Angiopathy as a Cause of Structural Organ Changes under Experimental Conditions in Diabetes Mellitus

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Abstract

One of the urgent problems of modern medicine is to understand and explain the structural rearrangement of organs and tissues in the course of diabetes. The article presents the results of a comprehensive morphological study of the structural organization of micro- and macrovasculators, as well as some organs under conditions of diabetes mellitus in the experiment.

The study material constituted 52 adult outbred white male rats, with the average weight of 150-180 g, 4,5-6,5 months old. The following research methods were applied: histological, electron microscopic, biochemical, experimental modeling of streptozotocin-induced diabetes mellitus, morphometric and statistical. It was found that diabetic microangiopathy develops after 2 weeks of experimental diabetes, while after 4 weeks of the experiment there were some structural changes in the aorta observed. Furthermore, after 6 and 8 weeks, some profound destructive changes in the mandible and testis were revealed.

The obtained results may be used as fundamental data for the development of new methods for diagnosis, prevention and treatment of pathology of the cardiovascular system, oral cavity and male reproductive system caused by diabetes.

Experimental article (J Int Dent Med Res 2022; 15(2): 935-941)

Keywords: Vessels, salivary gland, testicle, structure, diabetes mellitus, experiment.

Received date: 05 January 2022 **Accept date:** 14 May 2022

Introduction

One of the urgent problems of modern medicine is to understand and explain the structural rearrangement of organs and tissues in the course of diabetes. According to the WHO and the International Diabetes Federation (IDF), the global rates of diabetics are growing; in 2017 they made up 450 million people. It is worth mentioning that 50-80% of the patients with diabetes are estimated to die of cardiovascular disease[1]. Vascular complications in diabetes mellitus are called "diabetic angiopathies" and are characterized by generalized lesions of all parts of the hemomicrocirculatory tract, accompanied by degenerative changes of arterioles and capillaries (microangiopathy), as well as vessels of medium and large diameter (macroangiopathy)[2,3,4]. High cardiovascular risk in diabetes is manifested in the pre-diabetes stage. It is known that insulin resistance plays a leading role in the development of type 2 diabetes[5,6,7,8], which significantly increases the susceptibility to cardiovascular disease[9].

A universal morphogenetic sign of the development of complications of diabetes is pathological changes in blood vessels, which are characterized by different frequency, prevalence and features of manifestations for each patient. We still can not answer the question of what is the cause and what is the result in pathogenesis of angiopathy in diabetes. Feelings of thirst, dryness of the oral mucosa are one of the first clinical manifestations of diabetes. A number of studies in patients with diabetes mellitus show changes in the functions of the salivary glands and dental status in the form of: dry mouth, burrs in the corners of the mouth, scales on the lips, salivating of thick or cloudy consistency[10,11,12].

In addition, changes in the mineral and enzymatic composition of saliva may be observed[13,14,15,16]. However, there is not enough data on the morphology of the salivary glands in

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diabetes in literature. Clinical studies have shown that diabetes, as a generalized disease, also leads to the development of various dental pathological processes (periodontitis, periodontitis) with subsequent damage to the tooth and dental organ as a whole[17,18]. It was found that in patients with diabetes gum tissue is damaged, the mineral composition of tooth bone changes, the amount of collagen fibers of the periodontium decreases, which in turn leads to profound changes in the entire dental apparatus[19,20,21]. The problem of morphological features of the testis of humans and experimental animals in conditions of diabetes and other pathological conditions remains relevant and important for modern medicine. Information on the dynamics of the development of structural changes in the testis in diabetes is still quite insufficient[22]. This indicates the feasibility of a comprehensive study of the structural organization of micro- and macro vessels, as well as organs in general in diabetes mellitus. Therefore, the aim of our study was to establish the morphological state of the vessels of the hemomicrocirculatory tract, aorta, mandibular gland and testis in experimental diabetes mellitus.

