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STRUCTURAL AND FUNCTIONAL CHANGES IN THE TESTICLES AND EJACULATE OF INFERTILE MEN CAUSED BY EPIDEMIC PAROTITIS

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The purpose of the study was to establish the structural and functional changes in the testicles and ejaculate of 12 mature men with a history of epidemic parotitis. Ultrasound scanning and color ultrasound angiography of the testicles were performed in a clinical diagnostic center using a Siemens Sonoline G60S device (Siemens AW, Germany). Testicular volume and hemodynamic parameters were determined: peak arterial velocity, diastolic velocity, average blood flow velocity, and volumetric flow rate. In 7 testicular biopsies of infertile men with this pathology, taken in the urological department of the Regional Clinical Hospital of the Ivano-Frankivsk Regional Council, histostructural changes were studied. In the ejaculate, we studied the concentration of spermatozoa, their morphological forms, and motility according to generally accepted methods. According to ultrasound diagnostics, in infertile men with a history of mumps, the testicular volume decreased, on average, to $17.31 \pm 1.20 \text{ cm}^3$ against $19.50 \pm 1.40 \text{ cm}^3$ in control. Under these conditions, the peak arterial velocity in the testicular artery within the spermatic cord decreased to $14.90 \pm 1.20 \text{ cm/s}$ against $19.30 \pm 1.46 \text{ cm/s}$, and the volumetric flow rate decreased to $14.20 \pm 1.15 \text{ ml/s}$ against $16.50 \pm 1.20 \text{ ml/s}$ in the control. In the examined histological micro preparations made from testicular biopsies of infertile men, the diameter of convoluted seminiferous tubules decreased compared to the control. Laboratory tests of ejaculate in men indicate a decrease in both its volume and the concentration of spermatozoa in it.

Key words: epidemic parotitis, testicle, hemodynamics, spermatogenesis.

Б.В. Грицуляк, С.Б. Герашченко, В.Б. Грицуляк, І.Й. Случик, О.Я. Глодан, О.Є. Халло СТРУКТУРНО-ФУНКЦІОНАЛЬНІ ЗМІНИ В ЯЄЧКАХ ТА ЕЯКУЛЯТІ НЕПЛІДНИХ ЧОЛОВІКІВ, ЗУМОВЛЕНІ ЕПІДЕМІЧНИМ ПАРОТИТОМ

В проведеному нами дослідженні була поставлена мета вивчити структурно-функціональні зміни в яєчках та еякуляті 12-ти чоловіків зрілого віку, в анамнезі яких діагностовано перенесений епідемічний паротит. Ультразвукове сканування та кольорову ультразвукову ангіографію яєчок провели в клініко-діагностичному центрі на апараті Siemens Sonoline G60S («Siemens AW», Німеччина). Визначали об'єм яєчок та показники гемодинаміки в них: пікову швидкість артеріального кровотоку, діастолічну швидкість кровотоку, середню швидкість кровотоку, об'ємний кровоток. У 7-ти біоптатах яєчок неплідних чоловіків з цією патологією, забраних в урологічному відділенні Обласної клінічної лікарні Івано-Франківської обласної ради, досліджували гістоструктурні зміни, а в еякуляті – концентрацію сперматозоїдів, їх морфологічні форми та рухливість за загальноприйнятими методами. За даними ультразвукової діагностики у неплідних чоловіків, в анамнезі яких діагностовано паротит, об'єм яєчок зменшився, в середньому, до $17,31 \pm 1,20 \text{ см}^3$ проти $19,50 \pm 1,40 \text{ см}^3$ у контролі. За цих умов максимальна швидкість кровотоку в яєчковій артерії в межах сім'яного канатика знижується до $14,90 \pm 1,20 \text{ см/с}$ проти $19,30 \pm 1,46 \text{ см/с}$, а об'ємний кровоток зменшився до $14,20 \pm 1,15 \text{ мл/с}$ проти $16,50 \pm 1,20 \text{ мл/с}$ у контролі. В досліджуваних гістологічних мікропрепаратах, виготовлених з біоптатів яєчок неплідних чоловіків діаметр звивистих сім'яних трубочок зменшився в порівнянні з контролем. Проведені лабораторні дослідження еякуляту у чоловіків свідчать про зменшення як його об'єму, так і концентрації у ньому сперматозоїдів.

Ключові слова: епідемічний паротит, яєчко, гемодинаміка, сперматогенез.

