpses according to standard techniques in early stages after the death of the patient. Specimens were fixed in 10 % solution of buffered formalin over night at room temperature, washed and subjected to standard histological processing. From paraffin blocks histological preparations were made, stained with hematoxylin and eosin. For immunohistochemical investigation of biomarkers of Bcl-2 and Bax rabbit monoclonal antibodies (Spring Bioscience) were used. Visualization was performed with using indirect immunoperoxidase method with high temperature antigen retrieval, the study of specimens was carried out on microscope "Micros" (Austria), photomicrographs were made with the digital camera Olympus (Japan), the severity of expression of biomarkers was evaluated with using the Image Analysis program with subsequent morphometric analysis. Statistical processing of materials was performed using an application program Statistica 6.0, developed for biomedical research, the magnitude of the level of statistical significance was accepted for $p<0,05$.

RESULTS AND DISCUSSION

In lung specimens, stained with hematoxylin and eosin of the 1st group polymorphic changes were observed. In the lung parenchyma areas of emphysema with atelectasis were noted. Thickening of interalveolar septae was observed. In alveoli desquamated epithelial cells, macrophages and different amount of serous, hemorrhagic, and mixed exudate were present, most of macrophages was with pale cytoplasm. In addition, in lumina of alveoli caseous masses and tissue debris were observed.

The 2nd group (mono-infection TB) is characterized by the presence of pulmonary epithelioid cell granulomas type with giant multinuclear cells of Pirogov and Langhans, a significant number of small lymphocytes and different size foci of caseous necrosis. In group 1 productive inflammation, represented by foci of lymphohistiocytic infiltration, more localized in perivascular areas were detected. Foci of mild fibrosis and productive inflammation in subpleural departments with minor histiocytic infiltration and areas of emerging pneumoniogeic cavernas with different severity of inflammation were noted.

Outside areas of inflammatory infiltrate severe cytoplasmic expression (3-4 points) of immunoreactive material (IRM) in lymphocytes, alveolar macrophages, granulocytes, epithelial and endothelial cells were noted with using monoclonal antibodies to Bcl-2 in 1st group (HIV/TB). In areas of specific inflammation severe by mild (1 point) expression of the IRM was found.

In the 1st study group (HIV/TB) outside areas of inflammatory infiltrate moderate and severe (2-3 points) cytoplasmic expression of IRM was noted with using monoclonal antibody to Bax. In the same group in foci of specific inflammation severe and pronounced expression of the IRM (3-4 points) was detected.

In 2^{nd} group with using monoclonal antibodies to Bcl-2 (mono-infection TB) outside areas of inflammation in most cases mild and moderate (1-2 points) cytoplasmic expression of the IRM was found and weak (0-1 point) expression - in granulomatous infiltrates. With using monoclonal antibody to Bax moderate and severe (2-3 points) expression of the IRM was observed outside to specific areas of inflammation, and in areas of caseous-pneumonic lesions mild (1 point) expression of IRM was observed.

There were significant differences in morphometric parameters of the lung with using monoclonal antibodies to Bcl-2: outside areas of inflammation in 1^{st} group relative area of immunopositive objects was in 2 times higher ($p<0.001$) compared with the comparison group (2^{nd} group), and in foci of inflammation, this parameter increased in 5-fold ($p<0.001$) compared to mono-infection TB (Table 1, 2).

There is increase of the relative area of immunopositive objects in lung with using monoclonal antibody to Bax in foci of inflammation in 4 times ($p<0.001$) in the 1st group (co-infection HIV/TB), but at the same time there is reduction of the average area of immunopositive objects almost 3 times ($p<0.001$).

Thus, we identified increased levels of BC1-2 in different zones of the lung in co-infection HIV/TB with maximum expression in foci of inflammation, as well as increase the level of expression of Bax in caseous necrotic foci. Different protein expression Bcl-2 and Bax in mono-infection TB and co-infection HIV/TB, perhaps indicates a change in regulatory

mechanisms of cell death in persistence of co-infection, which leads to greater initiation of apoptosis and is most pronounced in areas of productive inflammation.

The marked increase Bax expression in macrophages, containing nuclei and dying epithelioid cells in foci of caseous necrosis is considered as a factor contributing to the expansion of tissue destruction. Alveolar macrophages are referred as fastly dying monocytic in origin cells, and with the absence of they activation by T lymphocytes, the number of which is reduced in later stages of HIV infection, their apoptosis occurs earlier [4,5,6], which possibly involves immature macrophages in the formation of caseous necrosis. This hypothesis confirms our earlier results, showing the expansion of foci of caseous necrosis in areas of productive inflammation in HIV/TB persons with drug dependence and low CD 4(+) lymphocytes according to morphometric studies [3].

CONCLUSION

Thus, in our study we revealed the increased expression of Bcl-2 in areas of granulomatous inflammation in the lung parenchyma in co-infection HIV/TB and increasing the change in the ratio of expression of Bcl-2/Bax (coefficient of apoptosis) indicates activation in infected cells anti-apoptotic mechanisms [5].

The intensity and number of Bcl-2 and Bax-positive cells in co-infection HIV/TB has been found out. The severity of apoptosis, dependent on the balance of pro- and anti-apoptotic proteins of the Bcl-2 family determined the sensitivity of cells to pro-apoptotic stimuli, was larger in foci of productive inflammation in lungs in co-infection HIV/TB .

Table 1

Morphometric parameters in the study of Bcl-2 expression in the lungs of the dead with TB mono-infection and HIV/TB co-infection

mono-infection TB (group 2)				co-infection HIV/TB (group 1)			
Sq., mkm^2	P, mkm	Perc. sq., %	CI, RVU	Sq., mkm2	P, mkm	Perc. sq., %	CI, RVU
outside areas of inflammation							
0,71 ±0,05	1,97 ±0,09	5,4 ±0,79	101,6 [107;128]	0,49 ±0,07	1,56 ±0,1	11,45 ±0,55	89,55 [79;105,5]
in focus of inflammation							
0,42±0,14	1,78±0,41	0,26±0, 01	100 [52;125]	1,06±0,35	2,0±0,37	1,29±0,07	106,8 [60;128]

Table 2

Morphometric parameters in the study of Bax expression in the lungs of the dead with the mono-infection TB and co-infection HIV/TB

Mono-infection TB (Group 2)				Co-infection HIV/TB (Group 1)			
Sq., mkm^2	P, mkm	Perc. sq., %	CI, RVU	Sq., mkm2	P, mkm	Perc. sq., %	CI, RVU
outside areas of inflammation							
0,56±0,05	1,99±0,11	11,5±0,76	141 [104;152]	0,6±0,05	2,24±0,16	11,5±0,7	86,8 [88;109]
in focus of inflammation							
0,64±0,05	2,4±0,14	3,18±0,13	133,4 [97;151]	0,26±0,02	1,27±0,04	12,1±0,9	152 [196;133]

S – average area of immunopositive areas mkm^2

P – average perimeter of immunopositive areas mkm,

Perc. sq. - Percent square of immunopositive areas, %,

RVU – relative value units

CI, – color intensity

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Authors:

Bykhalov Leonid Sergeevich - assistant to the department of pathological anatomy of the Volgograd state medical university, cand. med. sciences, doctoral candidate
Address: Volgograd, square of Fallen Strugglers, 1, 400131 leonby-vgd@yandex.ru <mailto:leonby-vgd@yandex.ru>

Smirnov Alexey Vladimirovich - head of the pathological anatomy of the Volgograd state medical university, doct. med. sciences, professor
Address: Volgograd, square of Fallen Strugglers, 1, 400131 alexey-smirnov@rambler.ru <mailto:alexey-smirnov@rambler.ru>

Makarov I.Yu., Zhukova E.N., Zhdanova R.A.

Pathomorphology of Regional Intrachest Lymph Nodes at Destructive Tuberculosis of Lungs with Account of Drug Resistance of Micobacteries

ABSTRACT

We studied morphological changes in the regional intra chest lymph nodes with drug-resistant fibro-cavernous pulmonary tuberculosis. Macroscopically all groups of lymph nodes were increased in sizes, indurated, with small foci of caseous necrosis. At the microscopic research of regional intra chest lymph nodes on the background of hyperplasia there was sharp narrowing and devastation of a T-zone with atrophy of a significant amount of lymphoid follicles. Thus, in the group with multidrug resistance the width of the paracortical zone is twice less than in the groups with monoresistance and remained constant drug resistance. Its bound with the cortical zone wasn't determined. The higher rate of pathomorphological changes were observed in the groups with polyresistance and multidrug resistance.

Keywords: tuberculosis, pathomorphology, drug resistant, lymph nodes.

INTRODUCTION

Annually more than 3 million people (6% of all causes of death) die of different forms of tuberculosis. In developing countries the fatal cases connected with tuberculosis make 25% of number of lethal outcomes which can be considered threatening. The special problem is drug-resistant pulmonary tuberculosis. According to World Health Organization, among patients with initial diagnosis 9,9% of mycobacterium strains of are steady at least against one drug, and resistant to tuberculosis mycobacterium, at least, to Isoniazid and Rifampicin is considered as multidrug resistant (MDR) [1,2,5].

The fact of existence of multidrug resistance of mycobacterium is the basis for preservation of epidemic intensity of tuberculosis, both in Russia, and in the Amur region in particular. There is rowth of incidence and mortality at tuberculosis with tendency to stabilization of the situation in the last years, higher increasing rate of acute progressive and generalized forms of the disease as well as high frequency (10-20% and more) polyresistant forms of tuberculosis. Social disadaptation of a certain part of the population (alcoholism, drug addiction, vagrancy) and high level of migration create a new situation in which the structure of mortality at tuberculosis changes towards prevalence the acute progressive and not revealed during lifetime of forms of a disease [3].

Strains of mycobacterium with MDR, cause tubercular process to be treated hardly, as the result of it severe chronic forms of the disease occur, not reacting to medicinal therapy [3,4,6]. Such process usually is widespread, is followed by severe destructive changes and has the progressing character with involvement in specific process of intra chest lymph nodes with frequent lymphohematogenous dissemination.

The aim of the research is to study pathomorphological changes in intra chest lymph nodes on THE autopsy material affected by medicinal and steady tuberculosis depending on a type of drug-resistance.

MATERIALS AND METHODS

The autopsy material covers 105 patients who died with drug-resistant fibro-cavernous pulmonary tuberculosis of Blagoveshchensk. During lifetime patients received standard schemes of treatment with use of the main antitubercular preparations (ATP) (an Isoniazid, Rifampicin, Pyrazinamid, Etambytol and Streptomycin), at development of the secondary drug-resistant (DR) of a combination of the main and reserve ATP.

All dead were divided into groups depending on type of drug-resistant of the activator (monoresistance - 15, polyresistance - 25 and multiresistance – multidrug resistant - 49). On age sexual structure of group were representative (middle age 45,86±11,73). Men aged from 25 till 70 years made 89,4%.

The comparison group included 16 patients died of pulmonary tuberculosis with drug-resistance.

As objects of the research regional intra chest lymph nodes were applied.

Slices were fixed in 10% formalin and filled in paraffin by a standard technique. The morphometric analysis is made on histologic cuts 5 microns thick. Cuts were painted with hematoxylin and eosin.

At macroscopic research the prevalence of defeat the regional intra chest lymph nodes was revealed by the specific process. Morphometric research was conducted by means of the research trinocular microscope with photonozzle and the automated digital system with software for the digital microscopy of VisionMorpho.

At microscopic research we determined a diameter of follicles, width of T-zones, quantity of lymphocytes of T - and B-zones on 1000 mcm2, with statistical processing of the obtained data with use of the MicrosoftExcel and "Statistika 6.0" programs.

RESULTS AND DISCUSSION

The obtained data testify to the fast, progressing course of destructive tubercular process in lungs, being followed thus by the expressed pathomorphological changes in lymph nodes.

Macroscopically all groups of lymph nodes were increased in sizes, dense, on a section with the small centers of a caseous necrosis. These pathomorphological changes are most expressed were observed in groups with polyresistance and multiple medicinal stability. The sharp atrophy of lymphoid follicles, the remained follicles small, with sites of a lipomatose is microscopically noted.

Average diameter of follicles in these groups it is reliable less, than in group of comparison and group with monoresistance (Pic. 1).

In most cases at patients with poly-and multiresistance of mycobacterium the paracortical zone is considerably narrowed. In all groups of patients with tuberculosis its average width reliable is less than in control group. The tendency to its reduction from group of comparison to group with mono - poly-and multiresistance is noted. Its border often doesn't decide on a cortical zone (Pic. 2).

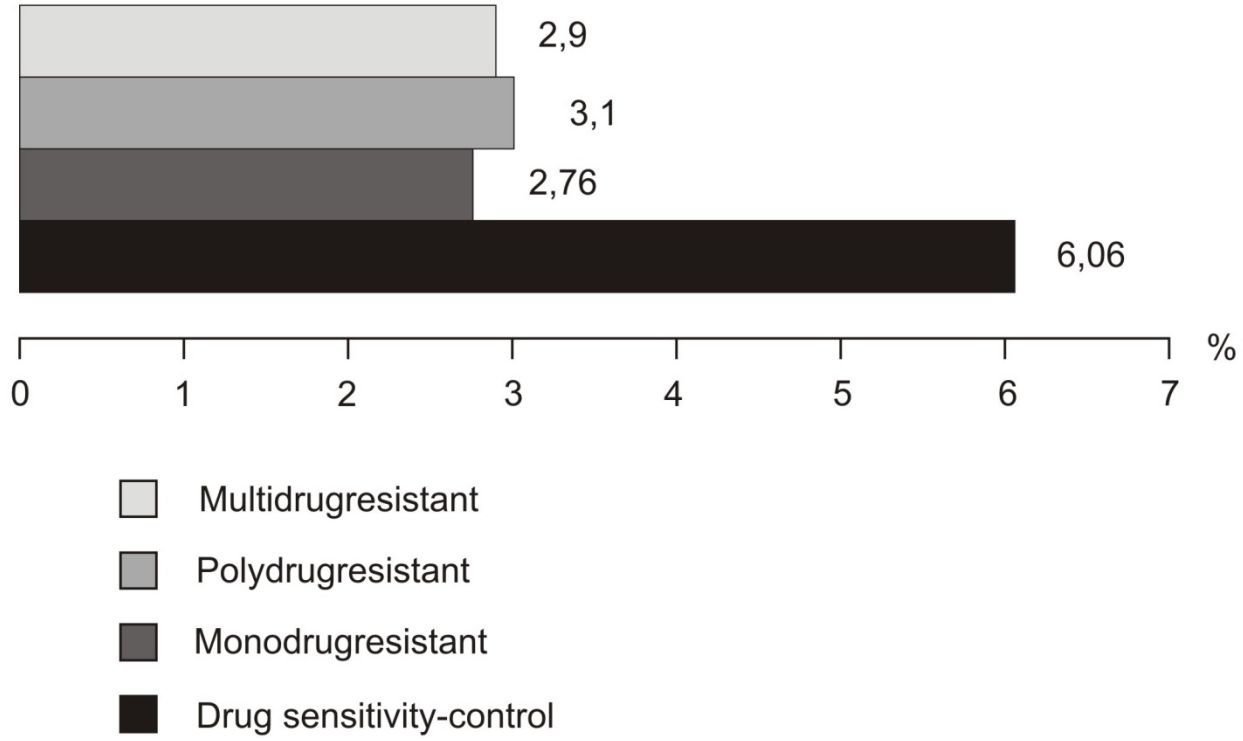
There is devastation of T-zones to decrease in quantity of lymphocytes. So patients with MDR have an average quantity of lymphocytes on the area of 1000 mkm2sostavlyaet 12,83±1,16, and in control group 21,13±1,83 ($P < 0,05$). It is resulted by an enlightenment of a paracortical zone with an exposure the reticular and the connective tissue of elements, with development of a plasmatisation (Pic. 3,4). In 65,3% of cases small epithelium-cellular granulomas with huge cells of Pirogov - Langkhans are found.

CONCLUSIONS:

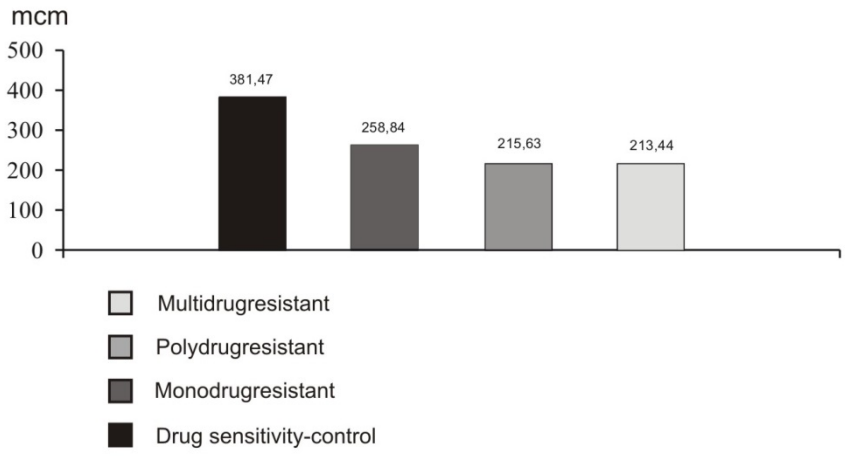
Thus we established that the patients with progressive fibro-cavernous pulmonary tuberculosis in the regional lymph nodes have acute devastation of T-zones with plasmatisation of the cortical substance and persistent responsive centers of follicles testifying to suppression of cellular immunity. The acute atrophy of primary follicles is noted as well as disappearance of secondary lymphoid follicles.

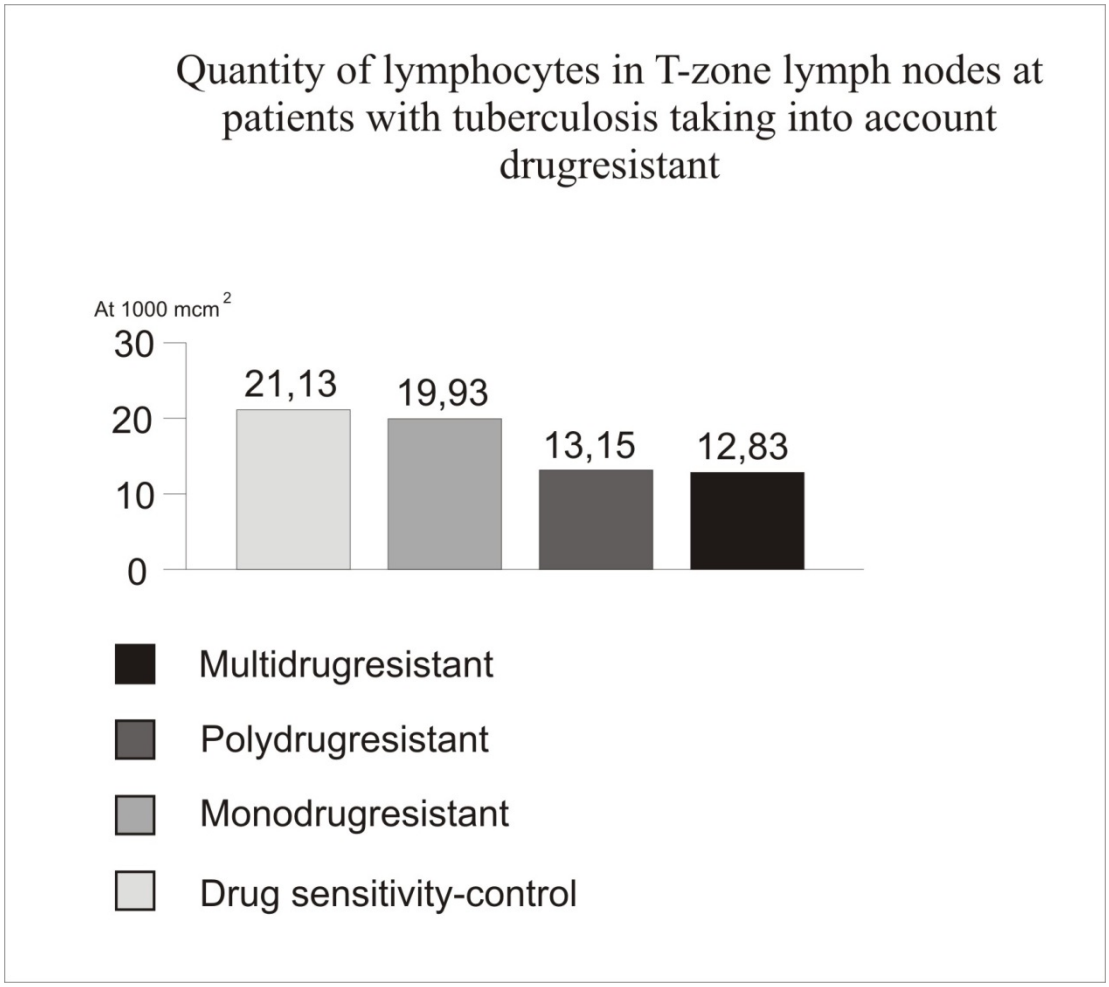
These changes are most expressed among the patients died from progressive fibro-cavernous pulmonary tuberculosis being affected by tuberculosis micobacteria with polyresistance and multidrugresistance during the lifetime .

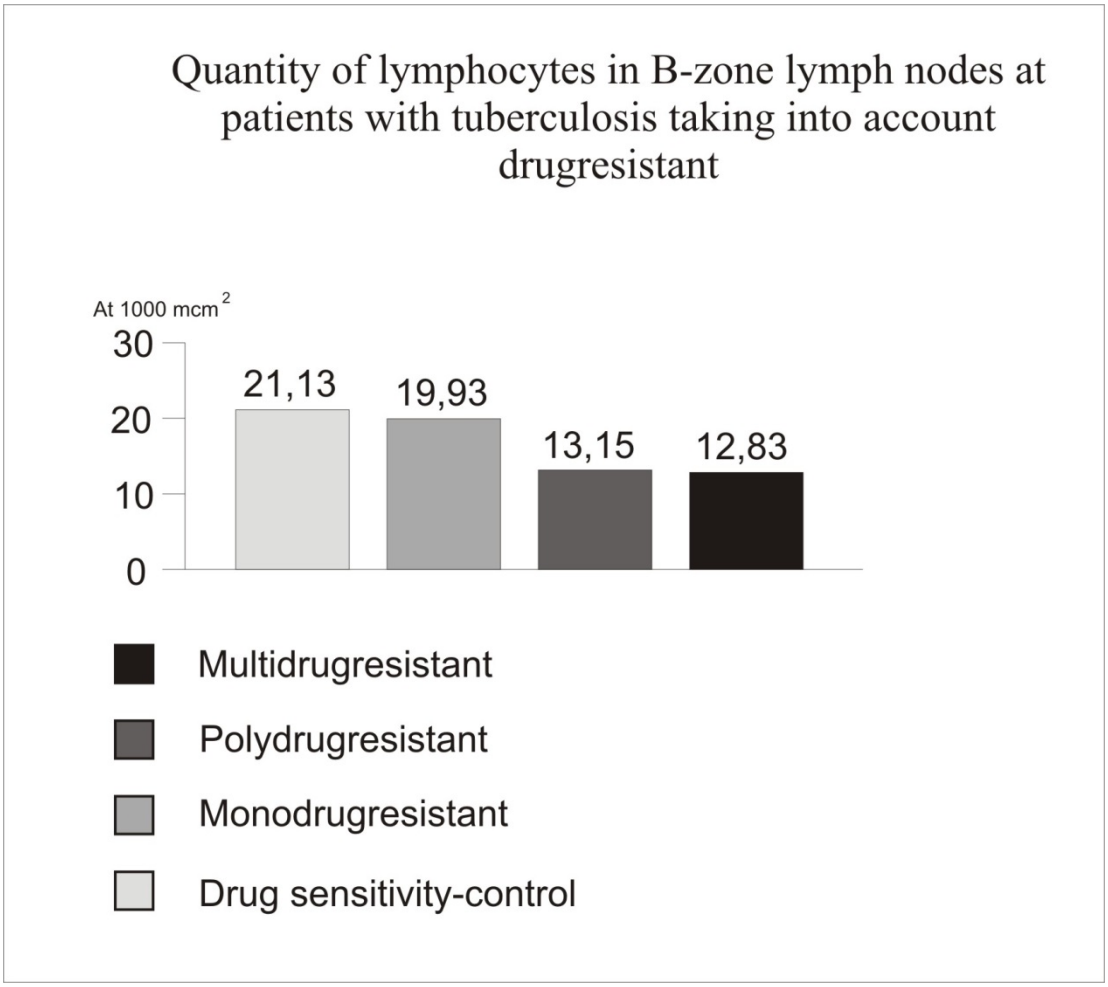
The relative volume of follicles in lymph nodes at patients with tuberculosis taking into account drugresistant



Average width of a paracortical T-zone in lymph nodes at patients with tuberculosis taking into account drugresistant




Quantity of lymphocytes in T-zone lymph nodes at patients with tuberculosis taking into account drugresistant
At 1000 mcm 2
30
20
10
0
21,13
19,93
13,15
12,83
Multidrugresistant
Polydrugresistant
Monodrugresistant
Drug sensitivity-control



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Authors:

Makarov I.Y. – doctor of med. sciences, professor , Amur State Medical Academy, Blagoveshchensk, 95, Gorkogo Str., Blagoveshchensk, 675000, Russian Federation. mail:prorektoragma@mail.ru

Zhukova E. N. - Amur State Medical Academy, Blagoveshchensk, 103, Go'kogo Str., Blagoveshchensk, 675000, Russian Federation. mail:zhukova1201@rambler.ru

Zhdanova R.A. Amur State Medical Academy, Blagoveshchensk, 103, Gor"kogo Str., Blagoveshchensk, 675000, Russian Federation. E-mail:regzhd@mail.ru

Tikhaya I.A., Gorbach T.V., Pliten O.N.

Consequences of Cyanobacterial Toxicity and Their Residues in an Organism

ABSTRACT

As a purpose there has been the study of water content with a «flowering» phenomenon, falling into the centralized water system from surface reservoirs and its effect on FAS-ligand content and anticardiolipin antibodies in blood of parturient women and newborns' cord blood from two cities

of the Kharkov region Bogodukhov (the artesian water-supply) and Chuguev (the water intake from Seversky Donets), they being characterized by prolonged «flowering» of the surface. We have found some facts indicating the negative effect of cyanobacteria and their metabolites contained in drinking water and organism as well.

Keywords: water; cyanobacteria; FAS-ligand; anticardiolipin antibodies; parturient woman; newborn.

INTRODUCTION

Reservoirs of Ukraine are mainly polluted by nitrogen compounds, petroleum products, heavy metals. Nowadays the leading suppliers of nitric compounds are dark blue-green algae, or cyanobacterias which are more than 2000 kinds. Thus ecologists notice that the reservoirs where these algae reproduce themselves, other ecological factors are not so important [1, 2, 4, 5].

In Ukraine in many settlements water intake is carried out from the open reservoirs with the phenomena of «water-flowering», that is numerous of reproducing themselves cyanobacterias. The degree influence of the consumption of running water having residues of cyanobacterias on human's health, including parturients has been estimated according to recommendations of Scientific Research Institute of problems of ecology (Kharkov). For this purpose the groups of habitants of two cities of the Kharkov area have been studied – Bogodukhov (artesian water-supply) and Chuguev (water intake from Seversky Donets, where

the long-time «flowering» of water is observed).