Materials and methods

The studies were performed on 52 adult outbred white male rats, with average weight of 150-180 g and 4,5-6,5 months old.

Animals were kept in the vivarium of Lviv National Medical University named after Danylo Halytsky, experiments were conducted in compliance with the main provisions of the Law of Ukraine № 3447-IV "On protection of animals from cruel treatment", EU Directive on protection of animals used for scientific purposes № 63 (from 22.09. 2010), orders of the Ministry of Health of Ukraine № 944 of 14.12.2009 and the Ministry of Education and Science of Ukraine № 249 of 01.03.2012.

In rats, insulin-dependent form of type I diabetes mellitus, similar to insulin-dependent form of type 1 diabetes mellitus in humans, was induced by a single intraperitoneal injection of Sigma streptozotocin at a rate of 7 mg per 100 g of body weight of the test animal (prepared on 0.1 M citrate buffer). = 4.5). The material for the study was collected after 2, 4, 6 and 8 weeks of the experiment under the control of blood biochemical parameters (glucose level, level of glycosylated hemoglobin and general hemoglobin). Euthanasia was performed by diethyl ether overdose. The material for the study were preparations of the aortic wall, salivary gland sections and testicular sections. For histological examination, fragments of the aorta, mandibular gland and testis were soaked in 10% neutral formalin for 24 hours. The material was poured into paraffin blocks, from which slices 5-7 μm thick were made on a sled microtome of the MS-2 model. Histological sections were stained with hematoxylin and eosin, or azan following Heidenhain method. We also used resorcinol-magenta by Weigert with the addition of picrofuxin using Van Gizon method according to conventional approach. The preparations were studied on a light microscope MBI-1 at magnifications of x200 and x400. The Aver Media computer system was used to photograph the micropreparations. Electron microscopic examination was performed on an electron microscope UEMV-100K at an accelerating voltage of 75 kV and magnifications x4000-x8000 in the Laboratory of Electron Microscopy of Danylo Halytskyi Lviv National Medical University. Standard laboratory techniques were used to perform a biochemical blood test. Excel software from the Microsoft Office application package was used for statistical analysis of source data, mathematical calculations, their graphical representation and analysis results.

The numerical values of the structural parameters were represented by sample averages (M) with standard error (m), standard deviation and level of confidence (p).

Results

After 2 weeks of experimental diabetes mellitus, the arterioles of all studied organs had a sharply swollen endothelium with the elements of proliferation, the wall of the arterioles was thickened, homogeneous, eosinophilic (Fig. 1). By this stage of the experiment, the outer diameter of the arterioles has increased significantly - (18.56 ±2.59) μm, compared with the control group - (10.39 ±2.21) μm, the difference was statistically significant ($p < 0.0001$). The wall of the venules had an uneven thickness, and its lumen was irregularly shaped. The shape of the lumen of the capillaries is preserved, but we observed a slight thickening of their wall due to edema of endothelial cells, the diameter of the

capillaries increased to (3.45 ±1.21) μm, while in the control group it was (3.22 ±1.18) μm, but the difference compared to the control group was not reliable p = 0.97.