The study is a fragment of the research project "Actual aspects of andrology and correction of spermatogenesis", state registration No. 0119U103671.

According to the literature, in recent years, there has been a tendency to increase the number of infertile marriages [1, 5]. Moreover, in almost half of the cases, the cause of their development is a violation of spermatogenesis in men caused by various factors, one of which is an infectious factor [9, 12]. Epidemic parotitis (mumps) is an acute infectious viral disease near the ear salivary glands, which often occurs not only in children and adolescents but also in men aged 18–25 [10, 13]. Infection occurs by airborne transmission; the entrance gates are the mucous membranes of the nasal cavity, mouth, and pharynx. The

mumps virus mainly affects organs and the nervous system, with manifestations of meningitis and meningoencephalitis [3]. One of the complications of parotitis is orchitis, which manifests itself by the phenomena of general intoxication, high body temperature and pain in the testicles, which increase in volume and may develop atrophy and azoospermia during puberty [6]. Therefore, the study of the state of hemodynamics and the nature of structural and functional changes in the testicles and ejaculation of infertile men of mature age who have suffered epidemic parotitis remain relevant.

The purpose of the study was to determine the features of hemodynamics, the nature of cytohistological changes in the testicles and the ejaculation of infertile men of mature age who suffered from epidemic parotitis.

Materials and methods. Ultrasound scanning and color ultrasound angiography of the testicles in 12 infertile men aged 22–35 years with a history of epidemic parotitis were performed in a clinical diagnostic center using a Siemens Sonoline G60S device (Siemens AW, Germany). Testicular volume and hemodynamic parameters were determined: 1) peak arterial velocity – PAV (cm/s); 2) diastolic velocity – DV (cm/s); 3) mean blood flow velocity – ABFV (cm/s); 4) volumetric flow rate – VFR (L/min). In 7 biopsies of the testicles of infertile men with this pathology, taken in the Urology Department of “Regional Clinical Hospital of the Ivano-Frankivsk Regional Council”, histological changes were studied. In the ejaculate, we studied the concentration of spermatozoa, their morphological forms, and mobility according to generally accepted methods [9]. The enzyme-immunoassay method investigated the testosterone concentration in the blood using the analytical analyzer IMMULITE-2000 (Siemens Healthcare Diagnostics Inc., USA). Statistical analysis of the obtained indices was performed using Statistica 10 software. The data of 7 practically healthy men of a given age served as a control. The Committee on Biomedical Ethics of Vasyl Stefanyk Prykarpattia National University found no moral and ethical norms violations during research work (protocol No. 2 dated 04.10.2022). The informed consent of each research participant was obtained for the conduct of the study, as well as the collection and processing of data.

Results of the study and their discussion. The condition of the bloodstream is essential in the functioning of the testicle and determines the nature of metabolic processes in the organ's parenchyma. According to ultrasound diagnostics, the volume of the testicles was reduced to $17.31 \pm 1.20 \text{ cm}^3$, against $19.50 \pm 1.40 \text{ cm}^3$ in the norm, in infertile men of mature age with a history of epidemic parotitis. Under these conditions, the maximum blood flow rate in the testicular artery within the spermatic cord was reduced to $14.90 \pm 1.20 \text{ cm/s}$ against $19.30 \pm 1.46 \text{ cm/s}$, and the volumetric flow rate was decreased to $14.20 \pm 1.15 \text{ ml/s}$ against $16.50 \pm 1.20 \text{ ml/s}$ in the control. The mean velocity of blood flow in the testicular veins decreased to $5.10 \pm 0.15 \text{ cm/s}$, and the volumetric blood flow to $6.20 \pm 0.30 \text{ ml/s}$ against $9.60 \pm 1.20 \text{ cm/s}$ and $8.17 \pm 1.30 \text{ ml/s}$ in men of the control group. In infertile men with a history of epidemic parotitis, a relationship between changes in the hemodynamics of the testicle and disorders in the spermatogenic epithelium of this organ was revealed (Table 1).

Table 1

Structural changes in the testicular parenchyma of infertile men of mature age with a history of epidemic parotitis (M±m)

Testicular parenchyma parameters	Control group	After the epidemic parotitis
Testicular volume (cm^3)	19.50 ± 1.40	17.31 ± 1.20
Diameter of seminiferous tubules (μm)	210.60 ± 5.30	$173.80 \pm 1.90^*$
Volume of Leydig cell nuclei (μm^3)	90.20 ± 5.60	$77.40 \pm 2.30^*$

Note: * – $p < 0.05$ compared to the control.