Comparative analysis of the data from the histories of births of obstetrician departments of Bogodukhov and Chuguev central district hospitals for the period 2000–2004 years have been conducted. Anaemia of pregnant, diseases of thyroid, frequent spontaneous abortions and premature births, the anomalies during delivery are more frequent, higher index of perinatal mortality are more frequently diagnosed in Chugueve, relative by big number of children with mass of body less than 1999 g are registrated in Chuguev in comparison with Bogodukhov, and number of newborns with body mass more than 3500 g is less in Chuguev. The detailed analysis of peculiarities of pregnancy duration and results of labors in central district hospitals of these two towns for some years is published [8].

Clinical and experimental researches have established, which have been conducted by us before, that levels of some hormones (estradiol, estriol, progesterone, prolactin) in blood of parturients are reliably substantially decreased in women, residents of the settlement with a water-supply from the open reservoir (Chuguev) [7], and also the increase of levels of urea, kreatinine, aminotransferases are observed in this group, which testify the activation of catabolism of proteins, and intensification of the processes of lipids and proteins oxidization against weakening of antioxidant processes activity [6], that indicates the development of oxidizing stress, which is possible as the result of chronic action of cyanobacterias and their metabolites, entering organism with water.

The purpose of the research was the study of influence of consumption of water, entering centralized water system from the open reservoirs with the phenomena of «flowering». Content of FAS-ligand and anticardiolipin antibodies in blood of parturients and umbilical cord blood of newborns has been studied.

MATERIALS AND METHODS

Venous blood of woman in childbirths was taken for research coming for delivery in 34 cases in Bogodukhov central district hospital and in 29 cases in Chuguev central district hospital. Umbilical cord blood in the same cases was taken at the cut of umbilical cord.

For the exception of negative influence of chlorinates water product on an organism,

including biochemical indexes of blood, in Chuguev water used for preparation of food was settled.

Content of FAS-ligand and anticardiolipin antibodies determined in blood by immunological method. Statistical information is processed by the methods of variation and cross-correlation analyses.

RESULTS AND DISCUSSION

Possible reason of previously found by us the increase of proteins catabolism is activation of apoptosis in connection with the toxic loading. For the estimation of level of apoptosis we studied content of FAS-ligand in blood of woman in childbirths. FAS-ligand or CD95-L is a membranous protein, known as a «factor of death», contacts with a FAS-receptor and induces cell death [3].

The sharp increase of level of FAS-ligand content is established in blood of Chuguev habitants (6,97±0,47 pg/ml, $p < 0,001$) as compared to the level of physiological norm (0,89±0,06 pg/ml) and the level of Bogodukhov habitants (0,95±0,04 pg/ml), that is one of integral indexes, testifying the state of chronic intoxication, that means sevenfold increase content in blood of apoptosis marker.

Anticardiolipin antibodies are antibodies to the cellular membranes phospholipids. A certain level of autoantibodies to cardiolipin is present in blood of healthy people, but at its increase develops qualitatively new condition in hemostasis system. These bodies co-operate with the phospholipids of thrombocytes membranes and endothelial cells of vessels, causing their destruction and assisting in thromboses and thromboembolism development.

Growth of concentration of antibodies is sensitive and specific laboratory test, characterizing the risk of thromboembolic complications origin. Death of fetus, abortion, placental separation, oligotrophy and fetus hypoxia at pregnancy due to the thromboembolic damages of trophoblast and placentas are possible [3].

The study of content of anticardiolipin antibodies in blood serum of parturients showed, that their content in woman in childbirths from Bogodukhov corresponds a physiological norm (index of reaction 0,64±0,03 at a norm up to 1,0), in parturients from Chuguev was noted considerable increase of antibodies concentration (1,69±0,11). Increased level of anticardiolipin antibodies for Chuguev habitants can be explained by impossibility of rapid and complete utilization of forming wreckages of the own died in the result of apoptosis cells due to, at first, sharp apoptosis activation and, secondary, weakening of phagocytic possibilities in organism. There is start of autoimmune reactions against died cells proteins. It should be noted that antibodies are absent in parturients from Bogodukhov in 45% cases. However antibodies are present in 100% cases in woman in childbirths from Chuguev, in a number of cases index of reaction is higher than 2,0. It is characteristic, that content of antibodies is higher in those parturients which have the higher FAS-ligand level. It is evident, that antibodies formation is not only to cardiolipin but also to the proteins of many other organs, including kidneys, liver, epithelium of mucous membrane of gastrointestinal tract etc.

Moreover, it is established that FAS-ligand content in umbilical cord blood newborns from Bogodukhov corresponds to physiological norm (1,34±0,09 pg/ml, in a control group – 1,29±0,11), antibodies to cardiolipin are absent in 100% of cases. FAS-ligand content is increased in umbilical cord blood of newborns from Chuguev (2,01±0,14 pg/ml), in 30% of cases antibodies to cardiolipin are found (reaction index is 1,48±0,07). A characteristic feature is that antibodies are present in umbilical cord blood in the cases when and the highest indices of antibodies content are in maternal blood.

COCNLUSIONS

The hemoanalysis of woman in childbirths using different drinking-water showed that increasing of toxic compounds content in an organism, oxidizing stress results to multifold apoptosis intensification found by us in woman in childbirths from Chuguev. It is possibly, that apoptosis activation is associated with the direct action of bioactive substances of algae, because it is found in experimental researches, that they have expressed cytotoxic action.

Oxidizing stress causes tension in immune system state, that, in the end, results in autoimmune processes development (significant increase of antibodies content to cardiolipin has been revealed in our researches).

The levels of content of the studied indexes in umbilical cord blood showed, that toxic action of bioactive substances of algae on a fetus is less expressed, than on the mother’s organism, that is related with protective role of placenta. The barrier function of placenta reduces the «level» of damaging factor, however, metabolic violations in an organism of newborn take place, that can become reason of

functional violations in future.

Thus, the data indicating negative influence on an organism of cyanobacterias and them metabolites contained in drinking-water have been found.

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Authors:

Tikhaya Inna Anatolyevna – candidate of medical sciences, docent, associate professor of Department of Obstetrics and Gynaecology №1, Kharkov Medical Academy of Postgraduate Education, Kharkov, Ukraine, E-mail: pliten_oksana@mail.ru*;* **Gorbach Tatyana Viktorovna –** candidate of medical sciences, docent, associate professor of Biological Chemistry Department , Kharkov National Medical University, Kharkov, Ukraine, E-mail: pliten_oksana@mail.ru*;* **Pliten Oksana Nikolaevna –** candidate of medical sciences, associate professor of Pathological Anatomy Department, Kharkov National Medical University, Kharkov, Ukraine, E-mail: pliten_oksana@mail.ru

Mustafin T.I., Kudoyarov R.R.

Ways of Optimization of Postmortem Studies of Nosocomial Pneumonia at Patients with Cerebral Stroke

ABSTRACT

Ways of optimization of the pathological research in nosocomial pneumonia at patients with brain strokes are presented in this article. In many respects, they are concerned to the development and practical application of new approaches in research methodology of lung tissue in a low-impact autopsy. The improvement of the low-impact technology autopsy, the widespread use of different methods of coloring histological sections, the use of bacteriological, bacterioscopic research methods of lung tissue and cerebrospinal fluid are considered of great importance. If there is a mobile X-ray installation in the postmortem department the plan radiography of the chest is supposed. For a more detailed study of the ventricular system, vascular network, location and size of the pathological process in the body for justified reasons the brain CT is carried out. The development of the mortem examination at nosocomial pneumonia caused by cerebral strokes is associated with lifetime analysis of clinical, laboratory, functional, instrumental data correlated to the autopsy results.

Keywords: nosocomial pneumonia, cerebrovascular disease, stroke, optimization mortem examination, new methods, low-impact autopsy.

At present mortality rate from pneumonia in Russia corresponds to 5% of a community-acquired form and 20% in hospitals (A.G. Chuchalin et al., 2010). Pneumonia in our country is found in 3.86 cases per 1,000 people [1,2,3,4], and each year at 2 million patients are registered [5]. There is undeniable tendency to increase the number of patients with severe disease and increased mortality [6,10]. Also there is steady increase in the incidence of nosocomial pneumonia complicating cerebrovascular disease [7,8,9]. The average annual incidence of cerebrovascular disease was 2.1 per 1,000 population, and the death rate from stroke - 0,62-1,28 per 1000 inhabitants per year [9]. In this case, as noted by some authors on the acute period accounted for 35% of mortality, which to the end increase by 12-15% [9].

The aim: To identify ways to optimize postmortem study at nosocomial pneumonia in patients with stroke.

A thorough study of medical records is preliminary to a section of organs. In this connection, a chief doctor confirms of performing the autopsy with use of low-impact technology. Clarification of

timeliness, accuracy and nosological principle of detection and design of a final clinical diagnosis are of great importance. The final clinical diagnosis is rubricated into three categories. A lot of information can be obtained by reading diary observations, stage epicrisis, consultant's conclusion, council of physicians , post-mortem epicrisis. Correspondence of appropriate lifetime research methods with those in the standard list on this nosology is revealed. Finally, objective and subjective causes of those or other deviations from the standards of diagnosis and treatment of internal diseases (B.I. Shumutko, S. V. Makarenko, 2009) are determined as well as the order of the Ministry of Health of the Russian Federation dated August 9, 2006 № 596 "On approval of the standard medical care of patients with subarachnoid hemorrhage "and other documents are studied. In this context, studying the results of computed tomography of the brain, chest X-ray, bacteriological sputum cultures and tracheobronchial aspirates in dynamics can be considered as necessary procedures. In the diagnosis of pneumonia it is important to consider laboratory parameters, functional and other studies.

Postmortem examination of a corpse with nosocomial pneumonia is carried out on a definite plan, which is reflected in the literature (G.G. Avtandilov "Fundamentals of pathology practices", second edition, Moscow 1998). To identify etiology of the disease during lifetime and dissecting table a biological material for subsequent implementation and bacterioscopic bacteriological research is extracted. A prosector pays special attention to a state of the tracheobronchial tree, pathological changes in a body, fluid presence in the pleural cavity, measurs digital indicators. Usually with lung tissue four pieces of the biological material are extracted, and two from visceral pleura, two - from organ root . The plan includes the study of microbial identification number in 1g of tissue or in 1ml of fluid. The bacteriological research involves the identification of microorganisms that occur in monoculture or in association with sensitivity to antibiotics and bacteriophages. In other equal conditions the data of bacteriological studies and bacterioscopic promote accurate and high-quality clinical and pathology assessment cases in which the final comparison of clinical and postmortem diagnoses is conducted and assessment of the quality of rendering help is carried out. Typically, histologic sections were studied at the light-optical device. Often the color of micropreparations reduced to a limited number of ways. In this case, histological sections were stained with hematoxylin-eosin, picro fuchsin of Van Gieson, Weigert for identifying microbial colonies - Romanovsky-Giemsa, May-Grunwald to modify M.S. Tverdynin, rarely others. The morphological study of the body is carried out taking into account the long-term presence of the subject under mechanical ventilation. The first evidence of direct relationship of disease severity, the prevalence of pathological process in the body of the length of stay under mechanical ventilation. With the accumulation of the test material, probably will be obtained data representing the scientific and practical interest.

During recovery incidence of respiratory viral infections, it is important to remember about the likelihood of viral and bacterial pneumonia with stroke. Along with bacterial swab test and bacterioscopy of biological objects polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA)are conducted, which facilitate the search for etiological factors of pneumonia.

Federal Law of 21.11.2011 №323-FZ (67 articles) provides for compliance with decent relationship to the body of the deceased person and the maximum preservation of its anatomical shape. In accordance with the Federal Law of the Russian Federation, in the order of the Ministry of Health of the Russian Federation from June 6, 2013 № 354n "On the procedure of autopsy" also emphasizes the need to care with respect to the body of the deceased person. In this context, it is important to improve in nearer future a low-impact autopsy technology in pulmonary pathology without compromising quality of mortem examination.

At present, we performed a patent search aimed to develop a low-impact way to autopsy in diseases of the respiratory, digestive, genitourinary system.

At the same time, we should recognize the need to improve methods of investigation of lung tissue with pneumonia, especially in low-impact autopsy. Particular attention is paid to the study of the body at the level of the segmental bronchus. In these circumstances, it is unlikely escape from view pathologist even small foci of pathological changes in the body. The comparative characteristics of morphometric parameters of organs is to be implemented for further development of the postmortem examination. In the study of lung tissue, including a segmental level special scissors are used. Valuable information can be obtained by a careful analysis of the results of postmortem radiograph of the chest. In a case of brain stroke the postmortem computed tomography of the skull, and later of the brain significantly expands our understanding of the disease tanatogenesis (T.N. Mills, J.A. Medvedev, N.A. Ananiev, A.V. Suhatskaya, Y.M. Zabrovskaya, A.O. Kaznacheeva, 2008). Before sectioning the lumbar puncture is performed to obtain liquor. Its morphological and bacteriological examination will also help to solve the key problems of improving diagnostic pathology of nosocomial pneumonia with stroke. Improving mortem examination at nosocomial pneumonia caused by cerebral strokes is associated with lifetime analysis of clinical, laboratory, functional, instrumental data in correlation with the results of the autopsy.

CONCLUSIONS:

Thus, the optimization of post-mortem examinations at nosocomial pneumonia in patients with brain stroke is largely connected with the development and introduction of new approaches in research

methodology of lung tissue in a low-impact autopsy technology. Improving the low-impact autopsy technology, autopsy techniques lungs, the widespread use of different methods of coloring histological sections body, optimization of bacteriological and bacterioscopic study are considered to be main components of the postmortem examination with pneumonia in patients with stroke. In terms the mortem examination should include morphological and bacteriological examination of cerebrospinal fluid. In turn posthumous chest X-ray, and possible brain computed tomography allow to reach a new level of research at nosocomial pneumonia in patients with stroke. Consequently, in nosocomial pneumonia caused by cerebral stroke the mortem examination is related to the lifetime analysis of clinical, laboratory, functional, instrumental data in the clear linkages with the autopsy results.

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Authors:

Mustafin Tagir Islamovich, doctor of medical Sciences, Professor, head of Department of pathological anatomy of the Bashkir State Medical University, Republic of Bashkortostan, city of Ufa, Lenina street 3.

Kudoyarov Rustem Ravilevich, assistant Professor of pathological anatomy of the Bashkir State Medical University, Republic of Bashkortostan, city of Ufa, Lenina street 3. Phone 89373328848. e-mail: xirurg19@yandex.ru

Zhurayeva G. B., Turdiyev M.R., Sokhibova Z.R.

Activity of pathomorphologic changes in intestinal lymphoid tissue caused by sharp intestinal infections

ABSTRACT

Results of morphometric researches showed that babies died from acute intestinal infections had initially the grown number of stromal cells in lymphoid tissue of both iliac and large intestines of reticular and macrofagal origin with development of destructive changes in them. Paralell with these changes increase of the number of average lymphocytes is noted as the reaction of the immune system of intestines to an infection of active average lymphocytes. In addition to the changes above mentioned, during the subsequent age periods of babies there is growth in the quantity of small lymphocytes in composition of lymphoid follicles, which increases on average in 10-15 times as compared to the norm.

Keywords: acute intestine infection, iliac and large intestines, lymphoid tissue

Prevalence of acute intestinal infections at children's age and high rates of mortality have caused to consider them as one of actual problems. At newborns and children of chest age with unsuccessful premorbid background, such as prematurity, hypotrophy, rickets, anemia and congenital malformations, due to activation of opportunistic microorganisms, there is predisposition to development of acute intestinal infections (AII). At artificial and mixed feeding of children the pathogenic micro florae often causes dysentery, salmonellosis and staphylococcal enterocolit, and others of acute intestine infection (AII) [1,2,3].

The aim of the research. Identification of morphological criteria, morphometric parameters of mucous membrane and lymphoid tissue of intestines of nursing infants, as well as pathomorphological changes which developed as a result of acute intestinal infections.

MATERIALS AND METHODS

The basis of this research was formed by data of autopsies which are carried out at nursing infants who died of different types of acute intestinal infections during five years – from 2003 to 2007 in the Bukhara regional patholologic bureau and the Republican pathologic center. When calculating weight of children, ratio of physical development the

criteria of the European bureau of the World Health Organization (WHO) (2000) were applied, presented in the methodical proceedings "Feeding of newborns and nursing infants". For the purpose of simplification of the calculation of a level of constant weight increase of children in mathematical formulas and in numbers, the below-brought coefficient of physical development – Q = real increase in weight / ideal increase in weight has been established. The criterion of weight of a child includes a condition of development of all internals, including a condition of the proceeding development of bodies of the digestive tract. In our materials, lag of development of digestive tract organs was noted in 84,6% of cases, as even in the period of nonutility a child could die due to any sharp intestinal infections. In all our cases, autopsy at children was carried out in 0,5-1 hour after death. At first 5,0-10,0 ml of blood was taken from heart and vascular system, plasma was emitted from it in the centrifuge, immunological researches were conducted with the help of a radial immune diffusive method of Mancini and with the use of nonspecific serum (The Moscow NIIVS to them I.I. Mechnikova). Immunoglobulin E is defined by radio immune methods. The number of T - and V-lymphocytes is counted on sensitivity to theophylline. For bacteriological researches the distal part of small intestine, and also the part of large intestine were used. When dissecting children in the course of the research the small intestine was excreted completely and the same length of one part of the large intestine, the excreted interiors were packed in a thin paper, rolled up by a parcel, fixed for 3 days in 10 percentage solution of neutral formalin. Each parcel including both bowels of intestines was dehydrated in alcohol, in chloroform and was filled in paraffin. Then received histological cuts of 5-8 microns thick were painted with hematoxylin-eozine according to Van-Gizona and CHIC reaction. Histological and histochemical painted cuts were studied under a microscope; informative sites of the cut were photographed. For the purpose of specification of quantitative indices of lymphoid tissue of intestines in walls of thin and thick intestines, morphometric researches were conducted by the method of Avtandilov G.G. with calculation of quantity of lymphoid follicles and their cellular structure. The calculation was carried out under the microscope lens 40 with imposing on lymphoid follicles of intestines of 10 times Avtandilov's grid, consisting of 100 points, only 1000 points. The reticular of cells, lymphoblast, large, average and small lymphocytes, and also the degenerating cells and macrophages are counted as wee as the quantity in lymphoid follicles and their relative percent. Quantitative indices were processed by statistical methods, size arithmetic averages, square errors of averages and indicators of reliability were calculated.

RESULTS AND DISCUSSION

Results of the microscopic researches showed, at autopsies of children with pnevmopatiya and craniocereberal injuries the mucous membrane of intestines had a uniform hystotophografic structure, but their thickness and length are various. It is revealed that in the first half of one-year-old life of newborns the mucous membrane of both thin and thick gut isn't completely undeformed. Thus mucous fibers are short, of different form and size, the quantity of scaphoid cells isn't enough and they in the process of vacuolization. Especially, hypoplasia is noted in its connecting tissue mucous membrane, but not in its lymphoid cells, there are not enough vessels, and connecting tissue fibers are located randomly. Such rare lymphoid congestions consist of small and average size of lymphocytes. In Strom, being in their basis, seldom reticular cells are also randomly scattered. At the edges of lymphoid cellular congestions, post-capillary venial in which wall migration of lymphocytes is observed. At this appearing lymphoid follicles the mucous membrane is equal without tissues. Lymphoid follicles are located in a sub mucous layer. Newborns at the age of 7-12 months in the iliac gut wall have normal morphofunctional conditions of mucous membrane and lymphoid tissues. The tissues are extended, some are branched out, in its membrane the quantity of lymphoid cells is increased. Integumentary cylindrical epithelium is equal, nearly of one form and size among them there are enough scaphoid cells. Together with it, in this age period lymphoid follicles are located lonely and at distance from each other. In some of them in the center there are germinate centers and they turn on secondary lymphoid follicles. At this age in the wall of the small intestine lymphoid follicles are observed which are located in proximity of a muscular layer. They are rather smaller in size, their reticular cells are located randomly, the lymphocytes are on the periphery of the follicle infiltration in surrounding tissues. In the thick gut wall in this time of the research there are enough primary and secondary lymphoid follicles. If the primary lymphoid follicles are located in its connecting tissue membrane, the secondary have places in a submucous layer of the gut wall. The basis of secondary follicles is wide, the top is truncated and ridges on a surface of the mucous membrane. In which there are centers of reproduction and they consist of reticular cells and lymphoblast. Thus, babies during the first 6 months, had lymphoid follicles of different size and form, distance between follicles is wide; the germinate center is defined only in 65% of cases. During the next 6 months almost all lymphoid follicles have identical cellular structure and structure. Morphometric research of the iliac gut wall showed that the quantity of lymphoid follicles changes depending on age. It means that newborns have on average 34,8±5,2%, 1-3 months - 38,5±6,3%, 4-6 months – 75,4±9,4%, 7-12 months – 78,3±8,5%. It is revealed that in

the thick gut wall the quantity of lymphoid follicles is even less in comparison with thin: newborns have 24,7±4,2%, 1-3 months – 28,3±4,3%, 4-6 months – 45,2±6,4%, 7-12 months – 58,3±7,5%. So it is possible to judge that in the wall of both thin, and large intestine in dynamics of age till one year the quantity of lymphoid follicles gradually increases and at the end of one-year-old life their number becomes twice more than at newborns. The carried-out calculation of morphometric indicators of relative percent of the organization of cellular structure of lymphoid follicles of children aged one year showed considerable differences depending on the lived months. At newborns the most part of lymphoid follicles comprise of small lymphocytes which is 87,8±8,6% in average. The reticular cells (2,1±0,2%) as the basis of lymphoid follicles and lymphoblast (0,9±0,2%) in functionally passive state also makes 3% of the total of cells. On the square of the germinate center meets in a small amount large (1,2±0,3%) and the average size (1,2±0,3%) of lymphocytes. As a part of lymphoid follicles of the thick gut the quantity of small lymphocytes is even less 84,5±6,6%, the reticular cells of 3,1±0,2% and lymphoblast 1,2±0,3%. In the germinate center big (2,2±0,5%) and average lymphocytes (6,6±0,7%) are noted. On the periphery of lymphoid follicles destructive cells (1,8±0,4%) and fagociting macrophages (0,9±0,2%) are noted. The quantity and structure of the cells of lymphoid follicles of the small intestine of newborns testifies to the lack of antigen influence and shows a condition of rest of immune system. On morphometric indicators the organization of structure of cells of lymphoid follicles of the mucous membrane of the large intestine, sharply differs from monthly relative percent of cells at children about one year. The small lymphocytes comprise the most part of lymphoid follicles at newborns 84,5±6,6%. The reticular cells (3,1±0,2%) and lymphoblast (3,1±0,2%) comprise the stroma of lymphoid follicles which also are functionally passive, the rest cells rated at 4,3%. As a part of lymphoid follicles destructive cells (1,8±0,4%) and phagocyte macrophages (0,9±0,2%) are noted. The development of high-quality and quantitative changes in the structure of cells of lymphoid knots of the large intestine of children after six months of life is evident. According to the microscopic researches, the number of lymphoid knots sharply increases; the set of their separate congestions of big sizes is revealed. It is known that such condition of local immune system is noted in histotopographic and the morphofunctional relation, generally in an organism of nursing infants in the second half of the first year of life. In congestions of lymphoid follicles of mucous membrane of the large intestine the volume of the germinate center extends, the number the reticular cells in it increases up to 4 times, lymphoblast – twice, and large lymphocytes – by 3 times, the same is observed and in quality as well. On the germinate center small lymphocytes

form the dense ring which is thickened and condensed in its structure number of small lymphocytes, in comparison with newborns, decreases by 17%, their morph functional activity comes to light. On the surface of mucous membrane, including in a layer of integumentary epithelium infiltration the degree of small lymphocytes in symbioses and epithelium of mucous membrane is very high. Results of the clinical-anamnestic analysis of these children with AII showed that from the total (94 cases), the first age group included 19,1% of cases, the second – 27,7%, the third – 28,8%, the fourth – 24,4%. These children often had premorbid diseases: it is revealed that 28,4% of children had prematurity, at 23,6% - a hypotrophy, at 18,5% - rickets, at 21,7% - anemia and at 7,8% - congenital malformations. At children died from AII in all age groups there are low indicators of body weight, noted as at the birth as at death. Low weight indicators of a body are proved by the ratio calculation of physical development of the child which was as follows: in the first group it is 25% lower than the norm, in the second – 17%, in the third – 12% , in the fourth – 19% lower than the norms. It is established that in etiologic structure of activators of AII opportunistic microorganisms, such as koliinfection (28,4%), protease (23,7%), klebsiyell (12,6%), enterobakter (4,3%), sitrobakter (3,6%) are prevailing ones. Sometimes AII were caused by pathogenic microbes: in 3,5% of shield, 12,4% - salmonellosis, 5,6% - staphylococcus. In 7,9% of cases the etiology of AII isn't established. Studying of immunological indicators of the children who died of AII showed that at newborn IgA averaged 0,27±0,06 g/l, IgG – 5,4±0,8 g/l. At the age of 1-3 months is even lower, IgA – 0,11±0,02, IgG – 1,86±0,4 g/l. Thus in blood the quantity of a cortisol (5487,62±34,5 nmol/l) and immunoglobulin E considerably increases (143,65±12,34 ¬¿/l). In the senior age groups some increase in indicators of immunoglobulin's was noted that apparently is connected with formation of immune system of an organism. At AII IgM caused by pathogenic microorganisms indicators and IgG were high and sometimes reached IgM – 5,6, IgG – 15,8 g/l. In 7-12 months the period when artificial feeding prevailed and AII were caused by gramm negative microorganisms, there were lower indicators of immunoglobulin. At this IgG decreased to 2,81 g/l, IgM raised to 1,94 g/l. Results of the pathomorphologic of researches showed that at children of AII caused by opportunistic microorganisms were exposed by mucous desquamative, mucous and necrotic and necrotic-ulcer inflammatory enterocolit. Such severe course of the infectious process and profound pathomorphologic change of mucous membrane of the intestines were caused by premorbid diseases as prematurity, hypotrophy, anemia, rickets and congenital malformations. Thus, morphologically in the intestines there was desquamation of integumentary epithelium, necrosis and ulceration of mucous membrane, diffusion of

inflammatory infiltration of its membrane generally with mononuclear and single polynuclear cells. Studying of lymphoid tissue of the intestines showed that in connection with the infections at the died newborns lymphoid follicles is undeformed, atypically and unevenly hyperplastic. Thus the main morphofunctional zones of lymphoid follicles are not revealed, the active lymphocytes are located round the post-capillary venules. Their borders aren't defined, lymphocytes infiltrate its own cover and submucous layer diffusively, among them there is a large number of macrophages and destructive cells. In more senior age periods of babies it is noted that if lymphoid follicles of the intestines is initially atrophic and hypolastic, under the influence of AII in them there is chaotic and uneven proliferation of cells and secondary changes in a type of hemorrhage and necrosis can be noted. Thus, in one cases the diffusion proliferation of all cellular elements of lymphoid follicles is noted, in other cases the hyperplasia of the germinative center with reticulosis is defined, in the third cases their main morphofunctional zones are not revealed. Apparently, these various patomorphologic changes of mucous membrane and lymphoid follicles of the intestines depend on the initial condition of lymphoid tissue of the intestines and on pathogenicity of AII activator. Results of the morphometric research of the cellular structure of lymphoid follicles of the gut wall of the babies who died of AII showed that since early months of life quantity the reticular cells, macrophages, degenerate cells and average lymphocytes considerably increases. Such increase of the quantity of cells of lymphoid follicles proceeds in the next months. In the age period of 7-12 months, in comparison with norm, increases their quantities, on average, on 10-15 times. If to interpret these morphometric changes depending on the clinic-anamnestic data, it is possible to assume that babies in initial stages of the life, the formation of lymphoid system of intestines falls off the norm and in development of response reaction the prevailing proliferation of reticular cells and macrophages with destructive changes in some of them is noted. The morphometric indicators of lymphoid follicles of the thick gut wall at AII change in almost identical dynamics as well as in lymphoid follicles of the iliac gut wall and 10/7 parts of them include small lymphocytes, 1,6 part to average lymphocytes, out of 100 1,5 part refer to reticular cells, 1 part to macrophages. These indicators proceed to accrue also during the subsequent age periods. As a part of lymphoid follicles the following proportion of cells is noted: small lymphocytes make 10/3 parts, reticular cells of 10/1,6 parts, macrophages of 100/5, degenerate cells of 100/3 parts.

