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PATHOMORPHOLOGICAL CHANGES OF THE OPTICAL NERVE INTRACRANIAL PART IN DIABETES MELLITUS

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The work is devoted to the study of morphological changes in the optic nerve, mainly its intracranial part, in diabetic microangiopathy. It has been shown that diabetic microangiopathy is accompanied by proliferation of capillary endotheliocytes, resulting in narrowing or obliteration of the blood vessels lumen. In its turn, this leads to ischemia of nerve fibers with the development of dystrophic changes and partial or complete destruction of astrocytes and oligodendrocytes. Obviously, in diabetes mellitus, these processes also occur in the optic nerve tissues. Severe form of diabetic optic nerve microangiopathy is similar to the proliferative form of retinopathy by its morphological features.

Key words: diabetic retinopathy, optic nerve, morphology.

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ПАТОМОРФОЛОГІЧНІ ЗМІНИ ІНТРАКРАНІАЛЬНОЇ ЧАСТИНИ ЗОРОВОГО НЕРВА ПРИ ЦУКРОВОМУ ДІАБЕТІ

Робота присвячена дослідженню морфологічних змін зорового нерва, переважно інтракраніального відділу, при діабетичній мікроангіопатії. Показано, що діабетична мікроангіопатія супроводжується проліферацією ендотеліоцитів капілярів, в результаті чого виникає звуження або облітерація просвіту судин. У свою чергу це призводить до ішемії нервових волокон з розвитком дистрофічних змін і часткового або повного руйнування астроцитів і олігодендроцитів. Очевидно, що при цукровому діабеті ці процеси відбуваються і в тканинах зорового нерва. Важка форма діабетичної мікроангіопатії зорового нерва схожа за морфологічними ознаками з проліферативною формою ретинопатії.

Ключові слова: діабетична ретинопатія, зоровий нерв, морфологія.

The work is a fragment of the research project "Morphogenesis patterns of organs, tissues and vascular-nervous formations in the norm, in pathology and under the influence of external factors",state registration No. 0118U004457.

In recent years, diabetes mellitus has become an epidemic of the XXI century [6]. The number of people with diabetes doubles every 13–15 years and is about 240 million people worldwide. In Ukraine, according to statistics, about 1 million people suffer from diabetes mellitus, but the number of undetected patients is by 2–3 times higher than this figure [17]. Ophthalmic complications of diabetes mellitus are the cause of blindness in 80–90 % of patients. Morphological disorders of the eyeball in this disease are accompanied by damage to the retinal microvessels. According to the classification (E. Kohner, M. Porta, 1991), approved by the WHO, there are the following forms of diabetic retinopathy: non-proliferative, preproliferative, proliferative ones [4, 5, 17]. In addition, in diabetes mellitus, neuropathic changes of the optic nerve are frequently observed, but they are understudied. The morphogenesis study of pathological changes occurring in the optic pathway in diabetes mellitus is of considerable interest to both ophthalmologists and pathomorphologists. They permit to determine the pathomorphological process localization in certain areas of the optic pathway and to detect ophthalmic manifestations of diabetes mellitus symptoms [6, 7, 11, 16,]. However, in the literature available to us, questions about morphological changes in the intracranial optic nerve part in type II diabetes mellitus are insufficiently covered. It is known that the pathogenesis of type II diabetes mellitus is associated with glycosylation. These very changes can cause features of histochemical changes both in microvessels, and in tissue elements of the optic nerve.

The purpose of the work was to study the pathomorphological changes in the intracranial part of the optic nerve in patients with type II diabetes mellitus.

Materials and methods. The material was sampled on the basis of the Poltava Forensic Medical Bureau and the Poltava Regional Anatomic Pathology Bureau.

The control group of studies consisted of serial sections of intracranial optic nerve's histological specimens, which were obtained from people aged 20 to 50 years (15 persons). All studies were performed on the material of people who died as a result of car injuries. According to the autopsy, they had no pathomorphological signs of type II diabetes mellitus.

The study group consisted of serial sections of histological specimens obtained from patients with type II diabetes mellitus who died due to complications of non-insulin-dependent diabetes (9 cases). The distribution of the studied material in the age aspect is presented in table 1.