In the examined histological micro preparations made from testicular biopsies of infertile men, the average diameter of convoluted seminiferous tubules was reduced to $173.80 \pm 1.90 \mu\text{m}$, against $210.60 \pm 5.30 \mu\text{m}$ in the control. 30.8 % of seminiferous tubules are in a state of devastation and degeneration of spermatogenic epithelium cells. The actual shell of such tubules is thickened (up to 45–50 μm) due to the growth of connective tissue elements. In some places, the contours of the tubules are blurred and difficult to distinguish. Partial or complete obliteration of the seminiferous tubules, sclerosis, and hyalinosis were found in the testicular parenchyma of infertile men.

The diameter of the part of the convoluted seminiferous tubules is sharply reduced, and their complete atrophy is observed. The tubule shell itself is sclerosed. The spermatogenic epithelium is necrotized. In some places, there are single Sertoli cells near their shell. Cells of the spermatogenic epithelium are absent. In the spaces between the tortuous seminiferous tubules, leukocyte infiltration is observed, and the proliferation of connective tissue elements is noticeable. In the lumen of the seminiferous tubules, the decay products of the cells of the spermatogenic epithelium are visible. Some of the tubules are completely empty.

A severe degree of cell damage was found in 34.8 % of the preserved seminiferous tubules. Some of the cells of the spermatogenic epithelium are displaced into their lumen and necrotic. In other drugs, there is a progressive desquamation and reduction of the layers of the spermatogenic epithelium of the seminiferous tubules. Adjacent to the basal membrane of the tubules are individual Sertoli cells with a deformed nucleus and granular cytoplasm and signs of active phagocytosis. 1–2 layers of spermatogonia and spermatocytes in a state of pronounced dystrophy are adjacent to the shell of other tubules. Spermatogenic epithelial cells are sparsely distributed. The actual shell of the tortuous seminiferous tubules is thickened due to edema and fibrosis. Its contours are wavy. Layers of peritubular myoid cells are separated from each other by bundles of collagen fibers.

In 26.3 % of convoluted seminiferous tubules, a mild degree of damage to the cells of the spermatogenic epithelium was detected. Their own membrane is thickened to 15–22 μm , with edema. Cells of the spermatogenic epithelium are placed unevenly on the basal membrane. Cavities are visible between them in individual tubules. The number of cells, particularly spermatocytes and early spermatids, decreases in the spermatogenic epithelium. Their nuclei are swollen and hyperchromic. There is cytoplasmic swelling and cytoplasmic vacuolation. Some of the spermatogenic epithelium cells are displaced into the tubules' lumen and are necrotized. In seminiferous tubules with impaired spermatogenesis, mature forms of spermatozoa are absent. Violations of spermatogenesis are directly dependent on blood circulation disorders; in long-term cases, they are irreversible.

Along with progressive atrophic changes in the tortuous seminiferous tubules, pathological changes in the interstitial tissue of the testis are also naturally detected. Areas of interstitial tissue between tubules expand due to swelling and proliferation of connective tissue elements, and their volume increases. Interstitial connective tissue is replaced by coarse-fiber, hyalinized, and sclerosed tissue, which leads to the deformation of blood vessels and tortuous seminiferous tubule separation. Hyalinization of the perivascular and peritubular layers is observed. The volume of interstitial endocrinocyte nuclei decreased to $77.40 \pm 2.30 \mu\text{m}^3$ against $90.20 \pm 5.60 \mu\text{m}^3$ in the control.

Laboratory studies of ejaculation in men who suffered from epidemic parotitis indicate significant pathological changes (Table 2).