Thus, the results of morphometric studies have shown that at children who have died of AII, since the beginning in the lymphoid tissue of both iliac gut and the colon there is growth of the number of stromal cells of as reticular as macrophageal origin with the development of

destructive changes. Simultaneously with these changes the higher number of lymphocytes in the medium is observed as an indicator in the intestinal immune response on the infection of medium active lymphocytes. In the next age periods babies in the content of lymphoid follicles, there is increase of the number of small lymphocytes, which as compared with the norm increases by 10-15 times in average attached to the above changes.

CONCLUSIONS:

At the nursing infants the number of lymphoid follicles in the gut wall and colon gradually increases and at the end of one year the parameters become twice higher. In the cellular structure qualitative and quantitative changes were observed, usually in the second half of the year. In the newborns the undeformed lymphoid tissue of the intestine is revealed simultaneously with atopic hyperplasia in response to the infection. When lymphoid follicles are initially in the condition of hypoplasia and atrophy, in response to the infection the uneven and random proliferation of the cells is observed. Thus, the main macrofunctional zones of lymphoid follicles are not revealed morphologically, and the secondary changes in the type of necrosis and hemorrhage are detected. The development of AII with conventionally pathogenic microorganisms, is primarily dependent on the existence premorbidial diseases and initial morphological and morphometric failure of lymphoid tissue of the intestine. Thus, catarrhal desquamative, catarrhal and purulent necrotic-ulcerative enterocolitis is revealed **pathomorphologically. The AII in the lymphoid follicles in the intestinal wall, morphometrically since the first months of baby's life, the number of reticular, macrophageal, degenerative cells and medium lymphocytes, in the period of 7-12 months increased in 10 -15 times. Thus, the dynamics of reticular cells was noted: 1-3 months in 5 times, 4-6 months in 10, 7-12 months in 15 times. The number of macrophages increased in 7 times, and on average lymphocytes in 5 times.**

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Authors:

Zhuraeva Gulbahor Bahshillaevna - cand. med sciences, senior lecturer, head of the pathological anatomy Bukhara state medical institute. Uzbekistan

Turdiev Mashrab Rustamovich - teaching assistant Bukhara state medical institute. Uzbekistan

Sohibova Ziyoda Rahmatovna – teaching assistant Bukhara state medical institute. Uzbekistan +998914484826

gavhar72@inbox.ru <mailto:gavhar72@inbox.ru>
Uzbekistan 200117
Buhara
5/6 sq. 21, K.Murtazaev Str.
Zharylkasynova G. ZH.

Avramenko J.N.

Some Features of Structural Change of Cerebral Microvessels in Diabetes

ABSTRACT

To identify specificity of microvascular damage of the brain and a degree of structural abnormalities of vessel walls in diabetes (D) types 1 and 2 the morphological, morphometric and immunohistochemical research of autopsy cerebellum of patients with diabetes mellitus has been conducted. The structural change of brain microvessels in diabetes is noted to be a long-term process and characterized by low levels of expression of proliferation markers and apoptosis in fibrosis and hyalinosis according to the immunohistochemical study. The staging of microvascular damage of the brain in DM is defined, 3 morphological stages are allocated.

Keywords*:* diabetes mellitus, microangiopathy, brain, pathologic changes.

INTRODUCTION

The most characteristic feature of diabetes mellitus (DM) is the development of microangiopathy, frequency of which is 62-89% according to different authors [4]. Trigger mechanisms of its development are the genetic defects of the vascular wall, disorders of hormonal regulation, breach of the rheological properties of blood, tissue hypoxia, overproduction of hormones of insulin antagonists. The nature and the severity diabetic microangiopathy is determined by a combination of all these factors with specific metabolic disorders [1]. Microcirculatory disturbances have a leading role in the development of late complications of diabetes.

Chronic hyperglycemia ha s great impact on on the development of diabetic neuropathy [3]. The significance of hyperglycemia is determined by identical frequency of neuropathy in patients with diabetes type 1 and 2, although the pathogenesis of these forms of diabetes is different [1]. Symptomatic neuropathy is more common in people with poorly controlled diabetes [1,2].

Dysfunction of the nervous system occurs in parallel duration of diabetes and severity of metabolic disturbance. Long-term compensation for diabetic neuropathy improves and helps to reduce the frequency of this complication. This is evidenced by the results of a multicenter study «The Diabetes Control and complications Trial» (DCCT) [1, 2].

The life expectancy of patients with diabetes is increasing due to the optimization of monitoring and correction of blood glucose that promotes to an increase in its late complications. Nervous system takes a leading place among them. To date, diabetic encephalopathy is the least explored area of neurodiabetology.

MATERIALS AND METHODS

Comparative characteristic of morphological changes of brain microvessels was performed on autopsy material of patients with diabetes type 1 - 15 observations and diabetes type 2 – 30 observations. The comparison group included 10 patients who died with hyperglycemia on the background of intoxication of different genesis (pneumonia, peritonitis). The average age of patients with diabetes type 1 was 34 years, the average duration of the clinical manifestations of the disease was 12 years. The average age of patients with diabetes type 2 was 56 years, the average duration of the clinical manifestations of the disease was 16 years. The average age of patients with hyperglycemia due to severe intoxication was 34 years.

Methods. 1. Morphological methods: The study of the cerebellum sectional preparations stained with hematoxylin and eosin, Masson tri-color method, PAS-reaction.

2. Immunohistochemical study: Indirect immuno-peroxidase method with visualization system DAKO EnVision (Denmark) proliferation markers (PCNA, Ki67) and activation of apoptosis marker p53.

3. Morphometric method: a) measurement of the diameter of microvessels DCIRCLE (2√~AREAF / π); b) form factor (degree of deformation of blood vessels) FCIRCLE (4πAREAF / PERIMCROFT2); c) the thickness of the vascular wall (middle index of 10 measuring of wall thickness), was performed using a microscope with a video camera Axioplan 2 DXC-151A (Sony, Japan) by computer analysis of a digital image using a KS 200 software package (Kontron Electronic, Germany).

4. The statistical method of assessing the results of research using the applications of statistics.

RESULTS AND DISCUSSION

We observed a significant thickening of the walls of the arterioles and the bundle with irregular accumulation of PAS-positive substances in the basement membrane of microvessels with the deformation of the lumen by microscopic examination. There is a local increase in the number of pericytes in myo-adventitial layer, the accumulation in the thickened vascular wall

collagen fibers, which is a sign of a perivascular fibrosis, impregnation of wall plasma proteins leads to hyalinosis microvessels at the end. We noted the formation of single microaneurysms different shapes. Perivascular edema varying degrees of severity and spongiosis of tissue of the brain was determined around the modified microvessels, which is a manifestation of stagnation. We found that the morphological changes of microvessels in diabetes mellitus type 1 and type 2 have similar manifestations, but their degree of varies.

Diabetic microangiopathy can be divided into 3 stages according to light microscopy:

1) reversible stage of suffusion with proteins and lipids of blood plasma (a microscopic phenomenon bundles and thickening of the walls of blood vessels);

2) stage of local increase in the number of pericytes and fibrosis of thickened vessel wall;

3) irreversible stage of lipohyalinosis of vascular wall.

Glucose in high concentrations has direct toxic effects on cellular structures of vessels, which affects the morphology features of proliferation and activation of these structures in patients with CD type 1 and type 2. Proliferative activity of cellular structures of microvessels in DM was determined by the level of expression of PCNA and Ki67. Proliferating cell nuclear antigen (PCNA) is a subunit of DNA polymerase. The maximum level of PCNA observed during the S phase of the cell cycle when it forms a complex with an inhibitor of p21 [5]. The expression level of PCNA in diabetes type 1 was assessed as very low and determined in nuclei isolated pericytes of cerebellar arteriolar with fibrosis (Figure 1).

Ki67 is a nucleoprotein, which is expressed in all cell cycle phases except G0. Expression starts in late G1, peaking in the mitotic phase of the cycle [5]. The expression of the proliferation marker Ki67 was defined as negative in microvessel cells of the cerebellum in diabetes type 1.

The damage level of vascular endothelium was evaluated by detecting a marker of apoptosis p53. P53 protein is a major determinant of the cellular mechanism that leads to programmed cell death [5]. P53 expression was assessed as very low in the cells of the cerebellum microvessels in diabetes type 1 and was determined in individual endothelial cells (Figure 2).

The low level of expression of the proliferation marker PCNA in cells of hyalinized microvessel of the cerebellum was noted, in the presence of its expression in perivascular microglia and oligodendroglial nuclei in DM type 2. Often, the expression of PCNA in cells hyalinized precapillaries was absent (Fig 3, 4).

The expression of the proliferation marker Ki67 in microvascular endothelial cells of the cerebellum is also defined as negative in diabetes type 2. The absence of a pronounced proliferation of vascular endothelium in diabetes type 2 is likely due to the toxic effects of excess amounts of glucose and insulin in a chronic state of insulin resistance, due to the peculiarities of glucose metabolism in the brain.

Expression of apoptosis marker p53 was defined as weakly positive in pericytes of arterioles and precapillaries cerebellum in diabetes type 2.

According to the computer morphometry the deformation of vessels is most expressed in the brain of patients with diabetes type 1 in the stage of lipohyalinosis. At this stage in patients with diabetes type 1 reduces the contractility and dilated possible of arterioles in the brain (according to the computer morphometry of small diameter arterioles have great indicators form factor r = -0,31 - inverse correlation average degree). Microvessels in diabetes type 2 have a larger diameter and the thickness of the vascular wall, which may be related not only to glucose toxicity, and toxicity to insulin, that is often observed in patients. Compensatory hyperinsulinemia in insulin resistance (depending on the degree of severity) are atherogenic factor due to increased vascular smooth muscle cell proliferation and extracellular matrix protein formation. Table 1 (indicators microvessel morphometry).

CONCLUSION

In the development of diabetic microangiopathy 3 stages can be identified: the reversible stage of impregnating vascular wall proteins and lipids of blood plasma, the stage of locally increasing the number of pericytes and vascular fibrosis, thickened wall, the irreversible stage of lipohyalinosis in the vascular wall.

Structural reorganization of walls of microvessels in the diabetes type 1 and type 2 is a long-term process and according to immunohistochemical study in the stage of fibrosis and hyalinosis characterized by low levels of expression of proliferation markers PCNA, Ki67 and p53 apoptosis in isolated endothelial cells and pericytes of cerebral vessels.

At the level of light microscopy pathological signs of microangiopathy in the diabetes type 1 and 2 do not differ significantly.

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AUTHORS:

Avramenko Julia Nikolaevna – teasching assistant of the Zaporojskiy State Medical University, juliya_0304@inbox.ru.

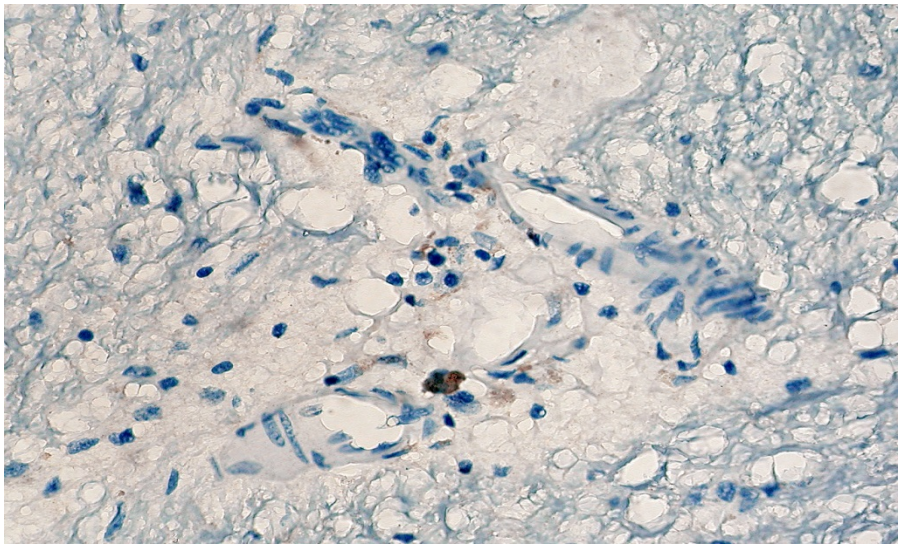

Fig.1. Expression PCNA pericytes of brain arteriolas at DM type 1

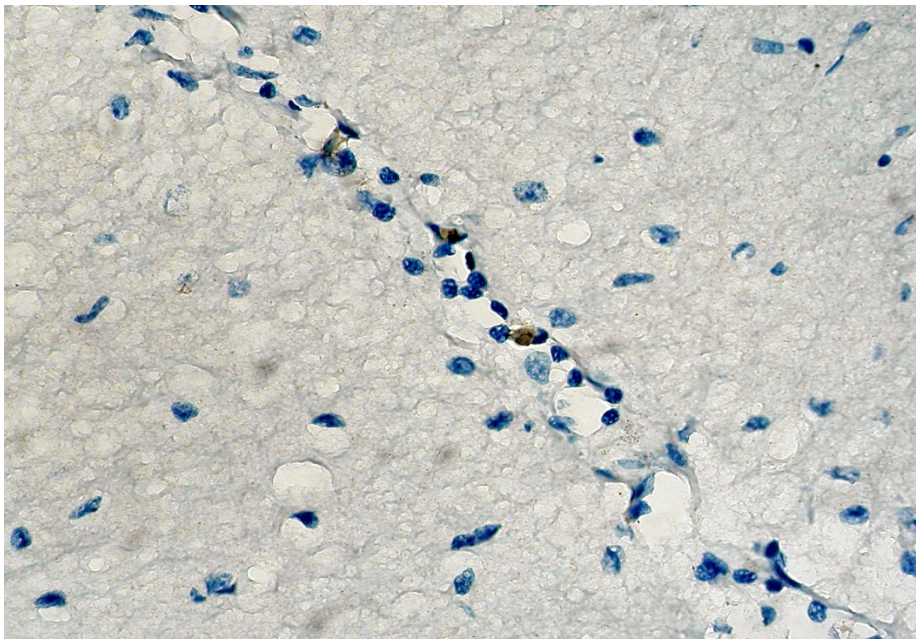

Fig. 2. Expression 3 in endothelia of brain precapillary at DM type 1

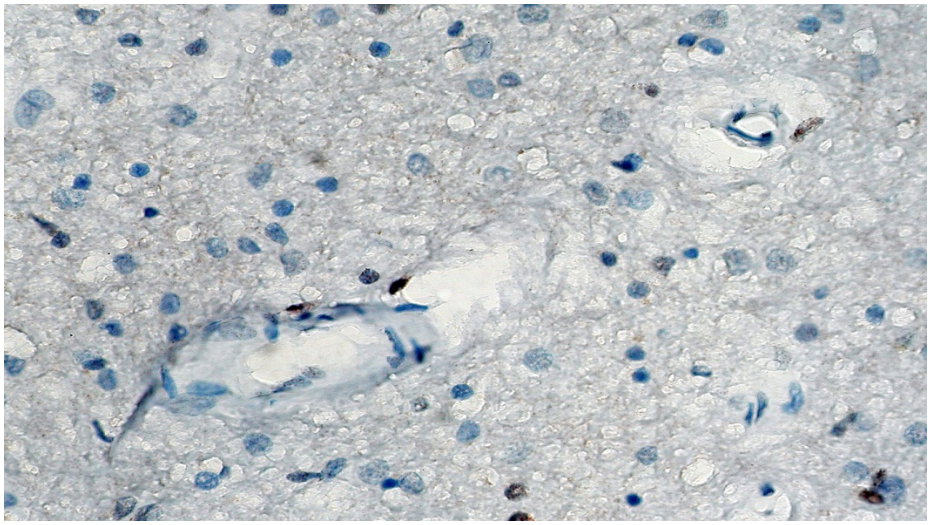

Fig. 3. Expression PCNA pericytes of brain arteriolas at DM type 2

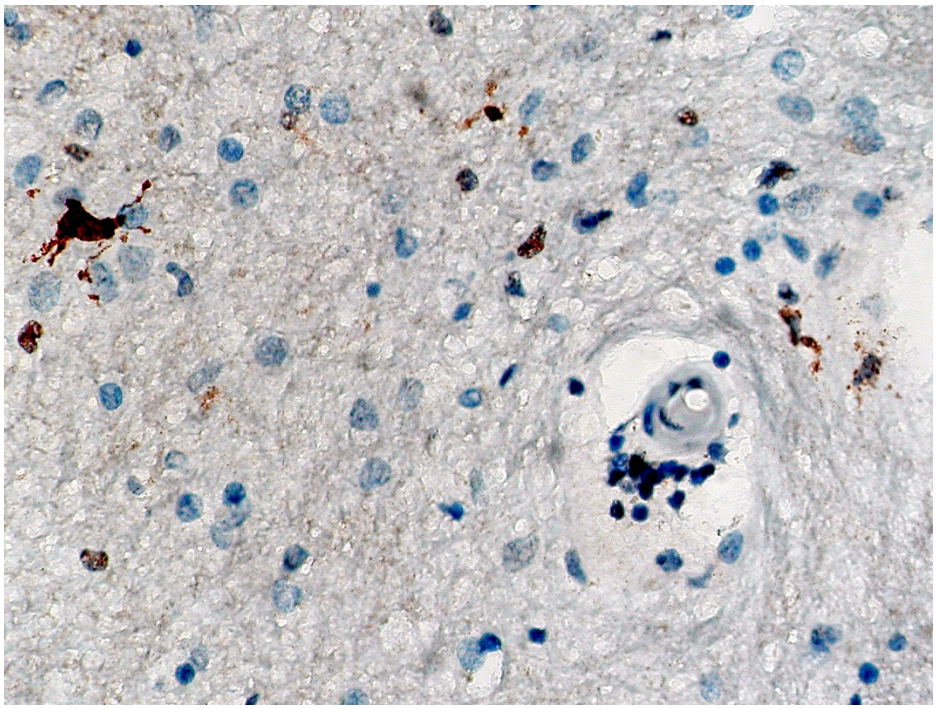

Fig. 4. Expression PCNA glial cells in the absence of expression of this proliferation marker Iin hyalinized precapillars of the brain type 2

Table 1

Showings of morphometry of microvessels

	Control (symptomatic hyperglycemia)	**DM Type 1**	**DM Type 2**
Brain			
1) diameter of vessels	22,75±1,86	26,28±2,45	29,668±3,03
2) value of form factor	0,89±0,012	0,77±0,028	0,83±0,02
3) thickness of vascular wall	3,44±0,16	6,30±0,37	7,43±0,40

Karanasheva V.A., Tlakadugova M.H., Pshukova A.A.

Pathomorphology of coronary heart disease at diabetes

ABSTRACT

Autopsy protocols with diabetes mellitus are studied with certifying causes of death, macroscopic and histological changes in a heart. Ischemic heart disease was considered to be the most common cause of death of these patients. Myocardial infarction at diabetes mellitus was noted more frequently at women, differed by frequency of atypical forms, aneurysm and breaks.

Keywords: diabetes mellitus, ischemic heart disease, myocardial infarction

The problem of diabetes mellitus remains universal for modern health protection in connection with steady growth of frequency in the whole world, by heavy clinical displays and vascular complications, resulting in death. Diabetes mellitus increases annually on 6-10%, in this connection the common amount of patients in many regions of the Russian Federation amounts to 2-4% of all population [1, 2, 5]. The medical social value of diabetes mellitus is determined by greater damage that it inflicts to a society as a lifelong chronic disease with higher degree of disability. In spite of plenty of researches, the separate aspects of this pathology continue to attract attention of scientific and practical doctors, including cardiovascular complications, particularly ischemic heart disease. [3, 4]. The defeats of heart at diabetes mellitus is observed so frequently, that it was named "Diabetic heart" [4, 5].

Taking into account the actuality of this pathology, **the aim of the research** was to study features of ischemic heart disease at diabetes mellitus.

This research is carried out in the Centralized pathoanatomical bureau of the Ministry of health in the Kabardino-Balkarian republic. 365 protocols of dissections of the dead with diabetes mellitus are studied in medical establishments of Nalchik city with the purpose to reveal causes of death, macroscopic and histological changes in a heart.

In the studied material ischemic heart disease was the most frequent reason of death at patients with diabetes mellitus (37,8%). Ischemic heart disease was observed, as well as usually, in age older 40, most often at 60-69 aged women (60,1%), that is explained by more frequent manifestation and other risk (high blood pressures and obesities) factors. In our material a hypertensive syndrome was present in 41,3% women and only in 30,8% of men. Combination of diabetes mellitus obesity is marked in 32,5% women and 15,4% men.

The analysis of frequency of ischemic heart disease at diabetes mellitus depending on its type showed that diabetes mellitus IHD type I was revealed in 13,1%, while diabetes mellitus

type II was in 86,9%. The amount of the dead with ischemic heart disease increased as far as longer duration of diabetes mellitus, although in 7,8% it was diagnosed as a new onset during the last hospitalization. However in the majority of them the morphological study of pancreas and buds testified to considerable duration of disease due to late diagnostics. Ischemic heart disease is more often observed at higher and middle severe forms of diabetes mellitus.

Forms of ischemic heart disease in the dead with diabetes mellitus were as follows: acute myocardial infarction in 29,1%; repeated and recrudescent myocardial infarction in 36,8%; chronic ischemic heart disease is in 33,3%. Chronic ischemic heart disease was presented by heart post-attack cardio sclerosis and chronic aneurysm.

Occlusive blood clots in coronal arteries at myocardial infarction were discovered in 54,3%. All of them arose up on background of expressed stenotic coronary sclerosis. More often blood clots were in downleg left coronal artery (22,8%), then in the right (18,4%) and rarer in the circumflex branch left (11,1%).

Transmural heart attacks were noted most often (68,1%). Of them intramural myocardial infarction amounted to 26,8% and subepicardial - 5,1%.

In 30,4% heart attacks are localized in the anterior wall of the left ventricle with involving in process between ventricular septum and apex; 27,3 - in the inferior and posterolateral walls of the left ventricle; 17,4% - in the anterolateral walls of the right ventricle.

The heart attacks of the anterior (6,5%) and inferior ((8,7%) walls of both ventricles were marked also with engaging in the process of papillary muscles and intraventricular septum. In addition the total heart attack of the left ventricle (of 5,4%) and isolated heart attacks were noted: intraventricular septum (of 3,2%) and papillary muscle (of 1,1%).

The high incidence rate of aneurysm in transmural myocardial infarction at diabetes mellitus was noted (36,9%). Of them acute aneurysm was in 21,7%, chronic in 11,9%, acute in combination with chronic in 3,3%. Acute aneurysm ruprures were observed in 8,1% of the total amout of myocardial infarction. Interior ruptures were revealed as well: a rupture of intraventricular septum and detachment of papillary muscle.

The clinical diagnostics of myocardial infarction at patients with diabetes mellitus often caused difficulties and at 18,4% they were not recognized. Probably, it was related to frequency of atypical forms of myocardial infarction (29,3%). Gastric form aws the most frequent (11,9%). In this case pancreatitis, food poisoning, gastro entoeocolit, dysentery were diagnosed. At this form heart attack was usually localized in the interior wall of ventricles.

In 9,8% myocardial infarction of the painless form was noted, quite often patients were operated concerning the moist gangrene of lower limbs. There were also cases of cerebrovascular (5), asthmatic (1) and unrhythmical (1) types of myocardial infarction.

The ischemic stage of myocardial infarction was found out at the 8 dead, 4 of them had acute coronary insufficiency developed on the background of heart post-attack scars. In all these supervisions there was coronary sclerosis, and 6 of them had occlusive blood clots.

Macroscopically in myocardium no fresh affected foci were revealed on plane cuts, areas of uneven plethora were revealed only.

The diagnosis of myocardial infarction was confirmed after histological and histochemistry research. In the necrobiotic stage (48 supervisions) the visible myocardial infarction as foci of yellow-pink areas with layers of crimson color, with mat on the cut were observed. At the microscopic research in this stage at ordinary histological colourings the large fields of myocyte necrosis were observed distinctly.

In the reparative stage (the 26 dead) the affected focal area was exposed to organization. The granulation fabric was developed from the side of endocardium and epicardium and from the foci of remained muscular fibres in the layer of infarciric area.

In the granulation fabric there was a plenty of capillaries, desmocytes, macrophages and lymphocyte. As far as cicatrization the zone of heart attack was filled with connective tissue of different degree of maturity, the amount of capillaries diminished as well as cellular elements.

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Authors:

1) Karanasheva Valentina Ahmedovna, cand. med. sciences, senior lecturer, head of the department of normal and pathological anatomy, Medical faculty KBSU, Ph. +7 (929) 8844383, e-mail: valentina.karanasheva@gmail.ru <mailto:valentina.karanasheva@gmail.ru> the Address: 360000, KBR, Nalchik, 3, I.Armand str.

2) Pshukova Albina Anatolevna, cand. med. sciences, teaching assistant of the department of normal and pathological anatomy, Medical faculty KBSU, Ph. +7 (909) 4905099, an e-mail: albina.75.75@bk.ru <mailto:albina.75.75@bk.ru> the Address: 360000, KBR, Nalchik, 3, I.Armand str.

3) Tlakadugova Madina Hazhismelevna, cand. med. sciences, teaching assistant of the department of normal and pathological anatomy, Medical faculty KBSU, Ph. + 7(928) 7166071, an e-mail: tla-madina@yandex.ru <mailto:tla-madina@yandex.ru> the Address: 360000, KBR, Nalchik, 3, I.Armand str.

Egorova A,I., Garmayeva D. K.

Morphological Assessment of Thyroid Gland at Native Men of the Republic Sakha (Yakutia) in different seasons

ABSTRACT

Morphological analysis of macro- and microstructures of thyroid gland at native male population of the Republic Sakha (Yakutia) in different seasons has been conducted. The males' thyroid gland appeared to be normoplastic mixed type of structure, indicators of outer and inner thyroid gland follicles diameter in winter were authentically bigger than in summer. The same tendency was observed when thyroid gland follicular - colloidal index was calculated. On a basis of the data obtained the assessment of impact of a season temperature factor on the thyroid gland structural indices was attempted, it being used as morphological equivalent of the body adaptation processes in northern regions.

Keywords: macromorphometry, micromorphemetry, thyroid gland, seasons.

INTRODUCTION

The Republic of Sakha (Yakutia) is one of the biggest territories of the Russian Federation by its territory. By its natural and territorial conditions the republic is not comparable to any other regions of the world. More than 40% of the territory is situated beyond the north of the Arctic Circle.

Almost all continental territory of the Republic Sakha (Yakutia) is based on integral centuries-long permafrost [7]. Natural environmental and climatic conditions of Yakutia are mainly characterized as extreme one. Sharply continental climate is specified by long winter and short summer periods. One of the main exogenous factors of the northern latitudes which might cause ambrosia of regulatory and compensatory mechanisms, dyscrasia, and metabolic disturbance of the body as the integral whole is cold. In these conditions, health preservation of the population of the Sakha Republic, theoretically based preventive measures directed to optimize the adaptation process in cold regions of the world are still urgent questions to be considered and worked out.