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Table 1

Material distribution in the age aspect depending on nosological units

Nosological unit	20–30 years	40–50 years	50–60 years
Died of car injuries	12	3	-
Diabetes mellitus (type II)		3	6

For histological methods paraffin sections were used with a thickness of 4–5 μm with their staining with hematoxylin and eosin according to standard methods. To study the parenchymal and stromal components of the optic nerve histochemical methods were used and their combinations with picro-fuchsin tissue staining by van Gizon, periodic acid Schiff (PAS) reaction with alcyan blue, fuchsin-picro-fuchsin by the Hart + van Gizon method, amido black 10B, Nile blue. These histochemical methods permitted to differentiate different structures of the optic nerve and blood vessel walls to determine pathomorphological changes in them characteristic of diabetic microangiopathy.

The study of stained microslides was performed with the Olympus BX-41 light microscope produced by Olympus Medical Systems Corporation using a ×10 ocular and ×10, ×20, ×40, ×100 field lenses, and their photography was performed with a digital camera produced by the "Olympus C 4040" company with "Olympus DP-Soft" software.

Results of the study and their discussion. Morphological changes of the microcirculatory bed of the optic nerve in patients with type II diabetes mellitus are manifested in the form of mild, moderate and severe changes in certain parts of the microcirculatorybed. Mild morphological changes are observed mainly in the interseptal arterioles, as well as in microvessels ramifying from them. These morphological changes include: thickening of the inner arteriole membranes in the form of dark deposits when stained with amido black 10B, partial destruction of the inner elastic blood vessel's membrane and the middle circular smooth muscle layer with a light brown color.

Perivascular connective tissue is swollen, sometimes single cellular elements are found in it. The optic nerve's nerve tissue adjacent to the arterioles is represented by small or medium-sized foci with clear contours in the middle of which there are nerve fibers (fig. 1).

In order to study the morphological changes of the optic nerve's septal arterioles in more detail, their microscopic examination at immersion magnification was performed. In the lumen of the arterioles erythrocytes were found, colored lilac, adjacent to the blood vessels' walls. In the septal arterioles of the intracranial optic nerve changes were detected, characterized by the loss of the inner elastic membrane's continuous structure, due to which it acquires a discontinuous appearance. Dystrophic changes were observed in the smooth muscle cells of the vascular wall, as a result of which light vacuoles of various sizes appeared in their cytoplasm. These vacuoles arose due to the accumulation of lipoprotein complexes, which is characteristic of the initial forms of microangiopathy. Perivascular edema was found in the tissues surrounding the vessel, which indicated an increase in vascular permeability (fig. 2).

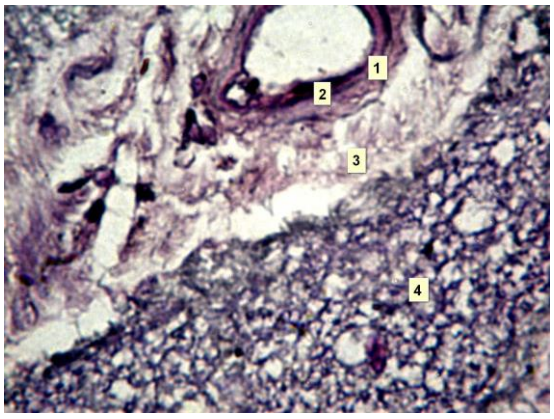

Fig.1 Mild degree of septal arterioles destruction in the intracranial optic nerve when stained with PAS+ alcyan blue: 1 – thickening of the inner coat of the vessel; 2 – destruction of the middle muscle layer; 3 – damage to connective tissue fibers around the vessel; 4 – nervous tissue –Magn. X800:

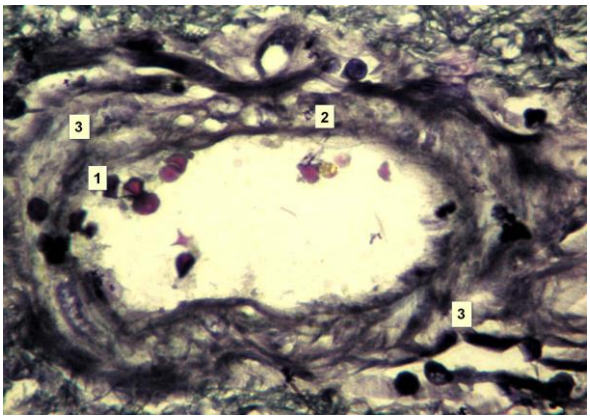

Fig. 2 Destruction of septal arterioles in the intracranial optic nerve: 1 – discontinuous appearance of the inner elastic membrane; 2 – vacuolation of smooth muscle cells; 3 – perivascular edema. amide black 10B staining. Magn. x1000.