Table 2

Ejaculate parameters of infertile mature men with a history of mumps (M \pm m)

Ejaculate parameters	Control group	After the epidemic parotitis
Volume (ml)	3.31 \pm 0.50	2.80 \pm 0.30
Color	grayish-white	grayish-white
Reaction (pH)	7.2	8.0
Sperm count in 1 ml (million)	70.25 \pm 8.60	13.70 \pm 0.60*
Active motile spermatozoa (%)	56.30 \pm 3.12	38.20 \pm 2.48*
Live sperm (%)	85.12 \pm 4.82	30.54 \pm 2.60*
Number of spermatozoa with a normal structure (%)	60.40 \pm 3.12	17.78 \pm 1.45*
Number of spermatozoa with head pathology (%)	15.46 \pm 1.36	29.17 \pm 1.24*
Number of spermatozoa with pathology of the mid part of the flagellum (%)	8.70 \pm 0.56	14.13 \pm 0.89*
Number of spermatozoa with pathology of the principal part of the flagellum (%)	10.17 \pm 1.02	17.30 \pm 3.30
Number of spermatozoa with combined pathology (%)	5.27 \pm 2.05	21.62 \pm 3.68*
Farris index (ng/dL)	765.50 \pm 18.20	340.20 \pm 10.35*

Note: * – $p < 0.05$ compared to the control.

The volume of ejaculate, dilution time, and viscosity are noticeably reduced. Its alkalinity increases. In particular, the concentration of spermatozoa in 1 ml of ejaculate decreases by five times. Its volume decreases to 2.80 \pm 0.30 ml against 3.31 \pm 0.50 ml in the control. The sperm concentration was reduced to 13.70 \pm 0.60 million/ml against 70.25 \pm 8.60 million/ml in the control.

Under these conditions, the number of morphologically normal spermatozoa decreases to 17.78 % against 60.40 % in the control. Among the pathological forms of spermatozoa, spermatozoa with head defects predominate in 29.17 % (small, vacuolized) compared to 15.46 % in the control. In a significant number of spermatozoa (14.13 %), pathological changes are found in the mid part of the flagellum (short, curved) against 8.7 % in control. In 17.30 % versus 10.17 % in the control, spermatozoa with pathology of the principal part of the flagellum were determined. All this indicates that men with a history of epidemic parotitis have a violation of the last stages of spermatids development and a violation of sperm motility. Characteristic disorders were deformity of the neck and tail, shortening of the tail, a significant cytoplasmic drop, and deformation or reduction of the sperm head. A 3-fold increase in the content of spermatogenesis

cells is observed. Under these conditions, the indicators of the content of spermatozoa with active progressive movement change significantly.

The concentration of testosterone in the blood of men after suffering epidemic parotitis decreased to 340.20 ± 10.35 ng/dL against 765.50 ± 18.20 ng/dL in the control group. Therefore, the obtained results indicate that infertile men with a history of epidemic parotitis have significant violations of testicular hemodynamic parameters, pathological changes in the structure of the testicular parenchyma, and disorders of spermatogenesis.

As is known, inflammatory diseases cause the narrowing of the lumen of testicular arteries and the thickening of their walls. The network of microvessels around the tortuous seminiferous tubules is compacted. Capillaries are significantly narrowed, and their focal reduction occurs. Expansion and tortuosity of all vessels of the vascular bed are observed [9]. First of all, pathological changes occur in the vessels of the venular link, which is explained by the absence of peritubular myoid elements in them and the anatomical tendency of the venous system to stagnant phenomena. A reduction in metabolic microvessels accompanies Ischemia of organs and tissues and in conditions of congestive venous fullness – their transformation into depositing vessels. Such transformations are monitored after chronic tissue inflammation and are accompanied, as our research and the works of other authors have shown, by changes in hemodynamics [4, 7]. Violations of hemodynamics are manifested to one degree or another by pronounced ischemia. The cells of the spermatogenic epithelium are most sensitive to hypoxia [2]. The decrease in the volume of the nuclei of interstitial endocrinocytes, which we observed in the testicles of infertile men, indicates a violation of these cells' metabolic and functional activity, which in turn also negatively affects spermatogenesis [11]. Degenerative changes in the spermatogenic epithelium and disruption of spermatogenesis lead to a decrease in the concentration of spermatozoa in the ejaculate, an increase in the number of pathological forms, a reduction in their mobility, and ultimately male infertility [1, 4, 8].

Conclusions

1. In infertile mature men with a history of mumps, testicular volume decreased to 17.31 ± 1.20 cm³, against 19.50 ± 1.40 cm³, the maximum rate of arterial blood flow in the testicular artery within the spermatic cord decreased to 14.90 ± 1.20 cm/s compared to 19.30 ± 1.46 cm/s in the control.

2. The diameter of convoluted seminiferous tubules decreased to 173.80 ± 1.90 μm versus 210.60 ± 5.30 μm in control, and the volume of interstitial endocrinocyte nuclei decreased to 77.40 ± 2.30 μm³ versus 90.20 ± 5.60 μm³ in control, which resulted in a decrease in blood testosterone levels to 340.20 ± 10.35 ng/dL, compared to 765.50 ± 18.20 ng/dL in control.