Neuroendocrine system where thyroid body is one of the considerable components that fulfills specific functions in adaptation coping reactions of the body. The importance of the latest is proved by the fact that under hypothyroidism of endogenous iodinated thyroid hormone the intensity of metabolism decreases as well as the body temperature when the hyper function of thyroid body causes opposite effect [4,5]. In accordance to this the thyroid disease is to be

studied as the marker of ecological trouble. In available scientific references the data referring to the study of body's season adaptation on the basis of morphological indicators of native male subjects' thyroid gland has not been found.

Aim of the study:

To give season histomorphological characteristics of thyroid gland structural organization of native men in the Republic of Sakha (Yakutia).

MATERIALS AND METHODS:

In order to support objectives of the research thyroid glands of 45 dead male bodies, representatives of indigenous nationality on the territory of the Republic of Sakha (Yakutia) have been evaluated for the period January 2007 to August 2012. According to human ontogenesis age periodization adopted at the 7th All-Soviet Union Conference on Age Morphology Problems, Physiology and Biochemistry (1965) the second adulthood age has been assessed.

The died as a result of violent and sudden death with no injuries of neck have been the subjects of the research.

Material samplings were performed in summer (June, July, August) time and winter (December, January, February) time on the base of State Budget Establishment "Bureau of Forensic Medicineand Mortem Department of Republican Hospital№1- National Medical Center of the Republic Sakha (Yakutia).

The autopsy was conducted during the first 12-24 hours after death. Information about the samplings was obtained from protocols, personal data. According to reportsnonelifetime and postmortem pathology of thyroid gland was diagnosed.

Thus, all studied bodies were divided into 2 groups:

1st group – persons who died in summer timefrom mechanic injuries incompatible with life and who did not have any pathology of thyroid gland during their lifetime;

2ndgroup – personswhodiedinwintertime from mechanic injuries incompatible with life and who did not have any pathology of thyroid gland during their lifetime.

Macromorphometrical method of research

During the postmortem examination of a corpse thyroid gland was properly separated, its topographic and anatomic characteristics were registered (perspective view and sectional). Thyroid gland was weighed on the scales VLKT-500 up to 0,01 gr. Further linear parameters of both lobes were measured (height, width, thickness). Absolute mass (AM) (gr) and relative

weight (RW) of thyroid gland were determined. The formula RW(TB) = Absolute mass (AM), gr/mass, kg x 100% was used. For thyroid gland dynamics study we determined thyroid volume with the formula: V= a·b·c·0,479,where a – length, b- width, c – thickness of thyroid body shares, 0,479–correction ellipsoid factor.

Histomorphometric method of the research

Pieces of thyroid gland tissue the size of 10 x 10 x 5,0mm were excised from middle part of both lobes(right and left at the same level). The material was fixed in 10% neutral formalin during 24 hours, then embedded in paraffin block with the unit «Leica EG 1150H». Four five micron thick sections were made from paraffin blocks with the luge microtome «LeicaSM 2010R». The sections were deployed in water bath and replaced to the micro slides. The sections for histologic research were colored with hematoxylin and eosin. When histologic specimen were studied the type of thyroid gland structure was determined, as well as its structural components which are morphological equivalents of thyroid gland functional state.

Thyroid gland morphometry was studied by its structural and functional components' quantitative assessment referring to recommendations given in scientific references [6]. Thyroid gland structural components' analysis (average outer and inner diameter of the follicle, the average height of follicular thyrocytes, follicular thyrocytes area, area of colloid, the core area of follicular thyrocytes)was conducted with licensed software, specialized for morphometric histologic research of specimen «Screen Meter». Secondary indicators were calculated basing on first indicators. Colloid accumulation index (CAI) indicator, follicular colloid-index (FCI) indicator, nuclear - cytoplasmic index (NCI) indicator were determined.

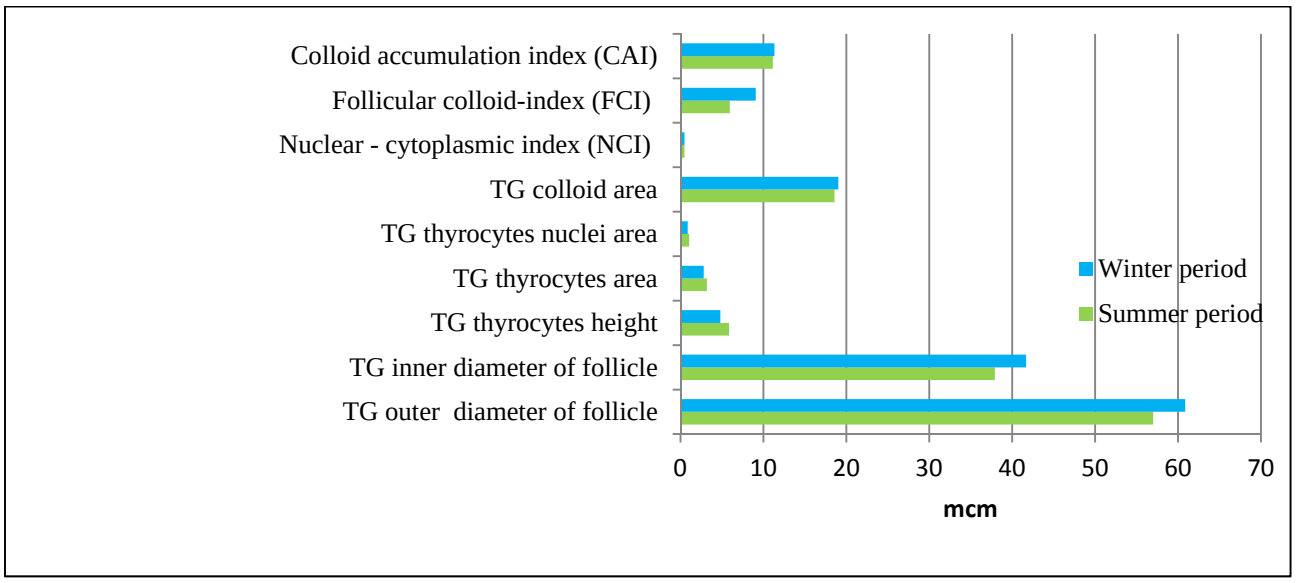
RESULTS AND DISCUSSIONS

Thyroid gland macro morphometric data of indigenous people in summer and winter periods: The thyroid gland is typically located on the front surface of the trachea and consisted of two lateral lobs connected by an isthmus. Sometimes pyramidal process was defined. The thyroid gland was surrounded by visceral fascia of the neck and enclosed in a dense fibrous capsule. The thyroid gland was smooth to the touch and had elastic consistence. The fabric on the cut was light chocolate brown. After linear dimensions of the thyroid gland were studied the average values were determine. As thyroid gland weight is one of the its morphologic state indicators, we determined average thyroid gland (TG) absolute weight (AW) indicator. So for indigenous people in summer period it was 24,90±1,96 gr and in winter period 22,48±0,61 gr.

The calculation of the average value of relative weight (RW) was in summer period 39, 00±2, 73%, in winter period 39,09±1,58 % [3].

Thyroid gland micromorphometric data of indigenous people in summer and winter periods: Microscopic examination of thyroid gland tissues in study groups showed that their histologic structure belongs to normoplastic mixed, middle follicular type. According to the author Grigorieva V.A. (1970) [2], thyroid gland of normoplastic mixed type is not fully mature and corresponds to inverse growth where there is regular development in one parts and suppression with negative environmental impact in other parts.

The shapes of the follicles were mostly round, ovoid and irregularly rounded shape. The average thyroid gland outer diameter of the follicle of indigenous people in summer period was 57,01±2,33 mcm, that is 1,05 times less than in winter period 60,87±1,42 mcm. The average thyroid gland inner diameter of the follicle in summer period was proved to be less 37, 92±1, 89mcm, than in winter season of the year41, 68±1, 38mcm.Thyroidgland follicular epithelium had cubic form. Average height of the latest in summer period was 5, 84±0, 19mcm, and in winter period 4, 80±0,21mcm ($p<0,05$). The study of medium-sized areas of follicular thyrocytes of indigenous people in summer period was 3, 19±0, 10%, that is 1, 14times bigger than in winter season of the year. Thyroid glandcell nuclei thyrocytes were predominantly round, oval, monochrome, located centrally in summer period 1,04±0,04%, in winter period 0,86±0,05% ($p<0,05$). The calculation of average indicator of nuclear - cytoplasmic index (NCI) showed equal values in summer and winter seasons of the year. Follicles were filled with homogeneous eosinophilic colloid. When the average indicator of colloid area was calculated, the result in summer period was 1, 05 times less than in winter period. Average indicator of thyroid gland follicular colloid index (FCI) that determines functional activity of thyroid gland among indigenous people in summer season was 5,94±0,52, that is significantly lower than in winter season (9,07±0,65) ($p<0,05$). When colloid accumulation index(Brown's index) of indigenous people was assessed, it was defined that this indicator does not change and constitutes 11,13±0,51(pic.1).



Pic.1. Diagram of indigenous people micromorphometric parameters of thyroid gland in summer and winter seasons.

CONCLUSION

The data obtained proves the statement that thyroid gland possesses specific plasticity when human body adapts to the environmental conditions [6]. When thyroid gland histomorphologic analysis of indigenous people of the RepublicSakha (Yakutia) was conducted we detected that thyroid gland microstructure reacts to the seasonal temperature. It was proved by increase of such indicators as height, thyrocytes nuclei area, thyrocytes inner and outer diameter follicles, follicular colloid index in winter time in comparison with summer time. Where in the increase of the follicular colloid index indicator can be explained as functional tension of thyroid gland which is necessary for maintenance of proper activity level in this season of the year [1, 7, 8].

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Authors:

Egorova Angelina I. – junior researcher of the clinic of the Medical Institute NEFU named of M.K. Ammosov, egorovaanil@mail.ru;

Garmaeva Darima K. – doct. med. sciences, professor of the Medical Institute NEFU named of M.K. Ammosov, dari66@mail.ru

Kazanov V.N., Garmaeva D.K., Khayrullin R.M., Ignatiev V.G.

The characteristic of Mammary Glands of Women of Yakuts in Age Aspect and Its Value in Multiplicating Mammoplasty

ABSTRACT

In this research the group of authors performed a topomorphometric analysis of a breast tissue of Yakut women who decided to change their breast shape. The data obtained allow to systemize a tactics in performing the surgical intervention.

Keywords: a breast tissue, Yakut women, mammoplasty.

INTRODUCTION

According to contemporary representations breast is considered to determine significantly interpersonal relations between genders and aspiration of young and adult women for breast maximally close for the common standards of size and form. [2] [5]

It is evident for our region that physical and pubertal development of girls who live in extremely continental climate has particular qualities which need detailed and differentiated study. It is proven that breast development, development of hairiness of the pubis and armpit, the first occurrence of menstruation of Yakut and Caucasoid girls outpace indicators of the girls of the other somatotypes in the studied periods of ontogenesis. Later development of secondary sexual characteristics of Yakut girls in contrast with Caucasoid girls is also detected. [1]

Thus, definition of precise standard diagnostics of the regional norm of breast and contiguous topographic layers and form of thorax is becoming increasingly important. The diagnostics significantly affects the choice of the optimal method of augmentation mammoplasty and determines positive final result.

Considering the foregoing and the lack of information on the individual variability of form, size and topometrical characteristics of breast of the women living in Republic of Sakha (Yakutia) in available Russian and foreign sources the authors determined the following **aim of the reseach:** revealing individual typological variability of form, size and topometrical characteristics of breast of Yakut women with taking into account age.

MATERIALS AND METHODS

Morphometry of breast was performed at 72 Yakut women aged from 20 to 40 years old who attended a private clinic "Victory Clinic" (Yakutsk) for correcting mammoplasty with the principles of voluntariness, individual rights and freedoms guaranteed by Articles 21 and 22 of the Constitution. The examinees were divided into the following age groups: I group from 20 to 25 years (14 women), II group - from 26-30 years (24 women), III group - 31-35 years (21 women), IV group - 36 -40 (13 women). The studied groups included persons with no subjective complaints on the state of the reproductive sphere, with no medical history of menstrual dysfunction and with no concomitant pathology of reproductive system. The large stout compass, centimeter linen tape and sliding dividers were used for morphometric measurement.

Measuring was carried out on the system “Body Logic” (Mentor Medical Systems B. V. - USA). The following indicators were recorded: Height, weight, age and ethnicity; The chest circumference at two levels; Height and width (vertical and transverse dimensions) compared with the left and right breast; Distance of the nipple-areola complex from the jugular fossa; Distance from the center of the collarbone to the nipple in comparison with the left and right sides of the breast; The thickness of the skin-glandular breast folds on the medial, lateral and upper poles; Dimensions of the nipple-areola complex, its vertical and transverse size; Extensibility of breast tissue in the lower pole; Fixing of the breast form, inspection for chest asymmetry, visual deformations, presence or absence of distortions of the spinal column. The results obtained in the study were entered into a spreadsheet Excel 7.0 and processed using standard statistical software package Statistica 8. The arithmetic mean - M, the average error of the arithmetic mean – m, were calculated for each of the studied parameters.

RESULTS AND DISCUSSION

Analysis of obtained data showed that the body weight of the surveyed women increases to the age of 35-40 years. Body mass index (BMI) was also higher in the older age group. The lowest BMI were noted in the first and the second age group (Table 1). Dimensional indicators of transverse diameter of the chest at the level of submammary folds and at the level of the nipples were also higher in the older age group. Among the surveyed contingent of Yakut women round shape breast is most often found in the third age group (12,5%); broad form of breast in the first group (22,70%); conical shape in the second group (18,0%); tubular form is found only in the first (1.3%) and the second (1.3%) age groups. Visual asymmetry between right and left sides of breast is observed in the first age group (26-35 years) (Table 2). The distance from the jugular fossa to the nipple increases to the fourth group, in comparison with the first group this indicator is 1,3 times greater, with the second group is 1.2 times greater and with the third group is 1.1 times greater. Also the difference in the average indicators of the distance from the middle of the collarbone to the nipple are noted in the studied groups, so the greatest magnitudes are marked in the fourth group, the lowest in the first group. Appreciable asymmetry of these indicators is found in the age of 20-25 years. The transverse dimension of the base of breast (from 12,2 to 13,4 cm) and a vertical dimension of the base of breast (from 11,5 to 12,5) are increased to 36-40 years. The thickness of the skin-glandular folds at the lateral, medial and upper poles also tends to increase with age, and the higher scores were recorded in the left side in all groups. Comparison of the indicators between the poles revealed the predominance of the thickness in the area of the upper pole in all age groups. There is a tendency of increasing of the distance between the nipple and submammary fold with age, from 5,5 to 6,5 without tension and from 7,5 to 8,4 with pulling the skin. However, a significant difference in the average indicators of the distance between the nipple and submammary fold is noted in the age groups of 20-25 years and of 26-30 years, and besides with the right side bigger than the left side. At the same time in the older age groups, these figures are relatively bigger on the left side (Table 3).

Submammary fold as the anatomical structure is a key part that defines the aesthetic breast augmentation and mastopexy, this structure is the foundation on which is based the construction of mammoplasty [4]. Thus, the asymmetry is less noticeable in the older age group, as well as

the outward signs of asymmetry of the chest or spinal column. In the other age groups, the incidence of asymmetries is comparable, while the maximum number of asymmetries of the chest is noted in the second group - funnel chest, keel chest, incorrect posture (Table 1).

Analyzing of the size of the areola on the vertical and lateral lines in comparison of the left and the right sides shows tendency of increasing of size with age (Table 4). Moreover, the size on the vertical lines has a marked tendency to increase, in comparison with the transverse line. Minimal transverse size of the areola is found in the first age group (3,14 cm.), maximal is found in the fourth age group (4,26 cm.). The minimal vertical dimension is also noted in the first age group (3,25 cm.) and maximal is noted in the fourth (4,38 cm.). The asymmetry between the right and left sides of the breast is fixed, it is more evident in its vertical dimension with increasing of the age (from 0,07 cm. to 0,15 cm.), although the maximal asymmetry in the transverse dimension is noted in the second age group of 26-30 years (from 0 to 0,08 cm.).

CONCLUSIONS

Thus, as a result of the study the research group determined topomorphometric indicators of breast of Yakut women in the different age groups. Among the surveyed contingent of Yakut women wide shape is most often found in the first group, conical shape – in the second group (18,0%), round shape of breast is more often in the third group (12,5%). Tubular form is found only in the first (1,3%) and the second (1,3%) age groups. More evident visual asymmetry of breast between the left sides is observed in the age group of 26-35 years. It is found that with sufficient symmetry of form of mammary glands in the majority of women in the studied groups there are many cases of asymmetry of the structure of the chest, probably due to rachitic factor widespread in our region. Proportional increase of the distance of the nipple-areola complex with increasing age relative to the jugular fossa and clavicle show ptosis of the breast tissue. The growth of the thickness of skin-glandular fold is more pronounced in the fourth age group (36-40 years), it indicates hypertrophy of breast tissue, this fact is important to be taken into account while calculating the volume of the future implant. Indicator of the stretchability of the lower pole of the breast is important for planning surgical intervention, because it shows the state of the skin pocket for the implant. This value also increases with age, and indicates the presence of breast ptosis, predominantly of the skin genesis. Also the great importance for the reconstruction of form of breast has asymmetry of the level of submammary fold, which is expressed in the third studied group (12,5%) of 31-35 years. Respectively, for this group the reconstruction of submammary fold, its symmetrization and strengthening are often. Naturally, the breast tissue susceptible to natural gravitational ptosis with the age, the same things happen with the nipple-areola complex, so in the fourth age group the maximal lateral and vertical dimensions are marked. While planning the surgical intervention it is appropriate to reduce these dimensions by periareolar annexation.

Table 1. Average indicators of some anthropometric data of Yakut women in different age groups (M±m (min-max)).

Parameters (mean values)	**20-25 years M±m (min-max)**	**26-30 years M±m (min-max)**	**31-35 years M±m (min-max)**	**36-40 years M±m (min-max)**
Body length (cm.)	164,0 ±5,4 (154,0-175,0)	162,1 ±4,4 (153,0-174,0)	165,2 ±5,7 (152,0-175,0)	162,8 ±5,6 (156,0-176,0)
Body mass index (kg/m^2)	19,4 ±2,1 (16,1-24,2)	19,0 ±1,6 (16,0-23,2)	21,0 ±2,0 (17,6-25,1)	22,6 ±3,5 (17,2-29,0)
Weight (kg.)	52,4 ±6,9 (43,0-70,0)	50,1 ±4,7 (42,0-61,0)	57,0 ±5,3 (50,0-60,7)	60,2 ±10,6 (48,0-83,0)
Chest circumference at the level of the inframammary fold (cm.)	71,9 ± 3,6 (66,0-78,0)	71,6 ±2,7 (66,0-79,0)	75,7 ±4,2 (69,0-84,0)	80,7 ±7,6 (72,0-92,0)
Chest circumference at the level of the nipples (cm.)	78,1 ±4,3 (72,0 -85,0)	77,2 ±4,3 (71,0-87,0)	82,7 ±5,0 (74,0-93,0)	88,3 ±8,4 (80,0-103,0)

Table 2. Forms of breast and qualitative indicators of asymmetries of Yakut women in different age groups.

№	Name of parameter	20-25 years	26-30 years	31-35 years	36-40 years
1	Shape of breast: Round	11,1%	8,3%	12,5%	2,7%
	Wide	22,70%	5,50%	5,50%	5,50%
	Tubular	1,30%	1,30%	0	0
	Conical	4,10%	18,00%	11,10%	9,70%
2	Visual asymmetry of the breast	9,70%	13,80%	13,80%	6,90%
3	Asymmetry of the level of submammary fold	11,10%	11,10%	12,50%	4,10%
4	Asymmetry of the level of the nipple-areola complex	8,30%	11,10%	13,80%	8,30%
5	Visual asymmetry of the chest and the spinal column (funnel chest, keel chest, incorrect posture)	11,1%	16,6%	12,5%	6,9%

Table 3. Parametric indicators of breast of Yakut women in different age groups (M±m (min-max)).

№	Name of parameter (mean values)	20-25 years M±m (min-max)		26-30 years M±m (min-max)		31-35 years M±m (min-max)		36-40 years M±m (min-max)	
		Right	Left	Right	Left	Right	Left	Right	Left
1	Distance from the jugular fossa to the nipple (cm.)	15,6 ±1,8 (11,0-19,5)	17,6 ±1,7 (15,5-21,5)	17,5 ±1,7 (11,8-20,5)	17,6 ±1,8 (11,8-20,5)	18,6 ±0,9 (16,0-20,0)	18,5 ±1,1 (16,0-21,0)	20,2 ±2,5 (17,0-25,0)	20,1 ±2,2 (17,0-24,0)
2	Distance from the level of the middle of the clavicle to nipple (cm.)	15,6 ±2,3 (11,0-19,5)	15,8 ±2,2 (11,0-19,5)	16,0 ±1,7 (10,0-19,0)	16,3 ±1,9 (10,0-19,5)	17,1 ±1,1 (15,0-19,5)	17,1 ±1,4 (15,0-20,0)	18,8 ±2,3 (15,0-23,0)	19,0 ±2,3 (15,5-23,5)
3	The transverse dimension of the base of the breast (cm.)	12,3 ±0,8 (11,0-14,0)	12,2 ±0,8 (11,0-14,0)	12,2 ±0,8 (10,0-14,0)	12,1 ±0,9 (10,0-14,0)	12,6 ±0,9 (11,5-14,5)	12,7 ±0,9 (11,5-14,5)	13,2 ±0,8 (12,5-14,5)	13,4 ±0,9 (12,5-15,0)
4	The vertical dimension of the base of the breast (cm.)	11,9 ±1,1 (10,0-14,0)	11,5 ±1,3 (9,5-14,0)	11,4 ±1,0 (10,0-13,0)	11,3 ±0,9 (10,0-13,0)	12,0 ±0,8 (10,5-14,0)	11,9 ±0,9 (10,5-14,0)	12,3 ±0,7 (11,0-13,0)	12,5 ±0,9 (11,0-14,5)
5	The thickness of the skin and glandular fold of the medial pole (cm.)	2,7 ±0,5 (2,0-3,5)	2,7 ±0,6 (1,5-3,5)	2,5 ±0,6 (1,5-4,0)	2,6 ±0,8 (1,0-4,0)	2,9 ±0,6 (2,0-4,0)	2,9 ±0,6 (2,0-4,0)	3,5 ±0,7 (2,0-4,5)	3,5 ±0,7 (2,5-4,5)
6	The thickness of the skin and glandular fold of the lateral pole (cm.)	2,7 ±0,6 (2,0-4,0)	2,8 ±0,7 (1,5-4,5)	2,6 ±0,8 (1,5-4,5)	2,7 ±0,7 (1,5-4,5)	3,2 ±0,6 (2,0-4,0)	3,2 ±0,7 (2,0-4,5)	3,4 ±0,4 (3,0-4,0)	3,7 ±0,6 (3,0-5,0)

7	The thickness of the skin and glandular folds of the upper pole (cm.)	2,9 ±0,6 (2,0-4,0)	3,0 ±0,8 (1,5-4,5)	2,8 ±0,8 (1,5-4,0)	3,0 ±0,9 (1,5-4,5)	3,1 ±0,6 (2,0-4,0)	3,3 ±0,7 (2,0-4,5)	3,7 ±0,3 (3,0-4,5)	3,8 ±0,5 (3,0-4,5)
8	Distance from the nipple to the submammary fold without tension (cm.)	5,7 ±1,6 (3,5-9,5)	5,5 ±1,5 (3,5-9,5)	5,6 ±0,9 (4,0-7,5)	5,5 ±0,9 (3,5-7,5)	5,8 ±1,1 (4,0-9,0)	6,0 ±1,2 (4,0-9,0)	6,3 ±1,0 (5,0-9,0)	6,5 ±1,2 (5,0-9,5)
9	Distance from the nipple to the submammary fold with pulling (cm.)	7,6 ±1,8 (5,0-11,5)	7,5 ±1,9 (5,0-11,5)	7,7 ±1,0 (6,0-10,0)	7,6 ±1,1 (5,5-10,0)	8,0 ±0,8 (7,0-10,5)	8,2 ±0,9 (6,5-10,5)	8,3 ±1,2 (5,0-10,0)	8,4 ±1,3 (5,5-11,0)

Table 4. Comparative parametric data of size of the areola of the breast of Yakut women in different age groups (M±m (min-max)).

№	Parameter (mean values)	20-25 years		26-30 years		31-35 years		36-40 years	
		Right	Left	Right	Left	Right	Left	Right	Left
1	The transverse dimension of the areola (cm.)	3,14 ±1,1 (1,5-5,0)	3,21 ±1,3 (1,5-6,0)	3,28 ±0,5 (2,0-4,0)	3,24 ±0,5 (2,0-4,0)	3,45 ±0,6 (2,0-4,5)	3,40 ±0,7 (2,0-5,0)	4,26 ±1,0 (3,0-7,0)	4,11 ±1,1 (3,0-6,0)
2	The vertical dimension of the areola (cm.)	3,25 ±1,0 (2,0-4,5)	3,25 ±1,1 (2,0-5,0)	3,28 ±0,6 (2,0-4,0)	3,20 ±0,5 (2,0-4,0)	3,42 ±0,7 (2,0-4,5)	3,45 ±0,7 (2,0-5,0)	4,38 ±0,8 (3,0-6,0)	4,30 ±0,8 (3,0-6,0)
3	Difference of the transverse dimension (cm.)	0		0,08		0,03		0,04	
4	Difference of the vertical dimension (cm.)	0,07		0,04		0,05		0,15	

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Authors:

KAZANOV Vladislav Nikolaevich – plast. surgeon JSC «Victory Clinic», kazavlad@gmail.com; **GARMAEVA Darima Kyshektovna** – doctor of med. sciences, prof. of MI NEFU of M.K.Ammosov; K**HAYRULLIN Radik Magzinurovich** – doctor of med. sciences, prof., head of the Department of the Ulyanovsk state university, prof.khayrullin@gmail.com <mailto:prof.khayrullin@gmail.com; **IGNATYEV Victor Georgievich** - doctor of med. sciences, prof., head of the Department NEFU of M.K.Ammosov.

Kalmin O.V., Khayrullin R.M., Kalmin O.O.

Correlative Interrelationship of Environmental Mineral Impurity and Thyroid Pathology of the Population of the Penza region

Abstract

We studied causation of frequency of thyroid gland pathology in the population of Penza and the Penza region and content of iron, nitrites and fluorine in water; cadmium, copper, zinc and lead in soil. High correlation of microelements in the water and in the soil is noted with the prevalence rate of diffusion craw, hypothyroidism and thyrotoxicosis. Effect of mineralization is manifested not only within the same year, but also there is a delayed effect.

Keywords: thyroid gland, thyroid pathology, ecology, soil mineralization, water mineralization, correlation.

The main role in the formation of thyroid function is given to the level of iodine sufficiency. However, despite the large-scale activities on providing the population with iodine, worldwide increase of prevalence rate of endemic goiter is marked. Moreover, there is evident tendency of leveling indicators of goiter in the male and female population, among the inhabitants of seaside and mountain regions, among the rural and urban population, and people living in non-black and black soils [1,2].

Not previously revealed endemic accidents in Tambov and Voronezh regions have the prevalence of goiter at 15-40% of schoolchildren in rural areas nowadays. In the Arkhangelsk region the frequency of pathology reaches 80-98%. Higher iodine deficiency is found in large areas of the Western (Tyumen region) and Eastern Siberia (Krasnoyarsk Territory, Yakutia), the Republic of Buryatia, the Republic of Tyva [3].