Histochemical staining with Nile blue was used to confirm the presence of lipoprotein deposits in the vessel wall walls, as this dye permits to differentiate fatty substances. It was found that in the small vessels' walls there were blue colored inclusions, which indicated the presence of complex fatty compounds. In addition, cerebrosides of nervous tissue were also colored blue. The septal arteriole's wall of the optic nerve was thickened. In the middle circular layer there was a deposition of lipoproteins, which

indicated their infiltration, which occurs due to dosorders of fat-protein metabolism and increased concentration of various lipoprotein complexes in the blood. It should be noted that the connective tissue around the arterioles was swollen, there were single macrophages observed in it. Nerve fibers around the septal arterioles are represented by individual bundles. The optic nerve tissue had the form of a fuzzy light trabeculae accumulation (fig. 3).

In order to determine the features of the optic nerve's microarchitectonics, its study was carried out using the combined histochemical staining with PAS+ amido black 10B.

It was found that the cytoplasm of astrocytes in the perivascular connective tissue turns red, and the nuclei of oligodendrocytes turn dark lilac by the method of PAS + amide black staining. However, in contrast to the norm, in the initial form of microangiopathy near the arterioles cavities of large enough size were present, where were torn processes of oligodendrocytes. The walls of the arterioles, which supply blood to the nervous tissue, are thickened due to hyalinosis (fig. 4).

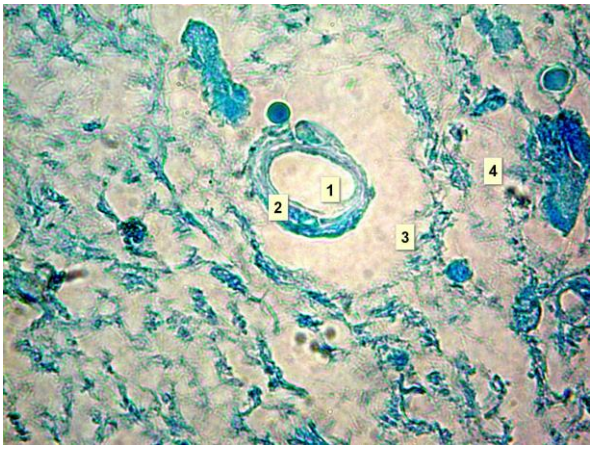

Fig.3 Accumulation of lipoproteins in the septal arterioles' wall of the optic nerve: 1 – lumen of the arteriole; 2 – lipoproteins in the muscle layer; 3 – perivascular edema; 4 – partial disorganization of nerve fibers. Nile blue staining. Magn. x200.

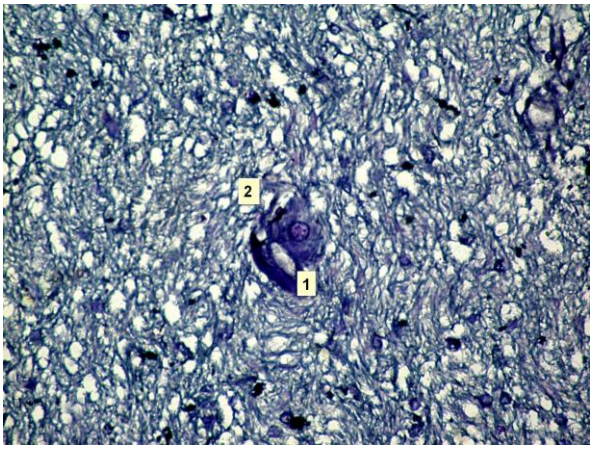

Fig. 4 Hyalinosis of arterioles in the intracranial optic nerve: 1 – hyalinosis of arterioles; 2 – accumulation of PAS-positive substance in astrocytes. Staining with PAS + amide black 10B.Magn. x200.

Thus, morphological studies of the optic nerve's microcirculatory bed in type II diabetes mellitus indicate a slight degree of the arteriole wall's and the optic nerve's structure damage, which is consistent with the literature data as a nonproliferative form of retinopathy [12].