3. In the ejaculate of men, the sperm concentration decreased to 13.70 ± 0.80 million/ml versus 70.20 ± 6.30 million/ml. The number of morphologically normal spermatozoa decreased to 17.78 % against 60.40 % in the control. The number of spermatozoa with head pathology increased to 29.17 % versus 15.46 %, and the number of actively motile spermatozoa decreased to 38.20 % versus 56.30 %.

Prospects for further research consist in the study of structural and functional changes in the prostate gland in men with a history of epidemic parotitis.

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DETERMINATION OF CHANGES IN THE CONTENT OF PRO- AND ANTI-INFLAMMATORY CYTOKINES IN PATIENTS WITH TRUE AND MICROBIAL ECZEMA

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The widespread, long-term chronic course of the disease, the complex nature of the disorders and insufficiently studied pathogenesis determine the interest in the problem of eczema. The purpose of this study was to study the features of changes in the content of peripheral blood interleukins depending on the clinical course, severity and duration of eczema to determine the role of the identified disorders in the development of the inflammatory process. In 40 patients with true and microbial eczema, anamnestic and clinical features, the nature of the disease, as well as the use of highly sensitive ELISA determined the features of cytokine status depending on the selected parameters. These features of cytokine imbalance, which depend on the clinical manifestations of dermatosis, suggest their role in the development of exudative-destructive process in eczema. The concentration of IL-2 in both groups decreased, and IL-4 increased, more pronounced in the 2nd group. The revealed disorders in microbial and true eczema of the level of pro- and anti-inflammatory IL, and the correlation of interleukin parameters have pathogenetic significance and determine the features of the clinical course of this disease.

Key words: true eczema, microbial eczema, immune system, cytokines, pathogenesis of eczema.

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ВИЗНАЧЕННЯ ЗМІН ВМІСТУ ПРО- ТА ПРОТИЗАПАЛЬНИХ ЦИТОКІНІВ У ХВОРИХ НА СПРАВЖНІЮ ТА МІКРОБНУ ЕКЗЕМУ

Широке поширення, тривалий хронічний перебіг захворювання, комплексний характер порушень і недостатньо вивчений патогенез зумовлюють інтерес до проблеми екземи. Метою даного дослідження було вивчення особливостей зміни вмісту інтерлейкінів периферичної крові залежно від клінічного перебігу, тяжкості та тривалості екземи для визначення ролі виявлених порушень у розвитку запального процесу. У 40 пацієнтів з істинною та мікробною екземою анамнестичні та клінічні особливості, характер захворювання, а також використання високочутливого ІФА визначали особливості цитокинового статусу залежно від обраних параметрів. Ці особливості дисбалансу цитокінів, які залежать від клінічних проявів дерматозу, свідчать про їх роль у розвитку ексудативно-деструктивного процесу при екземі. Концентрація ІЛ-2 в обох групах знижувалася, ІЛ-4 підвищувалася, більш виражена становила у 2-й групі. Виявлені порушення при мікробній та істинній екземі рівня про- та протизапальної ІЛ, співвідношення показників інтерлейкінів мають патогенетичне значення та визначають особливості клінічного перебігу цього захворювання.

Ключові слова: справжня екзема, мікробна екзема, імунна система, цитокіни, патогенез екземи.

The study is a fragment of the research project, "Optimization of disorders of adaptation mechanisms in acute and chronic dermatoses", state registration No. 0119U002905.

The problem of studying the mechanism of eczema is one of the most relevant in modern dermatology, due to the high level of morbidity, increasing the number of trigger factors, chronic recurrent course, causing psychosocial maladaptation of patients [4, 6, 7].

Numerous studies indicate the complex nature of disorders of many body systems in patients with eczema [3, 8, 10], but the leading role is given to genetically determined or acquired immune disorders [5]. The role of immune mechanisms in the development of dermatosis cannot be fully understood, because the state of the cellular and humoral parts of the immune defense is characterized by multidirectional changes. A special role in the development and course of exudative-destructive inflammation in the disease is played by cytokines, the biological action of which is very diverse. Cytokines are one of the main regulators of intercellular interaction, are actively involved in the implementation of the immune response, inflammatory reactions and others. [2, 9].