The Penza region, along with many others, can be considered a zone of endemic goiter, as the number of children with iodine deficiency reaches 85.9% [4].

The severity of endemic goiter can be resulted from (in the mode of plus or minus) excess or shortage of a microelement not as an etiological factor [5]. Imbalance of microelements caused by inadequate or excess intake contributes to the development of goiter: selenium (a component of the enzyme iodothyronine deiodinase - enzyme responsible for peripheral conversion T4 to T3 in liver and

kidneys), zinc (effect on thyroid stimulating hormone secretion, suppresses lead and copper toxicity), chromium, bromine, cobalt, copper, iron (involved in conversion of phenylalanine into tyrosine) molybdenum, cadmium, calcium, fluorine, phosphorus, potassium, lead (violates conversion of T4 to T3), mercury (violates metabolism of thyroid hormones), lithium, chlorine. In particular, it was found that the larger the indices of microelements in association links are: Mn: Co: Zn: Cr: Pb, the higher the prevalence of goiter in the population is. And people with increased thyroid gland in urine had higher parameters of I: Mn, I: Co , I: Zn, I: Pb, indicating worsening imbalance of trace elements [6,7].

The aim of this work was to study the relationship between frequency of thyroid pathology of residents of the Penza city and the Penza region and content of microelements in water and soil.

MATERIALS AND METHODS

Materials of the research included statistical data on incidence rates of thyroid disorders of residents of Penza city and the Penza region and the level of mineral contamination of the environmental components from 2000 to 2013. The analysis of the content of iron, nitrites, fluorine in the water; cadmium, copper, lead and zinc in the soil was conducted. All cases of thyroid pathology were divided into 5 groups: diffuse goiter, multinodular goiter, hypothyroidism, hyperthyroidism, thyroiditis.

Quantitative data were processed by variational-statistical methods, using the non-parametric correlation and dispersion factorial analysis using the software package IBM SPSS Statistics v22.

RESULTS AND DISCUSSION

The analysis of the incidence of thyroid pathology showed that the incidence of diffuse goiter was maximal in Bessonovsky and Nikolsky districts in 2006 (240.8 and 214.2 cases per 100 thousand population, respectively), Gorodishchensky (39.7) and Kamensky (327 1) districts - in 2008, in the Kuznetsky - in 2002 (285.9), Penza - 2001 (168.2).

The incidence of multinodular goiter was maximal in Bessonovsky district in 2004 (28.6) in Gorodishchensky - in 2009 (27.9), in Kamensky district - in 2008 (492.8) in Kuznetsky and Nikolsky district - in 2010 (115.2 and 153.2, respectively), in Penza - 2007 (52.63 cases per 100 thousand. population).

The incidence of hypothyroidism was maximal in Bessonovsky district in 2006 (114.5 cases per 100 thousand population) in Gorodishchensky district and the city of Penza - in 2009 (41.8 and 85.8, respectively), in Kamensky district - in 2008 (170.3) in Kuznetsky and Nikolsky districts - in 2011 (76

and 175.5, respectively).

The incidence of hyperthyroidism in Bessonovsky, Nikolsky districts and the city of Penza was maximum in 2004 (38.2, 67.3 and 23.1, respectively), Gorodishchensky district - in 2007 (77.1), in Kamensky region - in 2008 (80.6), in Kuznetsky - in 2000 (52.7 cases per 100 thousand population).

The incidence of thyroiditis reaches its maximum value in Bessonovsky district in 2008 (77.1) in Gorodishchensky - in 2011 (30), in Kamensky district - in 2008 (313.6) in Kuznetsky and Nikolsky districts and Penza - 2007 year (175.61, 97.06 and 35.4 cases per 100 thousand. population, respectively).

The analysis of the mineral composition of the environment showed that the content of iron in drinking water in Bessonovsky district reached its maximum value in 2011 was 0.88 mg / l, Gorodishchensky district - in 2013 (0.21 mg / l), in Kamensky - in 2007 (0.21 mg / L) in Kuznetsky - in 2012 (0.87 mg / l), in Nikolsky district - in 2008 (0.22 mg / l), Penza - 2003 (0.56 mg / l)..

Nitrite content in water of Bessonovsky district was the most in 2002 (1.74 mg / l), in Gorodishchensky district and Penza - 2007 (0.07 and 0.05 mg / L, respectively), in Kamensky district - in 2008 (0.12 mg / L) in Kuznetsky district - in 2006 (0.04 mg / l), and in Nikolsky districts - in 2010 (0.18 mg / l).

The level of fluoride in drinking water was maximal in Bessonovsky district in 2008 (2.56 mg / l), in Gorodishchensky district - in 2013 (0.16 mg / l), in Kamensky and Nikolsky districts - in 2011 (0, 88 and 0.17 mg / L, respectively), in Kuznetsky district and the city of Penza - 2009 (0.38 and 0.26 mg / L, respectively).

The content of cadmium in the soil reaches the highest value in Bessonovsky district in 2006 (0.12 mg / kg) in Gorodishchensky - in 2013 (0.2 mg / kg) in Kamensky and Nikolsky districts - in 2005 (0.11 and 0 12 mg / kg, respectively), in Kuznetsky district - in 2008 (0.19 mg / kg) in Penza - 2004 (0.19 mg / kg).

Copper levels in the soil was highest in Bessonovsky and Kamensky district and the city of Penza in 2007 (10.73, 9.48 and 9.75 mg / kg, respectively), in Gorodishchensky district - in 2009 (7.79 mg / kg) in Kuznetsky and Nikolsky districts - in 2012 (10.62 and 7.15 mg / kg).

The zinc content in the soil was maximum in Bessonovsky and Kuznetsky district in 2011 (27.93 and 56.69) mg / kg, Gorodishchensky district in 2010 (49.8 mg / kg) in Kamensky region - in 2008 (25 2 mg / kg 8), in Nikolsky districts - in 2012 (53.9 mg / kg) in Penza - 2009 (62.05 mg / kg).

The level of lead in soil is highest in Bessonovsky district and the city of Penza in 2008 (11.44 and 9.3 mg / kg) in Gorodishchensky and Kuznetsky districts - in 2009 (17.35 and 16.36 mg / kg,

respectively), in Kamensky district - in 2011 (10.19 mg / kg), in Nikolsky districts - in 2013 (19.2 mg / kg).

Analysis of the effect of the level mineralization of the water and soil on thyroid morbidity discovered that the zinc content in the soil in 2000 has a strong correlation with the incidence of diffuse goiter (r = 0,80), multinodular goiter (r = 0,75) and hypothyroidism (r = 0,81) 2000, cadmium content in soil in 2000 affects hypothyroidism morbidity in 2001 (r = 0,74) and 2002 (r = 0,72) year, and an influence on the incidence of diffuse goiter 2002 (r = 0,89) the level of copper in the soil in 2000 has a strong influence on the incidence of hypothyroidism in 2002 (r = 0,82).

The iron content in the water in 2001, has a strong correlation with the incidence of hypothyroidism in 2001 (r = 0,78). The fluorine content in the water in 2001 and 2002, has a strong correlation with the incidence of thyrotoxicosis in 2003 (r = 0,82). The iron content in water in 2004 relates to the incidence of hyperthyroidism in 2006 (r = 0,75), the concentration of iron in water, 2005 - with an incidence thyrotoxicosis 2007 (r = 0,85) the copper content in the soil in 2005 year has a proven relationship with the incidence of thyrotoxicosis in 2006 and 2007 (r = 0,75 and 0.78, respectively). The concentration of iron in the water in 2006, has a strong correlation with the incidence of thyrotoxicosis in 2006 and 2007 (r = 0,74 and 0.85, respectively). Nitrite content in the water in 2009 - with an incidence of hyperthyroidism in 2009 (r = 0,81). The lead content in the soil in 2011, is closely related to the level of hypothyroidism in 2012 (r = 0,86).

The One-way ANOVA test showed that there is significant effect of nitrite water and the soil cadmium on the incidence of hypothyroidism, as well as the iron content of water on the prevalence rate of thyrotoxicosis on 99% .

The identified patterns of influence of water salinity and soil on public health are consistent with the results obtained by Ya.G. Adamova (2003) in the Saratov region and L.M. Farkhutdinova (2007) in the Republic of Bashkortostan, in particular, confirmed the influence of copper and cadmium levels of hypothyroidism, fluorine and iron - to hyperthyroidism, which testifies to the unity of the pathogenetic processes occurring in thyroid gland of the inhabitants of different regions. Comparison of mineralization of water and soil of the Penza region with the state of the environment of the Saratov region and the Republic of Bashkortostan showed that the soil of the Penza region has a much higher zinc content than the soil of Bashkortostan (2 mg / kg), but approximately similar concentrations of iron, lead, zinc, copper of soil and water of Saratov region [8,9]. However, it should be noted that the dependence of the incidence of contamination was studied by Ya.G. Adamova and L.M. Farkhutdinova only within the same year, without taking into account long-term effects, which is undoubtedly present.

Conclusion

Thus, the study showed that there is direct relationship between the concentration of minerals in the water and soil and the incidence of thyroid disorders. It has been established that there is the strong correlation between the concentration of cadmium in the soil and the level of diffuse goiter and hypothyroidism, concentrations of iron, nitrite, fluoride of water and level of thyrotoxicosis. At the same time there is also a delayed effect of mineralization on the incidence of subsequent years.

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Authors:

Kalmin Oleg Vitalievich

FSBEI HPE «Penza state university», head of human anatomy, professor, doctor of medical sciences

ovkalmin@gmail.com <mailto:ovkalmin@gmail.com>

Khairullin Radik Magzinurovich

FSBEU HPE « Ulyanovsk state university», head of human anatomy, professor, doctor of medical sciences

prof.khayrullin@gmail.com <mailto:prof.khayrullin@gmail.com>

Kalmin Oleg Olegovich

FSBEI HPE «Penza state university», teachjing assistant of human anatomy

kalmin.o.o@gmail.com <mailto:kalmin.o.o@gmail.com>

Saharyuk A.P., Grigorenko A.A., Tarasyuk E.S., Shimko V.V., Verevetinov A.N.

Clinical and Pathologic Analysis of Venous Thromboembolic Complications in the Amur region

ABSTRACT

The study of mortality rate due to venous thromboembolic disorders (VTED) for 2009-2011 was conducted based on autopsy studies. The plan of the survey: age, gender, time of the year, disease, date of death, a source of primary thrombus formation, changes in lung tissue, a level of pulmonary venous obstruction by embologenic masses. The mean age of VTED deceased people was 58 years. The women were noted more frequently than the men. The VTED cases occurred mostly in winter months. The pathology structure treated: a postoperative period after multidisciplinary surgical interventions, of strokes, multidisciplinary therapeutic pathology. The venous sinuses of lower leg were one of the common causes of primary thrombi formation.

Keywords: venous thrombosis, venous thromboembolic disorders (VTED), pulmonary embolism (PE)

INTRODUCTION

Pulmonary embolism (PE) is one of three causes of sudden death along with stroke and heart attack. PE is the reason of death 0.1% of the Earth population, as a result of pulmonary embolism mortality rate is much higher than road traffic injuries, lung cancer and pneumonia, as well as causes 10-12% of all deaths in hospital [1,10]. In the hospital pulmonary embolism annually observed in 15 - 20 out of 1,000 treated patients. Often it complicated by injuries, post-operative, post-partum period [4, 5, 6]. The number of VTEC is constantly growing. The general cause of pulmonary embolism is thrombosis of the veins lower limbs. Deep vein thrombosis of the lower limbs - a common disease, the frequency of it is 100 - 160 cases per 100 000 population, with the frequency of fatal thromboembolism 60 cases per 100 000 population. In the United States fixed 8-20 million Cases of deep vein thrombosis. Venous thrombosis often have not symptoms, as are the nature of parietal character, does not violate the outflow of blood through the vein. So asymptomatic thrombosis, identify by radiometry with fibrinogen labeled with 125 I, and venography diagnosed with myocardial infarction in 5 - 20% of patients, brain stroke - 60 - 70%, diseases of internal organs - in 10 - 15% after orthopedic surgery - 50 - 75% of prostatectomy - 40% in the abdominal and thoracic surgery - a 29 - 30% of patients. Mortality

of thromboembolic complications varies between 2.1 and 6.2%. According to the American Medical Association, each year in the United States fixed 650 thousand Cases of pulmonary embolism, 356 of which end in death of the patient. 10% of patients with pulmonary embolism develops very fast and leads to death within hours after the first symptoms [2]. Most of these fatal cases was diagnose only at autopsy. [8] Early treatment of pulmonary embolism could have a very high effect. So, more than 90% of patients, who died of pulmonary embolism, are those who was not correct diagnosed, and not received right treatment [7,9]. Statistical analysis of patients with VTEC not so good, because the construction of VTEC diagnosis of the patient not pass on the statistical codes causes of death. This investigation dedicated to Actualization of VTEC problems.

The purpose of the investigation. Examine the role of venous thromboembolic events (VTEC) in the structure of sudden death. Create ways to prevent the development of VTEC and massive pulmonary embolism in hospitalized patients Amur region.

Objectives of the Investigation:

1. To determine the lethality of VTEC in Amur region per 1000 population per year.
2. To characterize the sex and age parameters of a patient who died of pulmonary embolism.
3. Analyze the seasonality and frequency of VTEC, depending on the time of year.
4. Identify the basic pathology trigger the development of venous thromboembolic complications.
5. Set the timing of death from VTEC since the beginning of hospitalization or venous thrombosis.
6. Examine the primary sources of thrombosis VTEC.
7. Determine the character of morphological changes in the lung tissue with VTEC.
8. SET level of thromboembolic venous obstruction with pulmonary embolism.

Materials and methods

Investigated the mortality of VTEC in three years on the results of postmortem explorations. Complied with ethical standards postmortem explorations, performed statistical processing of material. Patients, who died suddenly in Amur region and city hospitals in Blagoveshchensk was been investigated. Plan of exploration: age, gender, time of the year, disease, date of death, the primary source of thrombus formation, changes in lung tissue, the level of pulmonary venous obstruction embologenic masses.

The results of the study. For three years in Pathological Anatomy Department of the Amur Region Clinical Hospital made 1764 explorations of patients who died in ARCH and Blagoveshchensk city hospital. 96 (5.4%) patients the cause of death were venous thromboembolism. The average age of patients who died of VTEC was 58 years old. 56 women (58.3%), 40 men (41.7%). Most often VTEC occurred in the winter 28 (29.2%), in the summer of 24 (25%), in the autumn of 23 (24%), in the spring of 21 (21.9%). Pathology about which patients received inpatient treatment: postoperative period after multidisciplinary surgical interventions 30 (31%), strokes - 31 (32%), 35 (37%) - multidisciplinary therapeutic pathology. Thromboembolism of 18 (20%) patients developed until 24 hours after admission, in 23 (25%) after 2-7 days, in 55 (57%) more than 7 days. The primary source of thrombus in 50 (52%) were the venous sinuses of tibia, femoral-popliteal-iliac segments and inferior vena Vienna - 21 (22%), pulmonary heart 10 (10%), and the source was not detected in 19 (16%). Nonspecific changes as result of obstruction of pulmonary channel were find in 48 (50%) patients. Thromboembolism pulmonary trunk and pulmonary arteries were register in 69 (72%) patients. The cause of death in 27 (28%) was smaller thromboembolism of segmental arteries.

Discussion of the results. Right statistics on morbidity and mortality from PE is unknown, but about the prevalence of PE is 0.5 to 2 per thousand per year [1, 2]. According to our information, pulmonary embolism as the cause of death was 5.4% of the total number of postmortem explorations that is 0.8 per 1000 population per year in Amur region. The number of non-fatal asymptomatic thromboembolism of pulmonary channel is not yet possible to determine.

With age, the prevalence of VTEC is growing exponentially: from 0.05 per 1,000 children under the age of 15 years, up to 6 per 1,000 in the age group over 80 years. Increased frequency of pulmonary embolism with age can be explain by accumulation of comorbidities, which are the factors of risk. The average age of patients, who died of pulmonary embolism in the Amur region, was 58 years old.

Receiving oral contraceptives and hormonal therapy in women postmenopausal, increases the frequency of VTEC, which consistent with our information. 56 (58.3%) of women and 40 (41.7%) of men.

Most often VTEC occurred in the winter months 28 (29.2%), in the summer of 24 (25%), in the autumn of 23 (24%), in the spring of 21 (21.9%), which consistent with medical literature information, the number of VTEC in the winter 10-15% higher, due to a decrease in Humans motor activity during the winter months.

Structure pathology about which patients received inpatient treatment: postoperative period after multidisciplinary surgical interventions 30 (31%), Strokes - 31 (32%), 35 (37%) - multidisciplinary therapeutic pathology consistent with medical literature information and requires strict reference of Protocol prevention in these patients. Thromboembolism of 18 (20%) patients developed until 24hours after admission, 23 (25%) after 2-7 days, 55 (57%) more than 7 days. The time factor since the primary thrombotic events, of thromboembolism small, lobar and segmental arteries, allows provide preventive care to 80% of patients and prevent the development of a massive pulmonary embolism. The primary source of thrombus in 50 (52%) were the venous sinuses tibia, femoral-popliteal-iliac segments and inferior vena Vienna - 21 (22%), pulmonary heart 10 (10%), and the source was not detected in 19 (16%). Nonspecific changes as result of pulmonary infarction were found in 48 (50%) patients, which allows diagnosing embologenic venous thrombosis in these patients before develop of massive PE. According to various authors, embolization of the trunk and main branches of the pulmonary artery occurs in 50% of the lobar and segmental - 22%, small branches - in 30% of cases. In our investigation embolism of trunk and main branches was observed in 72% (69 patients), small, and segmental, lobar of 28% (27 patients). This allows prevents the development of massive PE of 2/3 suddenly death of patients [3].

Based on the Investigation results, Create methods of preventing the development of VTEC and massive pulmonary embolism of hospitalized patients in Amur Region. Innovations (№ 1880 from 06.08.2014 "Curation patients with thromboembolism lobar, segmental and small branches of the pulmonary artery»; № 1878 from 06.08.2014 "The organization of the prevention and treatment venous thromboembolic complications in the hospital»; № 1881 "The treatment of embologenic venous thrombosis »; № 1882 from 06.08.2014 "Screening ultrasound diagnosis asymptomatic venous thrombosis in patients with fractures of the femoral neck, the long bones of the lower extremities"; № 1879 from 06/08/2014 "Initiating the implementation of informed consent for the prevention of venous thromboembolic events (VTEC) in hospital»; № 1885 from 08.06.2014 "Mortality from venous thromboembolic complications in the Amur Region»; № 1888 from 07.08.2014 "The role of thrombosis and pulmonary embolism sources in patients with venous thromboembolic complications "; № 1887 from 07.08.2014 "Curation of postoperative surgical patients"; «№ 1889 from 08.07.2014" Surgical prophylaxis massive pulmonary thromboembolism in orthopedical patients"; № 1884 from 08.06.2014 "nonspecific changes of lung tissue as a result of lobar, segmental and thromboembolism of small branches of the pulmonary arteries"; № 1883 "Terms of mortality from venous thromboembolic complications in

the hospital"; №1886 from 07.08.2014 "Search asymptomatic venous thrombosis in the postoperative period"; № 1890 from 07.08.2014 "The structure of mortality from venous thromboembolic complications in the hospital"), which are entered in the work of center for cardiac surgery State Educational Institution of Higher Professional Education Amur State Medical Academy of the Ministry of Health and Social Development of the Russian Federation; Autonomous public health agencies of the Amur region Amur Regional Children's Hospital; State Organization of the Amur Region City Hospital; State Organization of the Amur region Amur Regional Oncology Center

CONCLUSION

1. Mortality from pulmonary embolism in the Amur region is 0.8 per 1000 population per year.
2. The average age of patients who died of pulmonary embolism - 58 years.
3. Gender were women 58% and 42%, respectively.
4. Every third patient died in the wintertime, every fourth summer and fall.
5. One-third of patients died in the postoperative period, the second third with strokes, and 30% were been hospitalized for severe multidisciplinary therapeutic pathology.
6. About 20% died of massive pulmonary embolism in the first days after receipt of 25% died in 2-7 days, 57% more than 7 days to several months.
7. The primary source of thrombosis in 52% of the venous sinuses were the tibia, femoral-popliteal-iliac segment and inferior vena Vienna - 22%, right heart 10%, was not detected in 16%.
8. Non-specific changes as result of obstruction of pulmonary channel found in every second patient.
9. 2/3 patients had an acute occlusion of the pulmonary trunk and main branches of the pulmonary artery.

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Sakharyuk A.P., Grigorenko A.A., Tarasyuk E.S., Shimko V. V., Verevetinov A.N.

An active surgical approach of treatment of embologenic venous thromboses in the basin of the lower vena cava

ABSTRACT

Surgical approach of treatment of embologenic venous thromboses in the inferior vena cava segment is presented. The most part of patients are operated in the first days of admittance. They were mainly in the subacute period of venous thrombosis that testifies to insufficiently effective work of primary surgical link and surgical departments. Operations were performed on iliac segment, inferior vena cava, including either thrombectomy from the suprarenal inferior vena cava or thrombectomy from the right heart or embolectomy from pulmonary arteries in temporary occlusion of vena cava, bandaging of the infrarenal vena cava segment, nephrectomy, alloprosthesis of the suprarenal vena cava segment. The active surgical approach of treating patients with floating thromboses of the inferior vena cava segment allows to save patients in 98% cases.

Keywords: venous thrombosis, venous thromboembolism, pulmonary thromboembolism (PTE).

The problem of venous thrombosis and pulmonary thromboembolism is an interdisciplinary problem, typical medical facility of any type. Frequency of venous thrombosis and thromboembolic complications is 100 - 160 cases per 100 000, with a frequency of fatal thromboembolism 60 cases per 100 000 population. Mortality from thromboembolic complications in the general population ranges from 2.1 to 6.2%. According to our information 5.4%, which is 0.8 per 1000 population per year [1, 2, 3, and 5]. According to the literature, 10% of patients develop massive pulmonary embolism caused by total occlusion of the pulmonary trunk, where the time factor does not allow saving the patient from death in our investigation 20% of patients died in the first day since the beginning of the disease [4]. Most of these fatal cases are diagnose only at autopsy [6, 7, and 8]. The timely prevention of migration floating thrombus can prevent sudden death from a massive pulmonary embolism.

The aim of the research: to reduce mortality from massive PE by developing scientific direction in the surgical treatment of thrombosis embologenic basin inferior vena cava.

MATERIALS AND METHODS

Based on innovations of Saharyuk A.P., Tarasyuk E.S., Verevetinova A.N. issued by the Medical University Amur GMA Russian Ministry of 08.06.2014 № 1878 "Organization of prevention and treatment of venous thromboembolic complications in the hospital"; №1881 "The

treatment of venous thrombosis embologenic"; № 1879 from 08.06.2014 "Initiate implementation of informed consent for the prevention of venous thromboembolic events (VTEC) in hospital"; № 1888 from 07.08.2014 "The role of thrombosis and pulmonary embolism sources in patients with venous thromboembolic complications"; № 1887 from 07.08.2014 "Curation of postoperative surgical patients"; № 1883 "Terms of mortality from venous thromboembolic complications in the hospital"; № 1890 from 07.08.2014 "The structure of mortality from venous thromboembolic complications in the hospital" we have investigated gender and age characteristics of patients, the timing of surgical treatment of venous thrombosis after hospitalization period, thrombus flotation level, the character of surgery, complications and outcomes.

RESULTS OF THE RESEARCH

Over 5 years - 2009-2013 at the Department of Vascular Surgery of the Amur Regional Clinical Hospital performed 4193 operations, while 147 (3.5%) patients were operate on for various localization of floating thrombosis. The number of patients by gender was the same: M - 72, W - 75, the average age was 52.4 years. 3/4 patients (75%) were operated in the first day of admmittance: 60 (41%) to 6:00, 50 (34%) to 24 hours. ¼ patients 37 (25%) were operated over a day or more after hospitalization. One-third - 42 (29%)were registered with the acute phase of venous thrombosis, two-thirds of 105 (71%), sub acute in 53 (35.8%) - more than a third of patients, floating thrombus was in the ilio-caval segment of basin inferior vena cava: inferior Vena cava - 9 (6.1%), common iliac Vena - 4 (2.7%), external iliac vena - 40 (27%) patients. IVC flotation level of suprarenal was present in 7 patients (4.7%), 2 patients (1.4%) on the renal level segment. Two-thirds of patients 93 (63.5%) flotation thrombus localized at the level of the femoropopliteal segment: common femoral Vena - 44 (30%), superficial femoral - 26 (18%), greater saphenous Vena - 12 (8.1%), popliteal Vena - 11 (7.4%). At one patient (0.7%) continued floating thrombosis reached the pulmonary heart.

Every third patient 51 (34.9%) had operation on the iliac segment, including ligation of the external iliac vein - 45 (31%), retrograde thrombectomy of iliac segment - 1 (0.6%), retrograde thrombectomy of iliac segment imposition of arteriovenous fistula at hip level - 1 (0.6%), isolated ligation of the common iliac vein - 4 (2.7%). Every fourth patient ligated superficial femoral Vein 38 (25.6%), including 3 (2%) in combination with crossectomy major saphenous vein, 1 (06%) crossectomy small saphenous vein opposite limb. As fourth patient 33 (22.8%) made thrombectomy common femoral vein, including 4 (2.7%) was isolated, 16 (10.8%) in

combination with ligation of the superficial femoral vein, 11 (8%) combined with crossectomy major saphenous vein and 2 (1.3%) in conjunction with ligation of the superficial femoral and major saphenous veins. Every fifth patient - 29 (20.3%) made crossectomy the major saphenous vein, including 13 (9%) in isolation. In 13 patients (9.3%) combined with a thrombectomy of the common femoral vein 11 (8%), and 2 patients (1.3%) and a superficial vein ligation. 3 (2%) patients only crossectomy combined with ligation of the superficial femoral vein.

Eleven operations (7.6%) were performed on the inferior vena cava. It includes 8 thrombectomy of the suprarenal region of the inferior vena cava, including in conjunction with: thrombectomy of the pulmonary heart - 1 (0.6%); embolectomy from the pulmonary artery in a temporary occlusion of the vena cava, ligation of the infrarenal region of inferior vena cava - 1 (0.6%); infrarenal ligation of the inferior vena cava - 1 (0.6%); nephrectomy - 4 (2.6%); alloprosthetics of suprarenal region of inferior vena cava - 1 (0.6%). Three patients (2%) were operate on again, on the retroperitoneal hematoma. 3 patients are die (2%) of re-thrombosis basin inferior vena cava and recurrent pulmonary embolism.

DISCUSSION

Experience of the Department of Vascular Surgery can recommend active treatment policy of floating venous thrombosis, which are the lot of young persons and working-age population in practical healthcare. Pathology is urgent, and 75% of patients operated at the first day of hospitalization, with this 71% of patients were in the sub acute stage of venous thrombosis, so we may talk about not effective work of the primary surgical care and surgical departments. 35.8% patients the level of the proximal thrombus flotation was at ileoCaval segment, 63.5% at the level of the femoropopliteal segment. One patient (0.7%) continued floating thrombosis reached the pulmonary heart. less than half of the operations 62 (42.5%) performed on ileokaval segment and 85 (57.5%) in the femoropopliteal. About embologenic complications of varicose veins performed on 29 (20.3%) operations. Eleven operations (7.6%) made on the inferior vena cava. This include 8 thrombectomy of the suprarenal of the inferior vena cava. Three patients (2%) were operate on again, on the retroperitoneal hematoma. 3 patients are dye (2%) of re-thrombosis basin inferior vena cava and recurrent pulmonary embolism.