Severe degree of morphological changes is manifested by significant proliferative changes. At the same time, due to endothelium proliferation, the lumen of both arterioles and venules narrows. The capillary network accompanying nerve fibers is characterized by multiple figures of mitosis in the endothelium. In considerable proliferation of endotheliocytes the lumen of capillaries and venules is almost completely closed. This leads to ischemia of the optic nerve tissue, accompanied by the destruction of the optic nerve fibers' bundles and to the almost complete absence of astrocytes and oligodendrocytes. As our studies have shown, morphological changes of the optic nerve, mainly intracranial, in diabetic microangiopathy, are accompanied by capillary endotheliocytes' proliferation, which leads to partial narrowing or obliteration of the blood vessels' lumen.

This process leads to ischemia of nerve fibers and partial or complete destruction of astrocytes and oligodendrocytes. These morphological changes in the intracranial parts of the optic nerve in type II diabetes mellitus are similar to the proliferative retinopathy observed in the retina.

As a result of our study, it was found that microangiopathy in type II diabetes mellitus was similar to retinopathy and was stepwise characterized by morphological changes first in the arterioles, accompanied by lipoproteins deposition, and, apparently, due to hypercholesterolemia and the occurrence of triglyceride and lipoproteine fractions, as well as lipoproteins of low and very low density.

With insufficient efficacy of diabetes mellitus treatment, which is a global medical and social problem of today, the development of complications is possible that limits the patients' quality of life, lead to early disability due to the development of vascular complications. One of the target organs in diabetes mellitus is the organ of vision [14]. Even with a short duration of type 2 diabetes mellitus, 35 % of patients have a non-proliferative form of diabetic retinopathy, and 14 % – a proliferative form [14].

It is known that chronic hyperglycemia is accompanied by accumulation of glucose in neurons, endothelial cells and other cells with the activation of pathological pathways of its metabolism. Excess glucose is used in the polyol pathway in insulin independent tissues with formation of sorbitol. Accumulation of the latter in the extracellular space leads to an increase in the osmolarity of the

intercellular matrix with the development of edema. Activation of the protein kinase pathway against the background of hyperglycemia contributes to the increased vascular permeability, disruption of the blood-brain barrier integrity and activation of lipid peroxidation processes [10]. This is confirmed by our studies, because even with minor changes in the tissues of the intracranial optic nerve, perivascular and intercellular edema was observed, in addition, fragmentation of the inner elastic membrane was observed, and hence it was the beginning of the blood-neuronal barrier destruction.

Resulting from activation of glucose utilization alternative ways, dystrophic changes in the vascular wall gradually develop, which leads to the nerve tissue damage. Despite the fact that microcirculation disorders are a classic manifestation of complications in diabetes mellitus, neurodegenerative changes develop much earlier and are accompanied by neurocytic apoptosis and glia dysfunction [15]. However, progressive endothelial cell dysfunction plays a leading role in further morphostructural changes of the vascular wall and nerve damage: thickening of the vessel wall, loss of perivascular cells, damage to the blood-neuronal barrier [14, 15].

It is established that the basis for the development of polyneuropathy chronic symmetrical forms are metabolic disorders and microangiopathy. It is the increased sorbitol content in the bodies of neurons, Schwann cells, endothelium and nerve processes that is associated with the main damaging effect of chronic hyperglycemia.

These are the consistent changes we found in our study. The obtained results indicate the correlation between morphological changes in the vascular wall, i.e. microangiopathy, and changes in the nerve tissue – neuropathy. Our data expand the knowledge about the morphogenesis of complications in diabetes mellitus, which can be used in the diagnostic process and in the treatment of patients, as the impact on certain pathogenetic links is a promising field of diabetic complications treatment [14].