CONCLUSIONS:

1. Embologenic venous thrombosis is the pathology of young, working-age people (average 52.4 years).
2. Three quarters of the patients, (75%) were operated during the first day of hospitalization, thus avoiding the massive, fatal pulmonary embolism in 98% of patients.
3. Not effective work of primary care confirmed that 71% of the patients were in the sub acute stage of thrombosis.
4. Interventions at ileoCaval segment of the inferior vena cava to 42.5% require high professionally of cardiovascular surgery and the development of targeted research direction in solving this problem.
5. Operations on the femoropopliteal segment were performed in 57.5%, they are positively predictable and can reliably prevent pulmonary catastrophe.

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Authors:

SBEI HPE «Amur State Medical Academy» MH RF:

Sakharyuk Alexander Petrovich – doctor of med. sciences, prof., fyubj@yandex.ru;

Grigorenko Alexey Aleksandrovich – doctor of med sciences, head of the department;

Tarasyuk Evgeniy Sergeevich − post-graduate student;

Shimko Vladimir Vasilevich – doctor of med. sciences, prof.;

Verevetinov Artem Nikolaevich − post-graduate student.

V.A. Tolokolnikov

Pathomorphologic Changes of Uterus with the Development of Alimentary Magnesium Deficiency and in Sulfate and Taurine Magnesium Correction States

ABSTRACT

In this research we established structural changes in all layers of rats' uterus due to alimentary magnesium deficiency accompanied by significant deviations of quantitative morphological indices that testify to multidirectional dynamics of changes with prevalence of impaired blood circulation and development of atrophic processes. When carrying out pharmacological correction of alimentary magnesium deficiency by magnesium sulfate and magnesium taurinat there has been positive dynamics of various on expressiveness with compensatory and adaptive changes in an uterus.

Keywords: alimentary magnesium deficiency, reproductive system, uterus.

INTRODUCTION

In the study of pathology of pregnancy and childbirth, researchers mostly take into account a problem of magnesium deficiency states [5]. In practice for the prevention and treatment of hypertonic uterine dysfunction of the fetoplacental system and generic activities can be used in a variety of magnesium-containing drugs [12, 7]. Study of the structural mechanisms of the influence of magnesium deficiency on the organs of the female reproductive system and the search for the optimal drugs used for pharmacological correction of pathological conditions associated with miscarriage and pathological conditions of delivery, is an important issue of obstetrics and gynecology [8]. In addition, data on the degree of compensatory changes in the uterus in terms formed deficiency of magnesium state and its pharmacological correction of various preparations of magnesium remains controversial [11, 9]. The search for regularities in the development of pathological changes is an important task and at the present time. Therefore, the purpose of this study is to identify major patterns of morphological changes in the uterus of rats at dietary deficiency of magnesium and its pharmacological correction in conditions of experimental simulation.

MATERIALS AND METHODS

The study was conducted at 70 outbred rats females, weighing 180-200 g at the age of 6 months. 1-St group [n=10] was intact females. To determine the phase of the sexual cycle for 10 consecutive days, each female was taken vaginal swab to determine the phase and the patterns of flow of the ovulatory cycle in selected into the experiment animals. In the 2nd group [n=10] included females who were at deficiency of magnesium diet for 12 weeks. Modeling dietary deficiency of magnesium was performed using a special deficiency of magnesium diet similar to the diet, manufactured by MP Biomedicals (Aurora, Ohio, USA) with a 3.5% mineral content of the mixture that does not contain magnesium. All diets were prepared using deionized water, the same water during the experiment was used as drinking water for animals on a diet. The speed and depth of the development of hypomagnesaemia controlled by defining the content of

magnesium in plasma and erythrocytes of animals, spectrophotometric method for color reaction with titanium yellow [6].

The 3rd [n=10] and 4th [n=10] group included females who had no magnesium diet for 12 weeks and since 9 to 12 weeks were treated orally through a feeding tube 50 mg/kg of body weight nutritional magnesium as magnesium sulfate (Magnesium sulphate), magnesium taurinate (Magnesium N-acetyltaurate), respectively.

Morphometric research was conducted with use of the program Video Test Morfo 4, statistical processing is carried out with use of the standard software package of Statistika 6.0 for Windows. For all types of the analysis as the statistically significant considered distinctions at $p < 0,05$ [1, 3].

RESULTS

Histological examination of the uterus experimental animals with non-magnesium diet (group 2), we found out that pathological changes were manifested in the form of nonspecific changes, vascular disruption of the microcirculation with subsequent pathological changes of endometrium and myometrium. Endometrial stroma was sealed with a small amount and uneven location of the uterine glands. From microvasculature of the endometrium were observed partial empty vessels. Myometrium was characterized by degenerative changes of myocytes with vacuolization of the cytoplasm, swelling intermuscular space and a plethora of microcirculatory vessels. In the stroma, compared with the control group there was a decrease in the number of eosinophils in 10.7 times ($p<0.05$) with the presence of isolated lymphocytes. According to a study it was found that the weight of the uterus of rats by without the magnesium diets decreased by 66% ($p<0.05$). When morphometric comparison with the control group, the thickness of the wall of the uterus was reduced by 70% ($p<0.05$), and the thickness of the walls of blood vessels increased by 35 % ($p<0.05$). Decreased the volume fraction of the endometrial glands, 40% ($p<0.05$), the height of a single layer of columnar epithelium and the volume fraction of the cores of the single-layer columnar epithelium, increased by 48% ($p<0.05$) and 50% ($p<0.05$), respectively.

When determining the duration of the estrous cycle in animals of the 2nd group there was an increase in the duration of the dioestrus phase 2 week 94,12 % ($p<0.05$), proestrus 100% ($p<0.05$), duration of estrus phase decreased by 46,94 % ($p<0.05$). On the 2nd month of the study significantly decreased the duration of the phase of the estrus at deficiency of magnesium animals on 43,33 % ($p<0.01$). When carrying out the correction of nutritional deficiency of magnesium has been a slight increase in the duration of estrus phase and decrease the duration of phase proestrus the infusion of magnesium-containing drugs.

In the correction of nutritional deficiency of magnesium with drugs such as magnesium sulfate and taurinate magnesium, endometrial thickness was significantly decreased by 36.6% ($p<0.05$) and 27% ($p<0.05$), respectively, relative to the control group, but noted that the increase in endometrial thickness in relation to the group nutritional deficiency of magnesium on 107,24% ($p<0.05$), and in the correction of taurinate magnesium 138.48% ($p<0.05$).

In the study of the myometrium in animals of the 3rd group (correction magnesium sulfate) there was increase of its thickness on 26,29% ($p<0.05$) in animals of the 4th group

(correction taurinate magnesium) thickness of the myometrium decreased by 10,48% ($p<0.05$) relative to the intact group.

According to the morphometric study in the 4th group (taurinate magnesium correction) ekzokrinotsit glands returned to normal, and the volume fraction of the cores of ekzokrinotsit glands significantly increased by 56,95 % ($p<0.05$), compared with the control group. When correcting magnesium sulfate height of ekzokrinotsit glands was significantly decreased by 42.45% ($p<0.05$), volume ratio (VR) of nuclei ekzokrinotsit cores glands was increased by 43,69% ($p<0.05$) as compared to intact animals (table 1).

DISCUSSION

We found out structural changes in the uterine wall at the nutritional magnesium deficiency and disruption of the estrous cycle indicate that inhibition of reproductive function developed as a result of electrolyte imbalance, pathomorphological changes in microcirculatory vessels, as evidenced by the decrease in the concentration of magnesium in the blood plasma of experimental animals and is consistent with the available experimental data, and typical age-related disorders of the reproductive system, and may be associated with impaired hypothalamic-pituitary regulation [4].

When correcting the deficiency of magnesium status with magnesium drugs, structural changes of the uterus is less expressed, as evidenced by qualitative changes: the reduction of the phenomena of swelling, disruption of the connective tissue of all layers of the uterus and its vascular system, evidenced by the results of morphometric studies, and literature data [10, 2], which appears to be associated with activation of compensatory mechanisms and vasoprotective and anti-inflammatory effects of various salts of magnesium.

CONCLUSIONS

Thus, it was found that the modeling deficiency of magnesium state observed structural changes in all layers of the uterus in rats, accompanied by significant deviations quantitative morphological indicators showing mixed dynamics change with the prevalence of circulatory disorders and the development of atrophic processes of nature.

When pharmacological correction of nutritional deficiency of magnesium taurinate or magnesium sulfate resulted in incomplete recovery of the structural components of the endometrium and myometrium, accompanied by increase in volume fraction of the uterine glands to control values when using magnesium sulfate, which indicates the existence of differences in drug pathomorphosis deficiency of magnesium condition and may explain the differences in the nature and dynamics of changes in reproductive function of rats.

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Authors
Tolokolnikov Vasiliy A. - assistant of the department of pathological anatomy of the Volgograd state medical university, post-graduate student
Contact phone: 8 960 883 20 84,
E-mail: ppeva73@yandex.ru

Morphometric indices	The control (intact)	Magnesium deficiency	Magnesium sulfate	Taurinate magnesium
Endometrial thickness, μm	1326,5±124,5	405,6±9,8*	840,6±22,1*#	967,3±24,4*#
The thickness of the myometrium, μm	559,1±12,3	168,5±4,20*	706,1±11,0*#	500,5±13,6*#
The wall thickness of the vessels in the myometrium, μm	26,2±0,2	35,5±0,7*	34,01±0,4*	30,3±0,4*#
The volume fraction of the endometrial glands, %	3,8±0,5	2,3±0,1*	4,1±0,3#	2,3±0,6*
The height of a single layer of columnar epithelium, μm	35,9±2,5	53,3±5,9*	30,5±0,4*#	54,4±0,6*
The volume fraction of the cores of the single-layer columnar epithelium, %	30,1±4,7	39,2±2,8*	46,2±2,8*#	43,4±1,3*#
Height of glands, μm	36,49±3,3	16,5±0,2*	21,0±0,2*#	36,6±0,3#
The volume fraction of the cores Associacao glands, %	30,9±2,1	39,4±2,7*	44,4±2,7*#	48,5±1,7*#

* the results are valid relative to the control group when $p < 0.05$;

- the results are valid for nutritional deficiency of magnesium at $p < 0.05$.

Rakov V.E., Artifeksova A.A.

A role of Ecologic and Industrial Intensity of the Region in Prevalence of Thyroid Gland

ABSTRACT

In this report the profile of thyroid glands diseases is detected subject to iodine deficient background and industrial development of the Vladimirskiy region RF.

Keywords: thyroid glands, iodine deficiency, oncological and nononcological pathology.

INTRODUCTION

According to the data over the past 15 years, the number of newly diagnosed cases of neoplastic diseases of thyroid gland (TG) has grown twice mainly due to young and middle-aged people [1].

According to domestic authors TG pathology is caused by: " Endocrine pathology is mostly environmentally-related including TG diseases, as the structure and function of TG are closely associated with extraneous iodine and other microelements" [2,4]. On the other hand, TG like any other organ is exposed to the effects of man-made factors, industrial and household poisons, some of which are specific features of a particular territory [3,5].

The Vladimirsky region includes areas which are characterized by normal iodine content in water and endemic iodine combined with varying degrees of technical and chemical effects on people. All this creates a multifactor environment influencing on a human-being, it allowing not only to undertake comprehensive assessment of their significance in the development of TG pathology, but also to create a program of preventive treatment and diagnostics of the diseases of this organ.

The aim of this work is to identify a spectrum of TG diseases, depending on the level of iododeficiency and effects of industrial factors in a particular area for the formation of a program of screening analysis and development of tumor and non-tumor pathology prevention.

MATERIALS AND METHODS

For research reports there were data from patients and biopsy materials having been taken for 10 years (2003-2013 years). In the statistical analysis of the results we used methods of epidemiological statistics (intensive and standardized incidence ratio, the chance1, the relative

risk), variation statistics (95% confidence interval, Kolmogorov-Smirnov test on normal distribution, Mann-Whitney test χ_2, exact Fisher test for the small number of observations), the Spearman correlation analysis method , a ROC-curves. We used program Statistica 6.0 for Windows, and Microsoft Office Excel 2003.

RESULTS AND DISCUSSION

Annual average intensive incidence rate of TG carcinoma in the Vladimirsky region for 10 years was noted in 2.7 cases per 100 000 population (93% CI 1.87 -3.71), which means 42 ± 4 new patients per year, due to increase in the absolute number of patients with this disease from 28 in 2003 to 59 in 2013. In 2013 year the incidence almost reached nationwide and the prevalence rate was higher than in 2012, but no reliable differences noted (Mann-Whitney, p> 0.06). OR of the disease was 1 in the regional center. Odds ratios indices (ORI) was significantly higher in the Kovrov on 2.22 (94% CI 1.09 -4.24) and in the Murom district on 4.9 (95% CI -7.76 3.69). At the same time we noted reliably poor ORI of TG cancer compared to the city Vladimir, but not identified in the area.

The attitude of the urban and rural population of TG cancer cases amounted to 84% versus 16%. At the same time, the average urban population areas in 2.4 times the average population areas throughout the research period. The relative risk of developing TG cancer among the urban population stood at 2.16 (95% CI 1.53 -3.07).

The ratio of women to men was 5 to 1. The number of men in the study group was 14.9%, women-85.1% (χ_2= 279.6, p< 0.001). Thus, TG cancer patients were female. What was more – women of middle age.

The average incidence rate among men was intensive 0.9 cases (95% CI 0.7 -1.06) at 100 000 male population a year, women-4.3 cases (95% CI -5.92 2.65) per 100 000 female population per year .Average morbidity during the entire observation period exceeded that of men in 4.8 times (t= 5.3, p< 0.001).

The concentration of iodine in water sources of the Vladimirsky region varies widely. In the areas where the water is taken from artesian sources the iodine content is higher and varies from small limits 0.218 g/l to moderate decline (0.164 -0.141 µg/l). In the areas with ground-based and soil and groundwater iododeficit has been pronounced with concentrations determined by standard methods (in 4 districts and regional center). The average concentration of iodine in

the water of the Vladimirsky region totaled 0.0982 ± 0.06 μg/dm³ (95% CI -0.1181 0.0758 average, median-0.109) (Kolmogorov-Smirnov test, p= 0.452). The minimum content of iodine (at least 0.125 g/DM^3) was determined in 3 districts.

To determine possible TG carcinoma incidence based on the severity of iodine deficiency in the areas we conducted the correlation analysis with the Spearman ‘s correlation coefficient. We found no direct correlation between the level of TG cancer incidence with the concentration of iodine in the water of the examined patients was weak and unreliable (Spearman R= 0.11, p= 0.48).

Next, we analyzed the dependence of the age level of patients with newly diagnosed of the TG carcinoma and iodine concentration in drinking water. This was identified prognostically significant concentration of iodine in the water that was > 0.120 μg/dm³. Based on this indicator, all patients were divided into 2 groups: the average age of patients in the group living on the territory with the level of iodine in water ≤ 0, 120 μg/dm³ totaled 42.4 ± 8.6. At the same time, the average age of the patients with TG carcinoma in a group with the concentration of iodine in the water > 0.120 μg/dm³, was 54.4 ± 9.7 (student's T-test, t= 2.3 (p= 0.042). Thus, with more pronounced iodine deficiency of TG carcinoma was first identified in patients was significantly younger than the group who are living in areas with normal or moderately reduced iodine concentration in drinking water.

It is known that non-tumorous TG pathology directly correlates with the level of iodine deficiency. Many changes to the structure of the thyroid gland in tumor pathology background form the process. Our research showed that 23% of all carcinomas (mainly papillary and follicular variants) as background processes have nodular and diffuse toxic goiter, and chronic thyroiditis and microadenoma. Medullary and anaplastic variants of carcinoma are more likely to develop in the absence of structural gland pathology de novo. Multifactorial analysis of the combinations iododeficit, normal concentration of iodine and non-tumor and tumor (background) pathology did not find significant dependency.

At 76.9% of patients TG cancer was diagnosed due to absence of the pathological process of non-tumor nature. At the same time the most frequent TG adenoma and nodular goiter were background in 10.8% and 6.3% of the cases respectively. TG adenoma and nodular goiter, as well as the absence of any background of pathology were not associated with a specific form of cancer .

The assessment of score range of histological forms of thyroid cancer found out that the relative frequency of papillary thyroid cancer decreased from 2003 to 2013, with 85.2% to 63.6%, however, a reliable decline does not reveal the criteria for χ_2= 1.948,(p)= 0.163. Follicular thyroid cancer rates increased from 11.5% to 35.3%, criteria for χ_2= 3.736,(p)= 0.053. Frequency of anaplastic cancer also did not change significantly (exact Fisher test, p= 0.111). Histological variant of medullary thyroid cancer was rare.

The study of the influence of external factors on TG cancer began with creation of a morbidity map covering all the Vladimirsky area. The data showed that the highest prevalence rate of TG cancer cases (32%) was identified in the regional center. But in some regions high levels were presented due to industrial plans influencing on the ecological status of the region with different intencity.

Thus, on the basis of these data, we found that TG carcinoma was noted mostly at young women in the ioddeficient regions, the malignant tumor with relatively high differentiation occurs on the background of benign processes - adenoma and nodular goiter; tumors with a low degree of differentiation have their own way of anaplasia, there are no background processes, they are more common in industrial centers according to the level of iododeficiency.

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Authors:

Rakov Vasily Evgenevich - correspondence post-graduate student, doctor pathologist of the Murom CRH, **Artifeksova** Anna Alekseevna - doctor med. sciences, prof., head of the Department, artifeksova@yandex.ru
SBEI HPE «Nizhniy Novgorod medical academy» MH RF

Guryeva A.B.

Age Features of Body Proportions of Yakut women in the Republic Sakha (Yakutia)

The study of anthropometric measures and body proportions of Yakut women from different age groups (I and II periods of middle-aged, elderly and senile age groups). The age variation of the length and proportions of women's bodies has been established (higher parameters of body length, lower index of pelvis width, higher index of trunk length at women of the I and II middle-aged period as compared with the women of older age groups). The assessment of body proportionality of the Yakut females of the RS(Y) has identified some not age-related features: rectangle body shape and relative leg shortness. Such anthropological characteristics show that there is age and regional variability of the physical status of the population of Yakutia promoting active life in the contemporary conditions of Yakutia.

Keywords: body proportions, indices, Sakha Republic (Yakutia)

INTRODUCTION

Long habitation of human populations under certain conditions of the environment determines the presence of specific morphofunctional characteristics of the population, peculiarities of vital activity of organism, allowing living and developing under these conditions. Accordingly, the characteristics of both external features and internal organization of humans of the population of different regions and ethnic groups are variable too. The singularity of the natural and climatic conditions of Yakutia (prolonged exposure to low temperatures, photoperiodism, geochemical situation), definite type of nutrition and other factors affected the structural and physiological set-up of people whose ancestors for generations lived in relatively slightly varied environmental conditions [10]. The history of anthropological studies of the peoples of Yakutia covers a period of over a hundred years [1,3,8,11,14]. Researchers were able to identify somatoscopic and somatometric features of the population of Yakutia. Aggregate natural and climatic factors of the Sakha Republic (Yakutia) along with current socio-economic and environmental conditions, and changes in dietary pattern of the population of Yakutia affect the morphofunctional status of the contemporary population, allow adapting to changing environmental conditions. In this regard, since 1997 in the Sakha Republic (Yakutia), the staff of Ammosov North-Eastern Federal University together with the staff of Voyno-Yasenetsky Krasnoyarsk State Medical University has been conducting comprehensive anthropometric study of the adult population of Yakutia [9], including the characteristics of overall dimensions, component body composition, and constitutional characteristics. However, up to the present the

assessment of the proportionality of the body build of the Yakutian adult population and the study of the variability of body proportions have not been carried out. The aim of this study was to establish the body proportions of women of Yakut ethnicity of different age groups, born and resident in Yakutia.

MATERIALS AND METHODS

We have analyzed anthropometric measures of 1227 women of Yakut ethnicity, born and resident in the Sakha Republic (Yakutia). Ethnic affiliation was determined on the basis of personal data (a survey of ancestors' ethnicity for three generations). This limitation to three generations is due to the difficulties in the responses of the surveyed. In accordance with the age periodization of human ontogenesis, adopted at the 7th All-Union Conference on Age Morphology, Physiology and Biochemistry of the USSR Academy of Sciences (1965), examined women fell into four age groups: adult age period I (21-35 years), adult age period II (36-55 years), elderly (56-74) and senile age periods (75-90 years). By social status, the examined women were full-time and part-time students of various faculties of higher and secondary specialized educational institutions of Yakutsk, manual workers, office workers, pensioners of various districts of the Sakha Republic (Yakutia). The study has been pursued after receiving a favorable decision of the local ethics committee subject to clear exclusion criteria, namely the presence at the time of the examination of acute diseases or acute exacerbation of chronic diseases, pregnancy, and refusal of examination survey. Anthropometric measurements were performed using the method of V.V. Bunak (1941) [2] adopted at the Research Institute of Anthropology, Moscow State University (1981). To characterize body proportions, the proportionality indices were calculated: the relative length of trunk, leg, arm, the relative width of pelvis, pelvic-brachial index. The analysis of proportions was made by comparison with average data [12]. Obtained material was processed using the methods of mathematical statistics and SPSS Statistics 17 software. We determined the pattern of each feature distribution with subsequent calculation of value M and its error m, the root-mean-square deviation δ, the coefficient of feature variation V. The Kolmogorov-Smirnov test was used to assess the normality of data distribution. We used methods of parametric and nonparametric statistics. Evaluation of group differences was made using the Student's t-test and the Mann-Whitney U-test [4].

RESULTS AND DISCUSSION

The results of the study have revealed that the average body length measures of women in the examined groups had age differences. Women of adult age periods I and II have higher measures of body length, senile women have lower ones. Higher values of height of women of adult ages I and II are caused by adequately higher rates of absolute measures of trunk length ($p<0,001$) and leg length ($p <0,05$) (Table 1). The pattern of age variability of women's body length is accounted for by the phenomena of secular trend found in various regions of the world [5], as well as age-related changes of the human musculoskeletal system.

The analysis of absolute measures of women's shoulders diameter, pelvis diameter has revealed age differences. The diameter of the shoulders of women of adult age periods I and II was not significantly different and was higher than similar measures of women of elderly and senile ages ($p<0,001$). The diameter of the pelvis of Yakut women had higher values in representatives of elderly and senile ages ($p<0,001$). Increase in shoulders diameter along with a decrease in pelvis size in girls and young women is observed in other regions too [6].

The analysis of the values of proportionality indices has found that the average value of pelvic-brachial index in all examined age groups accounted for more than 74.9, which indicates the rectangle shape of the trunk of examined women. The index of pelvis width has shown that women of adult age periods I and II have average (16.0–17.9) sizes of the pelvis and elderly women have a wide pelvis (index > 17.9). The analysis of the index of trunk length has found that women of adult age periods I and II had a long trunk (index > 52.9), and women of older age groups had an average trunk. The analysis of the index of leg length has shown that the index of less than 54.9 was found in all age groups, which indicates the relative shortness of leg. Relatively short legs of indigenous inhabitants of the continental zone of Siberia were also determined in other studies and explained by the adaptation of organism to low temperature conditions [1]. The index of arm length has shown that Yakut women of adult ages I and II are characterized by relative shortness of arm (index < 45.0), and longness of arm is typical of senile women (index > 46.9).

The study of age variability of anthropometric measures of the physical status of the population is given much attention in medicine, anthropology [7, 9, 13]. The analysis of proportionality indices in relation to the age has revealed significant differences. Thus, Yakutian women of Yakut ethnicity of adult age periods I and II, as opposed to women of elderly age, have lower index of pelvis width and higher index of trunk length.

CONCLUSIONS:

Thus, as a result of the study of anthropometric measures and body proportions of Yakut women of the Sakha Republic (Yakutia), age features have been revealed. The age variability of the length and proportions of women's bodies has been established (higher measures of body length, lower index of pelvis width, higher index of trunk length in women of adult age periods I and II as compared with those in women of older age groups). The assessment of proportionality of the Yakut women's body build has identified the features of proportions that are independent of age. Rectangle body shape and relative shortness of leg are typical of Yakut women of all examined age groups. Revealed anthropological characteristics show that there is age and regional variability of the physical status of the population of Yakutia promoting active life in the current context of Yakutia.

The impact of climatic and geographical, environmental and social factors determined a number of body build proportions peculiar to the female population of Yakut ethnicity of the Sakha Republic (Yakutia). Knowledge of the laws of morphofunctional status formation is the basis for assessing the health of the population. Identification of the regularities of the variability of the population's physical status at the level of individual groups (regional, ethnic and age, profile and others) is important to address the issues of human biology, clinical and preventive medicine, profiling and to achieve good results in sports.

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Table 1

Anthropometric measures of women of Yakut ethnicity of the Sakha Republic (Yakutia)

parameters	Women of adult age I (n=288)	Women of adult age II (n=475)	Women of elderly age (n=284)	Women of senile age (n=180)
Average age, years	28,80±0,23	41,75±0,17	66,58±0,28	80,70±0,26
Body length, cm	159,31±0,35	157,83±0,25	153,86±0,37	149,15±0,34
Body weight, kg	59,05±0,60	63,07±0,51	62,28±0,67	54,20±0,91
Trunk length, cm	87,68±0,26	87,18±0,22	77,97±0,40	78,60±0,25
Arm length, cm	70,15±0,22	69,98±0,22	70,92±0,36	71,12±0,43
Leg length, cm	82,11±0,35	80,52±0,29	80,02±0,41	79,53±0,38
Shoulders diameter, cm	34,89±0,08	34,82±0,06	33,23±0,12	32,14±0,14
Pelvis diameter, cm	27,43±0,08	27,94±0,08	29,14±0,13	28,25±0,13
Trunk length, %	54,88±0,14	55,32±0,06	51,00±0,24	52,66±0,13
Leg length, %	51,35±0,18	51,19±0,10	52,40±0,27	53,26±0,22
Arm length, %	43,90±0,11	44,41±0,05	46,42±0,22	47,61±0,23
Index of relative shoulder width, %	21,92±0,06	22,08±0,04	21,61±0,08	21,54±0,06
Index of relative pelvis width, %	17,23±0,05	17,71±0,05	18,96±0,09	18,95±0,09
Pelvic-brachial index	78,68±0,25	80,33±0.23	88,06±0,54	88,15±0,55

Authors:

Guryeva A.B. - associate professor of normal and pathological anatomy and operational surgery with topographical anatomy and forensic medicine of Medical institute of North Eastern federal university named after M. Ammosov. Yakutsk, Belinskiy street, 58.

O. G. Afanasieva, E.E. Egorova

Morphological Changes of Inguinal and Iliac Lymph Nodes with Modeling Tumor Process

ABSTRACT

Inguinal and iliac lymph nodes regional to a modeling tumor are investigated by methods of the light microscopy. With modeling of the neoplastic process some changes in inguinal lymph nodes connected with angiomegaly of cortical substance are noted. The hyperplasia of a cortical layer is regarded as a reflection of the antagonism of humoral and cellular immunity existing at certain stages of the tumor growth. The structural modifications in modeling of the experimental tumor are marked in a smaller degree in the iliac lymph nodes.

Keywords: lymph node, structure, tumor

INTRODUCTION

The annual increase in incidence and high mortality of patients from cancer of various localizations dictates the need of new effective methods of early diagnostics and treatment of malignant new growths, and also studying of the factors defining the course of oncological diseases and their outcome [4]. Tumoral process causes the morphological changes of regional lymph nodes to the tumor that is a reflection of organism's reaction in general [1,2]. The aim of this research was studying of features of the structural and cellular organization of inguinal and iliac lymph nodes when modeling tumoral process.

MATERIALS AND METHODS

The research experimental animal was outbred mice – males with the initial weight 17-20g. Animals were divided into two groups: 1) intact animals; 2) animals with tumor model. In each group there were 20 individuals, totally 40 animals. The experimental model of tumor was the hypodermic strain of Ehrlich tumor. Modeling of tumor was done by 0,1 ml ascitic liquid injection containing $3,0x10^{5}$ cells under skin of the external surface of the left hip of mice. The inguinal and iliac lymph nodes were taken as the objects of research. The animals of both groups were brought out of the experiment in 5 days after inoculation of tumor cells. All manipulations with animals were carried out under ether anesthesia. Lymph nodes were fixed in Telesnitsky's solution for 24 hours, dehydrated in a series of ethanol of the increasing concentration. Then the studied organs were concluded in paraffin blocks from which the serial cuts on the Reichert

microtome was done along a longitudinal axis of the organ. The cuts of 7-10 micrometers were prepared for a morphometry of structurally functional zones of lymph nodes, for the analysis of cellular structure of lymph nodes we prepared cuts of 7-10 micrometers, for the analysis of cellular structure of lymph nodes we prepared cuts of 3-5 micrometers thick. The histological cuts of inguinal and iliac lymph nodes done by Romanovsky's hematoxylin-eosin stain were studied by light microscope MBS-10 "Biolam". The principles of stereometry and method of imposing of dot morphometric grids have been applied to define the structural organization of lymph nodes. The grid test choice was considered sufficient if not less than 2 dots were the share of the structure occupying the smallest space of the cut. Imposing a grid we counted quantity of the dots which were dropping out on profiles of structures of the lymph node. The received quantity of dots was considered to a total dots of test system. We determined the area of all cut, the area of cortical and brain substance, a capsule, trabeculas, a regional sinus, primary and secondary lymphoid small knots, a cortical plateau and paracortical zone, corda medullaris and brain sinus in lymph nodes. We have counted a ratio of the specific area of cortical substance to the specific area of brain substance for lymph nodes in each experimental group. The cellular structure of lymph nodes was studied on cuts of thickness of 5 micrometers, 2 glemsa-stained slide. Lymphoblasts, average and small lymphocytes, macrophages, reticular cells, plasmablasts, plasmatic cells of different maturity level were differentiated in structurally functional zones of lymph nodes. The obtained data were processed by the method of variation statistics with a definition of average arithmetic, its mistake and confidential interval with reliability of R-95%.

RESULTS AND DISCUSSION

The results of our research testified that in the conditions of a normal lymph circulation the structure of inguinal and iliac lymph nodes was characterized by prevalence of cortical substance over the brain one. In the conditions of normal haemo-lymph circulation the difference of morphology inguinal and iliac lymph nodes consisted in prevalence of the relative sizes of a paracortical zone and cortical plateau in inguinal knot (17,86±0,13 and 20,17±0,98 respectively). The area of brain cords and sinuses in inguinal lymph node was 12,73±0,89 and 13,70±0.67 respectively, the area of brain cords in iliac lymph node was 14,96 ±0,74, the area of brain sinuses was 15,44± 0,64. The cellular structure of structures of cortical substance of both lymph nodes was presented by generally small lymphocytes, mature plasmatic cells prevailed in brain cords and in a gleam of brain sinus.

The main anti-gene charge was perceived by lymph nodes, regional to the place of developing of malignant tumor at the development of tumoral process [2]. Therefore the important criterion of immune protection was the assessment of the condition of the regional lymphatic course. Thus, inguinal and iliac lymph nodes in the normal conditions had some distinctions in microanatomic organization that structural changes would be different when modeling the tumor. 5 days later after a hypodermic inoculation of tumor, the most expressed changes happened in inguinal lymph node which size had increased in 3,3%, and the sizes of iliac lymph node authentically didn't differ from similar in control group. The area of cortical substance has increased in 11,7% (71,47±0,98), the area of brain substance has decreased in 17,1% in comparison with intact animals. In the structure of cortical substance of inguinal lymph node the relative sizes of secondary lymphoid small knots became bigger by 3,9 times because of the area expense of the herminative centers by 6,3 times (20,08±0,63). The area of iliac zone has increased in 2,1 times in comparison with the group of intact animals. The relative sizes of brain cords and brain sinuses have decreased in 7,3% and 26,2% in brain substance of inguinal lymph node when modeling the tumor (11,81±0,59 and 10,12±0,53). The regional sinus has extended in 84,7% in comparison with a similar indicator in group of intact animals. The expansion of regional sinus testified to the increasing lymph pressure in regional lymph node to the tumor due to the complicated outflow of the lymph and strengthening of biological processing [1,4]. The considerable changes happened in cellular structure of inguinal lymph node when modeling the tumor. In the herminative centers of secondary lymphoid small knots there was the increase in quantity of lymphoblast by 2,2 times, average lymphocytes by 3,5 times, macrophages by 1,5 times and reduction of relative number of small lymphocytes by 27% in comparison with group of intact animals. In paracortical zone the lymphoblast number has increased in 35,2%, small lymphocytes in 7,3% and macrophages in 38,3%, thus the number of average lymphocytes has decreased by 4,3 times (2,15±0,25). The increase in relative density of macrophages occured as protective reactions of the organism, and phagocytosis of macrophages of lymphocytes, neutrophiles and eosinophiles were considered as matrix material for synthesis of antibodies in the course of immunogenesis [2]. The number of plasmablasts has increased by 6 times in brain cords, the quantity of immature plasmatic cells by 2,8 times (25,60±0,40) that exceeded the corresponding value in group of intact animals. The number of macrophages has increased in 17,7%, average lymphocytes by 1,5 times in comparison with intact animals. The quantity of average lymphocytes has increased in brain sinus by 1,6 times (9,70±1,23), small lymphocytes by 2,6 times (51,50±1,76), macrophages by 1,2 times, the number of plasmablasts has increased

by 9,6 times (12,98±0,26) in comparison with the control group. The number of immature plasmatic cells has decreased in 32,7%, mature plasmatic cells by 3,4 times in comparison with a similar indicator in control group. The hypertrophy of structures of cortical substance and cellular changes in structures of inguinal lymph node testified the activation of lymphopoesis in inguinal lymph node [2].

Changes in structure of iliac lymph node were less expressed. The size of cortical and brain substance authentically didn't differ from the corresponding indicators in group of intact animals. In structure of cortical substance the area of secondary lymphoid small knots has increased due to considerable reduction of the area of primary lymphoid small knots. The area of paracortical zone has increased in 2,3% (17,10±0,72). There was the increase in the sizes of brain cords in brain substance in 12,7% and reduction of volume of brain sinus in 12% in comparison with these data in group of control animals. In the herminative centers of secondary lymphoid small knots percentage of lymphoblasts was made 2,32±0,43% that exceeded the corresponding data in group of intact animals by 2,4 times. The quantity of average lymphocytes and macrophages has been increased by 4,9 times and the number of small lymphocytes were decreased. The animals with experimental tumor in brain cords had density of plasmablast of 4,24±0,42 that exceeded similar data in group of control animals by 1,6 times. The number of average and small lymphocytes when modeling tumor has increased by 1,6 times and 1,4 times respectively. The number of mature plasmatic cells has decreased by 1,5 times (52,20±0,61). The number of average lymphocytes has increased by 4,7 times in brain sinus of iliac lymph node, small lymphocytes by 2,4 times, plasmablast by 5,8 times, macrophages by 1,1 times. The number of mature plasmatic cells has decreased by 1,9 times in comparison with intact animals. Antineoplastic immune reaction was carried out generally due to the expense of cellular link of immunity [2]. More expressed changes of cellular compound of iliac lymphatic node could be explained by the fact that part of lymph from the region of tumoral growth passing iliac lymphatic node, got in the iliac lymphatic node.

CONCLUSION

Additional data on the structurally functional organization of inguinal and iliac lymph nodes in the conditions of normal haemo - and lymphcirculation have been obtained. Various response of inguinal and iliac lymph nodes depended not only on extent of anti-gene influence and regionality of lymph nodes to a progression of tumor cells, but it could be caused by initially

different functional specialization. Structural transformations in inguinal lymph node when modeling experimental tumor were expressed more than in iliac lymph node.

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Authors:

Afanasieva Oksana Gavrilovna – cand. med. sciences, senior lecturer of the Medical institute NEFU of M.K.Ammosov: afanatomiya@mail.ru <mailto:kafanatomiya@mail.ru>,

Egorova Eya Egorovna - cand. med. sciences., senior lecturer of the Medical institute NEFU of M.K.Ammosov, eyaegorova@mail.ru.

Markovsky V.D., Sorokina I.V., Kaluzhina O.V.

Morphological Features of Kidneys at Rats' Posterity Affected by Chronic Prenatal, Acute Postnatal and Mixed Hypoxia (Experimental Research)

ABSTRACT

In this experimental study macroscopic and microscopic features of kidneys of fetuses and neonates affected by chronic fetal (CFH), acute postnatal (APH) and mixed hypoxia (MH) are established. The authors have shown that CFH and MH result in reduction of kidney weight in fetuses

and newborns, and APH does not affect the weight of the newborn kidney. In kidneys of fetuses and newborns there were signs of immaturity, degenerative, necrotic, inflammatory, sclerotic changes, hemodynamic disturbances and signs of edema, they mostly marked in modeling MH and CFH and moderately marked in modeling APH. The identified structural changes in the kidneys of fetuses and neonates affected by different types of hypoxia can lead to the development of nephrologic pathology in these children in the future ontogenesis.

Keywords: kidney, hypoxia, fetus, newborn, morphology.

Introduction. Chronic intrauterine hypoxia is severe stressor that implements its negative influence on not only mother's but the child' body [2]. It determines development of many diseases of organs and systems in such offspring with leading position of cardiovascular diseases [6]. There is data about chronic oxygen insufficiency influence on morphological status of fetal venosus ductus [7], aorta of chick [11] and rats embryos [10], development of pulmonary hypertension with pulmonary vascular remodeling in the experiment [13] in modern literature. However, in our opinion these data are at times contradictory and does not describe comparison of morphological changes in the pulmonary artery and the aorta in fetuses and infants under the influence of chronic intrauterine hypoxia.

The aim of research is to compare morphological changes of endothelial cover of pulmonary artery and aorta in fetuses and neonates undergoing chronic intrauterine hypoxia.

MATERIALS AND METHODS

The experiment modeling intrauterine hypoxia of newborn with asphyxia in labor was made on laboratory WAG rats. Pregnant female rats have been affected high-altitude hypoxic influence, which corresponded to 7500 m for 20 minutes each day at the same time, since pregnancy registration to delivery. Rats were divided into two groups: control group 1 – fetuses and newborns from mothers who were not exposed to high- altitude hypoxia (18 cases);

experimental group 2 – fetuses and newborns from mothers who suffered from high-altitude hypoxic exposure (16 cases). Female and offspring were euthanized. The autopsies of animals were made, the tissue pieces of pulmonary artery and aorta were cut for morphological investigation. They were fixed in 10% neutral formalin solution, then subjected to standard paraffin preparation through increasing alcohol concentration, Nikiforov solution (96% alcohol and diethyl ether 1:1), chloroform, then followed paraffin filling. Prepared blocks were sliced on microtome Microm HM-340 into serial sections $4–5\times10^{-6}$ m. The complex of histological, histochemical, immunohistochemical, morphometric methods was morphological processing. Morphological and morphometric studies were performed on microscope Olympus BX-41 (Japan) using Olympus DP-Soft (Version 3:1) and Microsoft Excel 2010 programs, and fluorescent microscope «Axioskor 40» (Carl Zeiss, Germany). The slides were stained with hematoxylin and eosin, van Gieson's Picric Acid Fuchsin, and according to Mallory. Immunohistochemical study was done on paraffin sections ($5–6\times10^{-6}$ m thickness) with direct Koons' method by Brosman's methodology [9]. Collagens III, IV type were defined by monoclonal antibodies (mAbs) to the respective collagens (Novocastra Laboratories Ltd.). Adhesive properties of the cells were defined by mAbs to CD34 (Novocastra Laboratories Ltd.). Optical density of endothelial and collagen immunofluorescence was measured by method of Gubina-Vaculik G.I. and others [5] with a microscope "Axioskor 40" and software Biostat.exe and was represented in conditional units of luminescence (cond. un. lum.). The findings were worked up statistically with the license application package «Statistica 6.0» («Statsoft, Inc») on the PC. The methods of variation statistics have been used, veracity was determined by the Student t-test [3]. All manipulations with animals were carried out according to the rules of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 18.03.1986), Directive Council of the European Society for Protection of Vertebrate Animals (Strasbourg, 24.11.1986).

RESULTS AND DISCUSSION

Macroscopic examination with a magnifying glass (×3, 8 diopters) showed that the intima of the pulmonary artery and the aorta was smooth and shiny without noticeable differences in both groups. Some microscopicall differences were revealed in blood vessels of the control group, though in the literature they described identical structure [8]. Endothelial cover both in the pulmonary artery and the aorta was represented by mononuclear cells layer, which are located on the basal membrane closely to each other. The average height of the cells in the first vessel was $3,20\pm0,04\times10^{-6}$ m, and in the second – $3,44\pm0,06\times10^{-6}$ m, which was reliably to each

other ($p<0,05$). The average width of endotheliocytes reached $7,12±0,15×10^{-6}$ m for the pulmonary artery and $7,25±0,18×10^{-6}$ m for the aorta, that had no significant difference between these values. The nucleus was stained evenly with hematoxylin and was located centrally, where there was a slight protrusion of cells into the vessel lumen. The cytoplasm was uniformly stained with eosin. There were determined 2-3 desquamated cells (2,40±0,09) in one field of view (×1000) in the pulmonary artery, and 1-3 cells (2,15±0,15) – in aorta, it had no significant difference between each other. Optical fluorescence density of endothelial cells was shown by CD 34 marker, which values were 0,495±0,01 cond. un. lum. for pulmonary artery and 0,476±0,01 cond. un. lum. for the aorta, which were not significant comparatively with each other. Well-defined basal membranes, on which the endothelial cells were localized, were stained evenly with eosin and accumulated evenly type IV collagen in the form of immunofluorescence moderate intensity (in the pulmonary artery – 0,526±0,02 cond. un. lum., in the aorta – 0,531±0,02 cond. un. lum.).

Thus, above-mentioned state of the vessels corresponded to the universally recognized notion of the norm and could be used as a control [1].

Microscopic examination of the same name vessels of *research* groups revealed the following differences. Endothelial cells were located tightly to each other on the basement membrane. Change of cells' width and height in both vessels indicated their flattening (Table 1).

The elongated oval nucleus of endothelial cells was located centrally in both vessels. The cytoplasm was evenly stained with eosin.

In one field of view (×1000) both in the pulmonary artery and in the aorta was detected size increase of desquamation fields at the analysis. Thus, this index is slightly higher (4,87±0,15 cells) in the first vessel than in the second (4,60±0,16 cells) one. That is significantly different from control values of corresponding vessels ($p<0,001$) and are not reliable between each other.

Endothelial cells of both vessels in groups with hypoxia accumulated marker CD 34 worse, which is proved by optical density decreasing. Thus, it was 0,397±0,02 cond. un. lum. in the pulmonary artery, and 0,379±0,02 cond. un. lum. in the aorta, which was significantly different from the control group values ($p<0,001$ and $p<0,01$, respectively).

Comparative analysis of data of optical fluorescence density CD 34 between the pulmonary artery and the aorta in groups with chronic intrauterine hypoxia were not found significantly different.

Basement membranes were slightly thickened in groups with oxygen deficiency in both

vessels. A downward tendency of type IV collagen volume in the study group compared with the control one has been established. The relative fluorescence density of the emission of collagen for the pulmonary artery was 0,497±0,02 cond. un. lum., and for the aorta – 0,495±0,03 cond. un. lum. Significant differences between the values of the optical density of the emission of type IV collagen in the vessels of the groups with chronic intrauterine hypoxia has not been established. Interstitial collagen type III was also determined in the structure of the vessels basement membranes, where as it is known, collagen type IV must be present. It may indicate presence of sclerotic changes [4]. These features can cause violations of metabolic processes in endothelial cover with development of degenerative changes with subsequent desquamation of cells [12]. This is confirmed by the significant increase in the area of desquamation fields in the vessels of the study group.

CONCLUSIONS:

1. Chronic intrauterine hypoxia causes the formation of significant morphological changes in endothelial cover of the pulmonary artery and the aorta in fetuses and newborns. It is manifested by trophic processes deterioration in endotheliocytes due to endothelial basement membrane thickening, which in turn results in cells flattening, reduction of adhesive properties, as evidenced by decreased expression of receptor CD 34 of endothelium, and increased desquamation. These changes are more significant in the pulmonary artery.

2. Basal membranes of the pulmonary artery and aorta have sclerotic changes as a result of interstitial collagen type III presence against type IV collagen deficiency.

3. The above-mentioned morphological changes reflect negative influence of chronic intrauterine hypoxia on the morphological status of the pulmonary artery and the aorta, which may be regarded as substrate for endothelial dysfunction formation in these people.

Literature.

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Markovsky Vladimir Dmitrievich – MD, professor, professor of Pathological Anatomy department, vice-rector for research and education, pathomorphology@ukr.net; **Sorokina Irina Viktorovna** – MD, professor, professor of Pathological Anatomy department, acting head of Pathological Anatomy department, soririna1@mail.ru; **Kaluzhina Oksana Vladimirovna** – graduate student of Pathological Anatomy department, kaluzhina24@ukr.net Kharkov National Medical University, Department of Pathological Anatomy, 4, Lenina avenue, 61022, Kharkov, Ukraine

Table 1

Sizes of pulmonary artery and aortic endothelial cells of fetuses and newborns (M±m)

	Width (10^{-6}м)	Height (10^{-6}м)	Width (10^{-6}м)	Height (10^{-6}м)
Control	7,12±0,15	3,20±0,04	7,25±0,18	3,44±0,06#
Chronic intrauterine hypoxia	7,94±0,11*##	2,96±0,03*	5,95±0,10*	3,06±0,06*

* – P<0,001 – the probability of the difference of two medium is reliable between the control and study groups;

– P≤0,05 – the probability of the difference of two medium is reliable between the corresponding values of the pulmonary artery and the aorta;

– P<0,001 – the probability of the difference of two medium is reliable between the corresponding values of the pulmonary artery and the aorta.

Osinskaya A.A., Fedorova A.I.

Structural Changes of Peripheral Bodies of the Immune System at Different Types of Antigenic Action in an Experiment

ABSTRACT

We studied the microanatomical organization of larynx lymphoid tissue and mesenteric lymph nodes of rats based on antigenic exposure. Changes in the larynx lymphoid tissues were analyzed after diamond dust exposure to a body in different periods of the experiment. Mesenteric lymph nodes were examined after experimental exotoxicosis through the intraperitoneal injection of 50% oil solution of carbon tetrachloride. We determined that the 3-day exposure to a larynx mucous membrane with diamond dust caused increase of the number of lymphoid tissue. The prolonged exposure (30 days) resulted in reduction of the amount of lymphoid tissue on larynx walls. In MLN as a reaction to exotoxicosis the hypertrophy of paracortex, cortex, lymphoid nodules, and growth of pulpous strands, marginal, cortical and medullar sinuses in lymph nodes were observed. In both cases the changes occurred due to the activation of a lymphoid apparatus focused on processing the toxicant.

Keywords: lymph nodes, lymphoid tissue, diamond dust, toxicosis

Over the last years there has been substantial interest in immune system structure and functions. This can be explained with the results of modern scientific papers indicating participation of the immune system in foreign substances inactivation and a body detoxication; violation of its capacity leads to development of various pathological processes in tissues and organs of the body [5]. An important role in organism abient responses under exotoxic states belongs to lymph nodes, which fulfill important regulatory-and-adaptive as well as barrier functions; they are largely in charge of drainage and detoxication of a lymphatic zone [1], as well as lymphoid tissue, which is one of the most sensitive body systems quick on contact with antigens at the earliest stages. Reaction of lymph nodes and lymphoid tissue of organs aims to treat arising disorders under the damaging factors action [3,7].

At present there is a significant progress in the study of certain organs of immune system [6]. Meanwhile, there is not due attention to the study of structural processes relationship on exotoxic exposure to a body in lymph nodes and lymphoid tissue.

In this paper we have attempted to combine data on structural changes in the peripheral organs of an immune system on different types of antigenic exposure.

The aim: to identify presence or absence of similar changes in the structure of the peripheral organs of the lymphoid system on different types of antigenic exposure.

MATERIALS AND METHODS

We selected 100 Wistar rats weighing 150-180 g. at the age of 3-4 months. For microanatomical study we chose larynx lymphoid tissue and mesenteric lymph nodes. Changes in the larynx lymphoid tissues were studied after exposure with diamond dust in different periods of the experiment. Mesenteric lymph nodes were studied after experimental exotoxicosis.

For exposure with diamond dust the animals were placed in the diamond hand-cut workshop of the lapidary plant of “Aurora Diamond" JSC of the Republic of Sakha (Yakutia). The animals were slaughtered on the 3^{rd} and 30^{th} days of the experiment. For formation of experimental exotoxicosis the animals were intraperitoneally injected 50% oil solution of carbon tetrachloride in a dose of 0.4 ml/kg. The animals were slaughtered by decapitation in the morning in 72 hours after the toxicosis formation.

The data was held in 10% neutral formalin solution for 24 hours and dehydrated in alcohols of increasing concentration and then put in paraffin. Paraffin sections of 5-7 microns thick were made with a sledge microtome. The sections were colored with hematoxylin and eosin. Morphometry of structure of the larynx walls and lymph node was performed by point counting with the use of a standard grid.

A relative area (in %) of the structural components – epithelium, mucosa, submucosa, glands and their excretory ducts, areas covered with diffuse lymphoid tissue and lymphoid cells nests - was measured on the microslides of the larynx walls.

We determined the area of the entire section and separate structures: capsules, cortex and medulla, sinuses in lymph nodes. We calculated the ratio of the specific area of the cortex to the specific area of the medulla (index C/M), the ratio of the cortical plateau area to the paracortical area (index C/P), and the ratio of the pulpous tissue area to the medullar sinuses area (index PT/CS).

All the received quantitative data were processed by the variation statistics method with definition of an arithmetic mean, its error and a confidence interval with the reliability of p-95% ($p < 0.05$); statistical significance between the parameters was indicated on the Student t-test.

RESULTS AND DISCUSSION

When studying microanatomy of the larynx walls, it was revealed that cells of the lymphoid tissue formed clumps located predominantly in a subepithelial zone of the larynx lamina propria and surrounded the glands' excretory ducts. The study found that on the third day of diamond dust exposure in the diamond hand-cut workshop there was a significant increase ($p < 0.05$) of the area covered with lymphoid tissue (13,7 ± 0,5) on larynx duct - by 1.14 times relative to benchmarks (12,0 ± 0,2). In interventricular larynx part, the area covered with lymphoid tissue was 15,4 ± 0,3, which was 1.2 times higher than the benchmarks (13,3 ± 0,4). The infraglottic cavity had also significant ($p < 0.05$) increase of lymphoid tissue - by 1.2 times (benchmark - 11,4 ± 0,2; experiment - 13,4 ± 0,4).

The study of the larynx structural components after prolonged diamond dust exposure (for 30 days) showed that the amount of lymphoid tissue was 1.08 times smaller ($p < 0.05$) than the benchmarks (11,2 ± 0,2) on the walls of the larynx duct; by 1,2 times - in the interventricular part (11,7 ± 0,07); and by 1.1 times - in the infraglottic cavity (10,9 ± 0,1).

Therefore, as a result of the study, we found that the 3-day exposure with diamond dust to the larynx mucosa caused lymphoid tissue increase. Such changes in the lymphoid tissue were estimated as the initial reaction of the immune system to the action of various toxic factors, which occurred as enhancement of lymphopoietic processes [2,4,5]. Prolonged exposure occurred with reduction of the lymphoid tissue on the larynx walls. We agree that these changes indicate the ongoing degradation processes resulting from chronic intoxication [2,5].

Under exotoxicosis induced by carbon tetrachloride in the mesenteric lymph node structure, the area of the cut nodes increases by 1.6 times (benchmark - 34,35 ± 1,79; toxicosis - 55,80 ± 3,97; $p < 0.001$) due to changes in intranodal zones as in the cortex and in the medulla. The cortex volume increased by 1.7 times (benchmark - 18,42 ± 1,06; toxicosis - 31,70 ± 2,13; $p < 0.001$) due to the significant increase in the area covered with the cortical plateau (benchmark - 4,27 ± 0,19; toxicosis - 6,60 ± 0,70; $p < 0.001$), paracortex (benchmark - 7,45 ± 0,32; toxicosis - 16,20 ± 0,80; $p < 0.001$), Marginal sinus (benchmark - 4,25 ± 0,41; toxicosis - 5,20 ± 0,21; $p < 0.05$), and cortical sinus (benchmark - 1,90 ± 0,15; toxicosis - 2,90 ± 0,21; $p < 0.001$). The area of lymphoid nodules tends to increase. The share of medulla in the lymph node structure

increases by 1.3 times (benchmark - 8,55 ± 0,28; toxicosis - 11,40 ± 1,17; p <0.001). At that, the area of pulpous strand increases by 1.4 times (benchmark - 3,85 ± 0,36, toxicosis - 5,30 ± 0,42; p <0.05), the area of medullar sinus - by 1.3 times (benchmark - 4 70 ± 0,20; toxicosis - 6,10 ± 0,75; p <0.001). Evaluation of lymph node sinus system indicates its growth. Extension of sinus system is mainly achieved through the cortical and medullar sinuses. It is known that the lymph nodes can deposit a large amount of liquid. In the early stages of venous stasis the volume of lymph node increases by 40 - 50% compared to the normal volume due to accumulation of a large mass of liquid therein [8]. Index C/M is 2,78 ± 0,91, which characterizes the node as a compact II type. The value of the index C/P is 0,40 ± 0,14, which indicates the prevalence of paracortex in lymph nodes. Index PT/CS of 0,86 ± 0,28 indicates the prevalence of sinus share in the lymph node medulla.

Thus, hypertrophy of paracortical zone, cortical plateau, and lymphoid nodules, growth of pulpous strands, Marginal, cortical and medullar sinuses indicate immune activation aimed at the toxicant processing and the lymph node's transport function improving. The observed changes in the structure of the mesenteric lymph nodes are a response to the introduction of the toxin and they signify that lymph nodes are in a state of structural and functional stress.

COCLUSIONS:

1. The immune system peripheral organs exhibit high reactivity in response to various antigens. Changes in the lymphoid organs depend on duration of a foreign agent exposure.
2. Structural changes on the larynx walls after short antigen exposure, as well as changes in the lymph nodes after experimental exotocsicosis are qualitatively similar and occur through activation of the lymphoid apparatus aimed at the toxicant treatment.
3. Prolonged antigen exposure causes suppression of functional capabilities of lymphoid tissue, resulting in a reduction of its quantity.