Conclusion

Microangiopathy in type II diabetes mellitus is similar to retinopathy and is characterized by morphological changes first in the arterioles with deposition of β-lipoproteins in the latter. This process is caused by hypercholesterolemia and the occurrence of triglyceride fractions in the bloodstream. The moderate degree of morphological lesions observed in diabetic angiopathy is characterized by a further process of disorganization in venules and capillaries. This reveals significant circulatory disorders with the sludge syndrome, as well as numerous diapedetic hemorrhages into the brain tissue. Morphological changes of microvessels, as well as circulatory disorders resemble preproliferative retinopathy. Finally, the severe degree of morphological lesions in diabetic angiopathy is characterized by high proliferative activity of endothelial cells in both capillaries and venules. Due to this, the lumen of these vessels is partially or completely obliterated. As a result, there occurs ischemia of nerve fibers, their partial destruction, as well as neoangiogenesis of the destruction site. Severe form of diabetic optic nerve microangiopathy resembles the proliferative form of retinopathy by its morphological features.

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INFLUENCE OF PROLONGED TRIPTERELIN-INDUCED CENTRAL DEPRIVATION OF TESTOSTERONE SYNTHESIS ON MORPHOLOGICAL STRUCTURE OF RAT'S LIVER

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In recent years, the incidence of prostate cancer has increased worldwide. For example, in 2012, the GLOBOCAN project found that prostate cancer was the second most commonly diagnosed cancer and the fifth leading cause of cancer deaths among men worldwide. Insufficient testosterone can exacerbate liver damage caused by obesity. The aim of our study was to identify morphological changes in the liver of rats at the tissue and cellular levels, to study the processes of formation of reactive oxygen species and the intensity of lipid peroxidation during prolonged central deprivation of testosterone synthesis caused by triptereline. Triptorelin-induced central deprivation of testosterone synthesis leads to oxidative damage to hepatocytes due to increased production of reactive oxygen species and decreased activity of antioxidant enzymes. Oxidative damage to liver cells begins at the molecular and cellular levels and becomes apparent at the tissue level on day 180 of the central deprivation of testosterone synthesis.

Key words: liver, triptoreline embonate, NO-synthase, superoxide dismutase, malon dialdehyde, superoxide anion radical, rats.

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ВПЛИВ ПРОДОВЖЕНОГО ЦЕНТРАЛЬНОГО БЛОКУВАННЯ СИНТЕЗУ ТЕСТОСТЕРОНА ТРИПТЕРЕЛІНОМ НА МОРФОЛОГІЧНУ СТРУКТУРУ ПЕЧІНКИ ЩУРІВ

В останні роки рак простати збільшується у всьому світі. Наприклад, у 2012 році проект GLOBOCAN показав, що рак передміхурової залози був другим за частотою діагностики раком та п'ятою провідною причиною смертності від раку серед чоловіків у всьому світі. Недостатня кількість тестостерону може посилити пошкодження печінки, спричинені ожирінням. Метою нашого дослідження було виявлення морфологічних змін у печінці щурів, вивчення процесів утворення активних форм кисню та інтенсивності перекисного окислення ліпідів під час тривалої центральної депривації синтезу тестостерону. Індукована трипторелін центральна депривація синтезу тестостерону призводить до окислювального пошкодження гепатоцитів внаслідок збільшення виробництва активних форм кисню та зниження активності антиоксидантних ферментів. Окисне пошкодження клітин печінки починається на молекулярному та клітинному рівнях і стає помітним на рівні тканин на 180-й день центральної депривації синтезу тестостерону.

Ключові слова: печінка, трипторелінoвий ембонат, NO-синтаза, супероксиддисмутаза, малоновий диальдегід, SAR, щури.

The study is a fragment of the research project "Experimental morphological study of cryopreserved placenta transplants and diphereline, ethanol and 1% methacrylic acid action on the morphofunctional status in a number of internal organs", state registration No. 0119U102925.

Prostate cancer has been increasing worldwide in recent years. For instance, in 2012 GLOBOCAN project showed that prostate cancer was the second most frequently diagnosed cancer and the fifth leading cause of cancer mortality among men worldwide. This trend even affected Asian countries like Japan Korea, since the lifestyle of the population of these countries became similar to the Western World [10]. The increased prevalence of prostate cancer leads to economic loses even for the most developed countries. In Sweden, the total annual costs extrapolated to Sweden were calculated to be 281 000 000 € [4]. Therefore, prostate cancer treatment and diagnostic of prostate cancer is an important problem for modern medical science.

One of the approaches to the prostate cancer therapy is either chemical or surgical castration, since testosterone and other androgens are viewed as key risk factors of prostate cancer progression [5]. However, recent scientific discoveries provided a background for reconsideration of this paradigm [9]. The

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