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Authors:

Alena A. Osinskaya, Associate Professor of the Department of General and Pathologic Anatomy, Operative Surgery with Regional Anatomy and Legal Medicine of NEFU Institute of Medicine; contact phone number - 89241689844, osin_alen@rambler.ru

Aida I. Fedorova, Associate Professor of the Department of General and Pathologic Anatomy, Operative Surgery with Regional Anatomy and Legal Medicine of NEFU Institute of Medicine; contact phone number - 89241696871, fed.aida@rambler.ru

Teryutin F.M., Barashkov N.A., Kunelskaya N.L., Pshennikova V.G., Solovyev A.V., Klarov L.A., Kozhevnikov A.A., Vasilyeva L.M., Fedotova E.E., Romanov G.P., Gotovtsev N.N., Pak M.V., Lekhanova S.N., Morozov I.V., Bondar A.A., Solovyeva N.A., Rafailov A.M., Alekseev A.N., Posukh O.L., Dzhemileva L.U., Husnutdinova E.K., Fedorova S.A.

Variability of auditory threshold at deaf patients with splice site c.-23+1G>A mutation in GJB2 gene (Konneksin 26)

ABSTRACT

In this article results of the audiological examination testifying to auditory threshold variability at deaf patients with splice site c.-23+1G>A mutation in *GJB2* gene in homozygous state are presented. According to this study this *GJB2*-genotype is characterized by (horizontal) flat orsloping audioprofile. There are recommendations for applying the results obtained in practice.

Keywords: deafness, c.-23+1G>A, *GJB2* gene, audiometric analyses, hearing thresholds.

INTRODUCTION

According to preliminary data of the audiological screening in the Republic of Sakha (Yakutia), the frequency of congenital hearing loss is higher than world average value (1: 1000 of newborns) [8, 9] and it is approximating to 1 per 700-900 infants [3]. At the same time, the prevalence of autosomal recessive deafness caused by homozygous c.-23+1G>A mutation of the *GJB2* gene is estimated as 16.2 per 100,000, and the carrier frequency of this mutation is one of the highest in the world (3% - 11% of the indigenous population of Yakutia are heterozygous c.-23+1G>A carriers). Thus, the territory of Eastern Siberia is known as the territory of extensive accumulation of c.-23+1G>A mutation in *GJB2* gene [3].

Several studies of *GJB2* gene mutations in Europe and Asia show a large variability of hearing thresholds in individuals with different mutations in the *GJB2* gene [2, 4, 7, 10, 11, 13]. However, in present time studies of hearing status in individuals homozygous for the c.-23+1G>A mutation in *GJB2* gene are rare. From available data of audiological analysis of three siblings from Bangladesh with c.-23+1G>A mutation in homozygous state are known, that two siblings had a moderate hearing loss, one – profound hearing loss, and audiological curve had a flat and sloping form [2]. Also, we had previously described audiological characteristics of 40

homozygous patients with splice site c.-23+1G>A mutation in *GJB2* gene from Yakut population [3].

Data on hearing thresholds in extended sample of deaf individuals with homozygous c.-23+1G>A mutation in *GJB2* gene are presented in this study. Results of this study will be useful for audiological and rehabilitating aid.

MATERIALS AND METHODS

Patients

108 deaf Yakut individuals (216 ears, the average age of 14.32±4.7 years) with a confirmed genotype c.-23+1G>A/c.-23+1G>A were included in studied sample.

Mutation analysis of GJB2 gene

A total 108 samples of genomic DNA, extracted from leukocytes of peripheral blood, were used for *GJB2* gene mutation analysis. Amplification of the coding (exon 2) and noncoding (exon 1) and flanking intronic regions of *GJB2* gene was conducted by PCR on thermocycler «MJ Mini» (Bio-Rad) using appropriate primers [5,6-15]. The PCR products were subjected to direct sequencing using the same primers on ABI PRISM 3130XL (Applied Biosystems, USA) («Genomics» Core Facility; Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia).

Audiological examination

Audiological examination was conducted on all 108 individuals (216 ears). Air conduction thresholds were measured on frequencies 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and bone conduction thresholds – on frequencies 0.25, 0.5, 1.0, 2.0, 4.0 kHz with audiometer «MAICO ST20» (MAICO, Germany) in the same conditions for all participants. The results were obtained separately for each ear on air conduction thresholds as on all measured frequencies, and also on speech range of frequencies – PTA 0.5,1.0,2.0,4.0 kHz separately.

Ethical approval

Written informed consent was obtained from all individuals. This study was approved by the local Committee on Biomedical Ethics of Yakut Scientific Center of Complex Medical Problems, Yakutsk, Russia (Yakutsk, Protocol No 16, April 16, 2009).

RESULTS AND DISCUSSION

Data from hearing thresholds audiograms of all studied patients (108 individuals, 216 ears) were summarized and presented in Figure 1.

The upper limit (minimum audiometric values) of hearing thresholds in the range of «low» frequencies (0.25 - 0.5 kHz) was estimated at 20.0 dB, «medium» frequency range (1.0 - 2.0 kHz) - 35.0 dB, «high» frequencies (4.0 - 8.0 kHz) - 40.0 dB. The lower limit (maximum audiometric values) was limited to 105.0 - 120.0 dB (Figure 1).

In the majority of studied ears (83.79%) hearing sensitivity was preserved at all measured frequencies (up to 8.0 kHz inclusive) and only 16.21% of the cases had a loss: 16 ears (7.41%) - 4.0 kHz, 6 ears (2.78 %) - 2.0 kHz and 13 ears (6.02%) - 1.0 kHz (Figure 1).

Hearing thresholds percentiles were examined to determine the audioprofile characteristic for presence of mutation 23+1G>A. The 25th, 50th and 75th percentiles were calculated. The audiological curves at the 25th and 75th percentile were presented by sloping curves, while the 50th percentile (mean) was close to the flat curve, and a wide interval (from 35.0 dB to 45.0 dB at different frequencies) between the 25th and 75th percentiles (Figure 1) confirmed the variability of hearing loss.

The results of audiological analysis of three individuals with a homozygous splice site c.-23+1G>A mutation in the *GJB2* gene from Bangladesh [2], are comparable with our results. Preservation of the remaining hearing of the majority of studied individuals with genotype c.-23 +1G>A/c.-23+1G>A, is consistent with the results of a multicenter study [13], where compound-heterozygous c.35delG/c.-23+1G>A individuals presented significantly less severe hearing loss than homozygous c.35delG/c.35delG patients [13].

Clinical recommendations

The flat audioprofile in individuals with *GJB2*-genotype c.-23+1G>A/c.23+1G>A should be considered in logopaedic and speech training programs. For selecting and programming of hearing aid for children more effective habilitation and rehabilitation is possible considering results of this study.

CONCLUSION

The results of this study demonstrate variability of hearing thresholds in individuals with homozygous c.-23+1G>A mutation in the *GJB2* gene. In addition, auditory sensitivity in the measured frequencies (0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz) was remaining intact. Calculation of the 25th, 50th and 75th percentiles showed that presence of mutation c.-23+1G>A is reflected in flat or sloping audioprofile.

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Authors:

FSBSI «Yakut scientific centre of complex medical problems»: **Terjutin** Fedor Mihajlovich - junior researcher, rest26@mail.ru, **Barashkov** Nikolay Alekseevich – cand. biol. sciences, head of lab., barashkov2004@mail.ru, **Pshennikova** Vera Gennadievna - researcher, **Gotovsev** Nurgun Naumovich – junior researcher, **Solovieva** Natalia Alekseevna – cand. med. sciences, junior researcher; FSAEI HPE «Northeast federal university of M.K.Ammosov»: **Soloviev** Aisen Vasilyevich - engineer, **Romanov** Georgiy Prokopevich - lab. researcher, **Pak** Maria Vladimirovna - post-graduate student, **Lekhanova** Sargylana Nikolaevna - к.м.н., senior lecturer, **Rafailov** Adjum Mihajlovich – cand. biol. sciences, senior lecturer, **Fedorova** Sardana Arkadievna – doctor biol. sciences, head of the scientific lab. of molecular biology; **Kunelskaya** Natalia Leonidovna – doctor of med. sciences, prof., deputy director on scientific work SBEH «Moscow research clinical institute of otolaryngology of L.I.Sverzhevsky» of the Department of public health of Moscow, nlkun@mail.ru; FSBSI «Institute of biochemistry and genetics SC SB RAMS » (Ufa): **Dzhemileva** Lilia Useinovna – docror med. sciences, senior researcher, **Husnutdinova** Elza Kamilevna – doctor of biol. sciences, prof., head of the Department og genetics; FSBSI of chemical biologies and fundamental medicine SB RAMS, the Center of collective using "Genomika" (Novosibirsk): **Morozov** Igor Vladimirovich – cand. biol. sciences, senior researcher, **Bondar** Alexander Anatolevich – cand. chemical sciences, senior researcher,

Posukh Olga Leonidovna – cand. biol. sciences, senior researcher. FSBSI of cytology and genetics SB RAMS (Novosibirsk); SBE RS (Y) RH №2 - Center of urgent medical aid: **Klarov** Leonid Aleksandrovich - doctor radiologist, **Kozhevnikov** Andrey Aleksandrovich - doctor, professional pathologist, chief non-staff profathologist of MH RS (Y); **Alekseev** Anatoly Nikolaevich – doctor of historical sciences, prof., director FSBSI on humanitarian researches and problems of small numbered North people SB RAMS; SBI RS(Y)« RH №1- National Cantre of Medicine: **Vasilieva** Lena Maksimovna – teacher of the deaf and hard of hearing, **Fedotova** Elvira Egorovna – cand. med. sciences, doctor, surdologist -otolaringologist, chief non-staff surdologist MH RS (Y)

N.I. Douglas, T.Yu.Pavlova, Ya.G.Rad

New Methods of Teaching Ultrasound Diagnosis

ABSTRACT

At the present time new methods of teaching ultrasound diagnosis using a virtual simulator are needed to be applied. The ultrasonic simulator
has many advantages in developing practical skills and thus increasing training of doctors.

Keywords: ultrasound diagnosis, virtual simulator, obstetrics, gynecology.

INTRODUCTION

Each prospective or practicing obstetrician gynecologist and hospital physician should possess practical skills of ultrasound (ultrasound). The ability to conduct independently ultrasound and interpret its data are necessary activities for any obstetrician gynecologist. It is also necessary to carry out high-quality diagnostic ultrasound in pregnancy, it is very important because in the structure of infant mortality congenital malformations of a fetus in the Russian Federation and the Republic of Sakha (Yakutia) take the second place. This means that fetal malformations are not detected or detected in the later stages of pregnancy. One of the main measures to reduce infant mortality is to improve the quality of prenatal diagnosis. Therefore, the training of doctors in obstetrics ultrasound diagnosis is becoming the most important issue in reducing the infant mortality rate in the region.

Teaching of ultrasound (ultrasound diagnostics) in the clinic has a number of difficulties:

depending on a patient and a clinic;

depending on availability of affordable pathology;

lack of rare options of ultrasound picture in gynecological pathology;

time limit;

observance of ethic principles

On the basis of the abovementioned data, there is need for new methods of teaching ultrasound diagnosis, what are the simulation technologies in obstetrics and gynecology. To implement such teaching methods the ultrasound simulator is applied that allows training and professional development in ultrasound diagnostics in realistic conditions.

The main purpose of the use of virtual simulation is to develop and enrich the skills of scanning and interpretation of ultrasound images without the participation of patients. Advantages of practical training in ultrasound using simulation technologies:

realistic training without any patient;

a variety of patients and pathologies;

independence on a clinic working hours;

no constant teacher required;

objective method of assessing skills and abilities;

students' mistakes do not lead to adverse clinical consequences.

To achieve this goal in December 2014 the ultrasound simulator "Shelli" (Germany) was obtained and has been used in the training of interns, residents and practicing physicians (Photo 1).

Ultrasound simulator "Shelli" has two torsovyh dummy and four ultrasonic sensors Photo 2 (Convex - for study of the abdominal cavity and retroperitoneal, pelvic organs and fetal linear - for studies of surface structures, such as the study of the thyroid gland, blood vessels, a sector - used in cardiology and transvaginal gynecological research and studies of the fetus), provides a realistic B-scan image of the investigated organs and tissues of high quality resolution that accurately reflect the fact that we get during the ultrasound examination in patients in real-time. Full database ultrasound simulator "Shelli" distributed thematic modules adapted to the level of "novice" to "with experience" physician ultrasound diagnosis. Each module 12 comprises a documented clinical example of patients. The system can be upgraded with additional modules from a constantly updated library databases provided by the leading clinics of Europe. The data library supplied with the extended completion ultrasound simulator, contains more than 200 different clinical observations of patients and more than 500 reference photos.

From December 2014 till January 2015 42 students got thematic improvement on prenatal diagnosis in obstetrics (Photo 3,4)

Forms of learning:

- Lectures with presenting own videos;
- Development of practical skills in the modern ultrasound simulation training device "Shell" (transvaginal and transabdominal technique);
- Participation in the study with a physician performing a real ultrasound diagnosis;
- Review of archives of the Department with analysis of the most interesting cases of diagnostic;
- Teaching of writing a protocol and conclusions in ultrasound diagnosis;
- Analysis and discussion of the most interesting cases of diagnostic clinical cases.
- Step by step control of theoretical and practical knowledge in the learning process.

Considering the fact that in the database of this device there are various thematic modules and other specialties, training opportunities are available for doctors of various profiles, which will undoubtedly improve the quality of ultrasound diagnostics in general.

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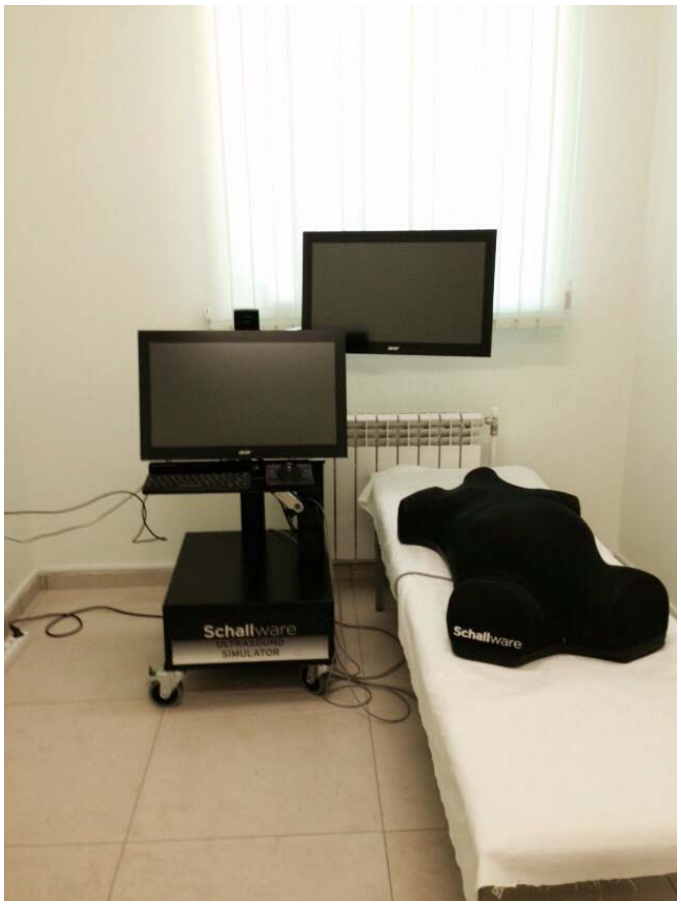
Authors:

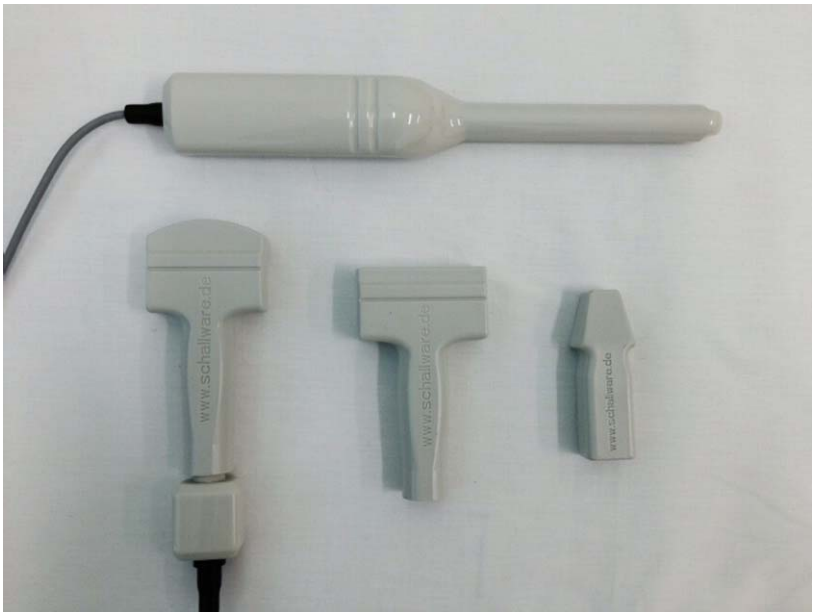
Duglas Natalya Ivanovna – doctor of med. sciences, head of the department of obstetrics and gynecology, MI NEFU of M/K/ Ammosov, nduglas@yandex.ru

Pavlova Tatyana Yurievna – cand. med. sciences, associate prof., tatyanaupavl@mail.ru

Rad Yana Gennadievna – cand. med. sciences, associate prof., rig787@yandex.ru



Picture 1. The ultrasound simulator "Shell"



Picture 2. Ultrasound sensing devices

Picture 3. A training course of Duglas N.I.

Picture 4. The students during the lecture of thematic improvement "Issues of prenatal diagnostic in obstetrics

V.S. Petrov, A.V. Tobokhov, V.N. Nikolaev

Characteristics and Technical Aspects of Laparoscopic Cholecystectomy at Acute Cholecystitis

ABSTRACT

Results of the analysis of cases of surgical treatment of gallstone disease based on the National Medical Center of the Republic of Sakha (Yakutia) for 2008 - 2013 are presented. Due to inefficiency of conservative treatment during 12-24 hours the urgent laparoscopic cholecystectomy (LCE) was conducted. For patients with complicated forms of cholecystitis a two-stage treatment with LCE on the second stage was conducted. The study showed that the combination of acute cholecystitis disease with gepatoduodenopancreatic zone the urgent LCE can be successfully applied.

Keywords: acute cholecystitis, choledocholithiasis, laparoscopic cholecystectomy.

INTRODUCTION

Acute cholecystitis makes its adjustments and features in the technique of laparoscopic cholecystectomy and still remains one of the urgent problems of abdominal surgery [1]. Severe infiltration and increased bleeding tissues, inadequate visualization and poor differentiation of the structures that were observed during surgery in patients with acute cholecystitis, significantly increase the risk of surgical intervention and dictate the need for development of several techniques that contribute to the successful and safe implementation of laparoscopic surgery for acute cholecystitis.

MATERIALS AND METHODS

This paper presents the analysis results of 2778 surgical treatment cases of gallstone disease according to materials of the National Center of Medicine of the Republic of Sakha (Yakutia) in 2008 – 2013. The Average age of the patients was 49.2 years. Among patients, women made up the vast majority, forming 2303 (82,9 %), men 475 (17,1 %).

Indications for urgent laparoscopic cholecystectomy (LCE) mostly did not differ from indications for "open" emergency surgery: under absence of effect from conservative treatment within 12-24 hours, we resorted to urgent LCE, when over a 2 day-period had passed from the beginning of the attack [1]. It

should be noted that the conservative attempts to control an attack of acute cholecystitis was conducted only under absence of patients with diffuse peritonitis. The main reason-why factor that may tempt us to conduct or refuse performing urgent laparoscopic surgery, along with the inefficiency of conservative treatment, was duration of the disease from the beginning of the attack.

Basing on this indicator, as well as on a clinical picture of the disease, we judged on the extent of possible changes in pericholecystic tissues and the probability of executing the urgent LCE. In a number of cases of obstructed acute cholecystitis, the necessity occurred of a two-stage surgical approach: external or internal drainage of bile ducts with the following delayed LCE. To perform the first stage decompression of the gallbladder, we preferred the technique of percutaneous perhepatic micro cholecystostomy under ultrasound control (PPMC) as the most gentle and optimal way. The main advantages of PPMC before laparoscopic micro cholecystostomy were minimally invasive intervention, no need for imposing pneumoperitoneum and special training of the patient, the possibility of its implementation in terms of perivascular infiltrate. After puncture and drainage of the gallbladder antegrade cholecystocholangiography was performed. The study conducted under fluoroscopy, gave the opportunity firstly to get information about the location of a catheter in the gall bladder. The study of extrahepatic bile ducts was performed with the help of antegrade cholecystocholangiography. Under less informative antegrade radiographic contrast study ("blocked" cystic duct), under jaundice or anamnesis pointing to frequent acute pancreatitis episodes of jaundice, we performed endoscopic retrograde cholangiopancreatography. When determining the second stage timing of surgical interventions we primarily focused on data of the study's special methods. Results of the dynamic ultrasound and antegrade cholecystocholangiography allowed objectively assess the dynamics of the inflammatory process, the condition of the gallbladder and extrahepatic biliary tract, perivascular tissues, the functional state of the liver. In acute cholecystitis complicated by mechanical jaundice, we usually tried to do surgery in 3 weeks after normalization of the liver pigment metabolism.

RESEARCH AND DISCUSSION

At 123 (4.4%) patients with complicated forms of cholecystitis the two-stage treatment was used in the second phase of LCE. The age of patients ranged from 48 to 76 years, with patients of elderly and senile age accounting the vast majority - 91 (74,0%) persons. The most patients, 109 (88.6%) ones, had diseases of circulatory and respiratory systems. The combination of complicated acute cholecystitis and related therapeutic pathology in these patients was characterized by development of the mutual aggravation syndrome, which required the comprehensive corrective therapy before invasive surgery.

The combined study of the bile ducts has enabled to identify choledocholithiasis at 77 (2,8 %) patients, in one case combined with stenosis of large duodenal papilla. All 77 patients received endoscopic papillosphincterotomy (EPST) with correction of the extrahepatic bile ducts pathology. 19 patients got endoscopic lithoextraction along with EPST, the rest had spontaneous passage of stones after EPST.

LCE was performed in the period from 7 to 35 days after imposition of PPMS. The average length of preoperative hospitalization made up 23.5 ± 1.8 days.

Adhesive preprocess around the gallbladder was identified during surgery at the vast majority of patients with acute cholecystitis, and 45 (10,5 %) patients had the gallbladder in the infiltrate of various severity extent formed, as a rule, by greater omentum, stomach and duodenum. The selection of the gallbladder from adhesion and infiltration began with capture of the visible area of the gallbladder wall and cautious offset of pericholecystic tissues with soft clip or peanut. This was quite easily managed with loose infiltrate and "soft" adhesions. Under more dense growths we used electric anchor, electric scissors or electric dissector. While separating adhesions, infiltration and excretion of the gallbladder we evaluated the nature and extent of its changes, topographic-anatomic relationships, visualization and access to the bladder's neck, hepatoduodenal band, the gate of the liver. To that end, we changed position of the operating table (lifting the head end, rotation along the longitudinal axis to the left, extension of subhepatic operating roller), adjusted the location and type of used trocars, took the decision on necessity of entering an additional trocar.

Indications to introduction of the 5-th additional tool - endoscopic retractor Endo Retract - were inadequate excretion and visualization of the bladder neck, the liver entry, preventing the safe manipulation and dissection of tissues in this area. Most often this operating situation occurred under perpicholyctic infiltrate with markedly edematous and infiltrated tissues, under location of the Hartmann pocket below hepatoduodenal ligaments. In addition, the need to enter an additional tool occurs during surgery at obese patients with hypertrophic great seal, closing the surgical field. We chose place of entering the endoscopic retractor individually accounting the topographic-anatomical picture. Usually the endoscopic retractor was entered via an additional 10-mm trocar, which was installed in the left hypochondrium by 3-4 cm below the costal arch on midclavicular line.

When analyzing topographic-anatomical and technical aspects of preparation and identification of the cystic duct and artery in acute cholecystitis a number of regularities were established. In 75 % of cases, the cystic duct connects with the common hepatic one under acute angle of 45°, in 17-20% of cases it can pass parallel to the common hepatic duct and in 5-8 % cases it crosses the space in front of or behind the common bile duct, falling from the left side. Despite the fact that in none of 430 cases of

LCE in acute cholecystitis we have not met the true anomalies of the biliary tract, in general, adhesions in the form of infiltration in the field hepatoduodenal ligament created quite unusual topographic-anatomic relationship of the choledochus and the cystic duct. The most common mistake when selecting the cystic duct, which leads to damage of the common bile duct was medial and upper dorsal tension of Hartmann pocket, which contributed to its bias to the gate of the liver. This leads to the convergence of the gallbladder neck with the common hepatic duct, thereby causing a decrease in the angle of the cystic duct confluence. By using laparoscopic optics with a tilt angle of 0°, the common bile duct seemed to be a continuation of the gallbladder's one and located with it on the same plane. Accounting this mechanism of common bile duct injury, traction of Hartmann pocket of gall bladder was made in the lateral or lateral-ventral direction. This contributed to the increase of the angle between the cystic and common hepatic ducts, as well as location of the Calot triangle plane along axis camera view of the laparoscope.

First of all we tried to isolate and identify the cystic duct. After clipping and crossing the cystic duct the neck of the gallbladder became mobile that allows select the cystic artery at a sufficient period. In case of bleeding this technique allowed adequately visualize the area of artery passage and detect the source of bleeding and to conduct targeted hemostasis.

Considerable difficulties can arise at the stage of selecting the gallbladder from the bed of the liver. A layer of loose connective tissue between the gallbladder wall and liver tissue in result of inflammatory process underwent significant changes. Along with coarse fibrous degeneration infiltration was marked. Selection of the gallbladder should be done with careful coagulation of the bed, being careful to avoid damage of the right hepatic artery of the right hepatic duct, which passed in the immediate vicinity of the gallbladder bed. After separation of the gallbladder, a section of connective tissue should be left at the site of attachment of the bottom of the gallbladder to the liver. Holding this section by the clip inserted through a lateral trocar, we raised the liver up for examination of the gallbladder bed.

Thus, the study showed that combination of acute cholecystitis with hepatobiliopancreatic area diseases urgent LCE can be successfully applied. Its execution was the most worthwhile when the uncroped attack, in the absence of gross infiltrative scar pericholecystic changes and pathology of the biliary ductal system. Speaking about the security of running LCE, features and technical aspects of its implementation at patients with acute cholecystitis, it was necessary to consider that with development and introduction of endoscopic surgery only method of removing the gallbladder had changed, and the nature of cholecystectomy remained the same. In this regard, the main way to improve the LCE results,

the most careful adherence remained to principles of surgery on the gallbladder and bile ducts operating for over 100 years.

CONCLUSIONS:

1. The combination of acute cholecystitis with diseases of hepatobiliopancreatic area urgent LCE can be successfully applied. At that, it is the most appropriate to execute the surgery in the absence of gross infiltrative scar pericholecystic changes and pathology of the biliary ductal system;

2. With development and introduction of endoscopic surgery only method of removing the gallbladder has changed and the nature of the surgery, cholecystectomy, remained the same.

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Authors:

Valeriy Sergeyevich Petrov – Cand. of Med. Sciences, director of Republic Hospital N1 – National Center of Medicine, Yakutsk

Address: 677019, 4 Sergelyakhskoye Road, Yakutsk

Phone: 8 (4112)395000

Alexander Vasilyevich Tobokhov – Professor, Doctor of Medical Science, head of hospital surgery and radiology chair at the Medical Institute of the North-East Federal University named after M.K. Ammosov

Address to contact: 677010, 4 Sergelyakhskoye Chaussee, Yakutsk

Phone: 8 (4112)395644

e-mail: avtobohov@mail.ru

Vladimir Nikolayevich Nikolayev – Cand. of Med. Sciences, associate professor of the hospital surgery and radiology chair at the Medical Institute of the North-East Federal University named after M.K. Ammosov