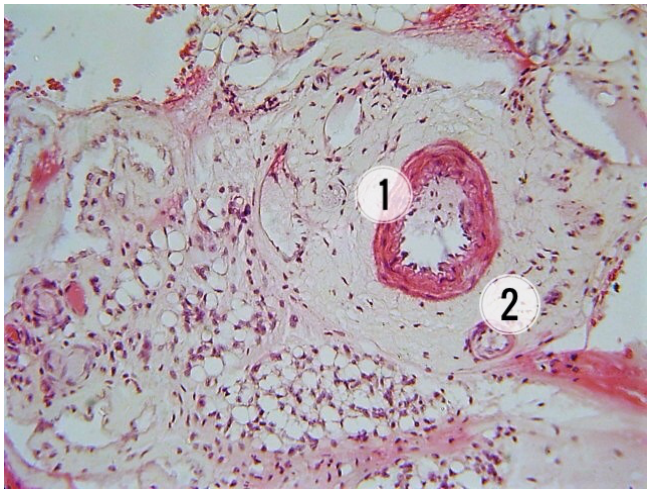

Figure 1. Edema of the endothelium of the arterioles with elements of proliferation, thickening of the tunica media (1), slight thickening of the capillary wall (2) of tunica externa of the aortic wall of white rat 2 weeks from the onset of streptozotocin-induced diabetes mellitus. Photomicrograph. Hemotoxylin and eosin staining. Image: x200.

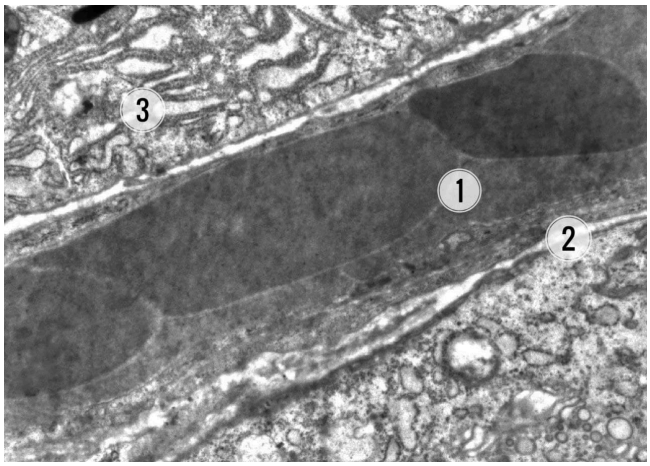

Figure 2. Erythrocyte-filled capillary lumen (1), fluffy basement membrane (2), dilated granular endoplasmic reticulum (3) of the submandibular gland of the white rat 2 weeks from the onset of streptozotocin-induced diabetes mellitus. Electronic microphotography. Image: x14000.

Electron microscopic examination of the submandibular gland after 2 weeks from the onset of experimental diabetes showed reactive changes in the vessels of its hemomicrocirculatory tract. The nucleolemma of endothelial cells in some places lost a clear contour, the perinuclear space was uneven, had wide and narrow areas, the cytoplasm was swollen, there were few organelles, including dilated channels of granular endoplasmic reticulum (Fig. 2), small, with transparent matrix and partly reduced number of cristae, with polysomes and lysosomes. The lumenal surface of endothelial cells was characterized by visible protrusions of the cytoplasm in the form of microvilli into the lumen of microvessels.

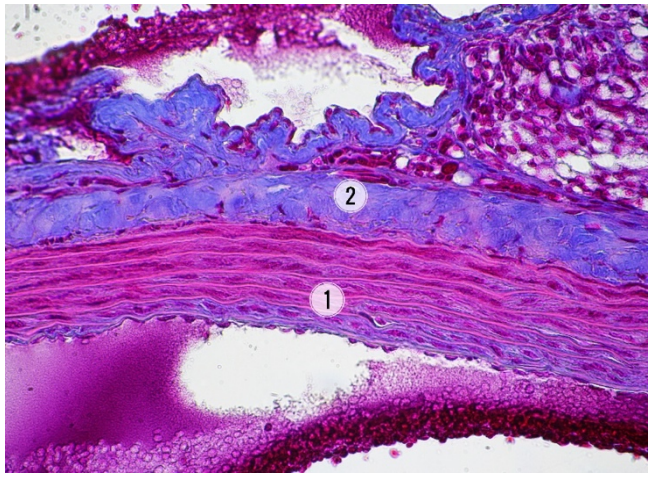

Figure 3. Increase in thickness of the internal elastic lamina of the tunica media of the aortic wall (1) and edema of the tunica externa of the aortic wall (2) 4 weeks from the onset of experimental diabetes mellitus. Photomicrograph. Azan staining by Heidenhain. Image: x200.

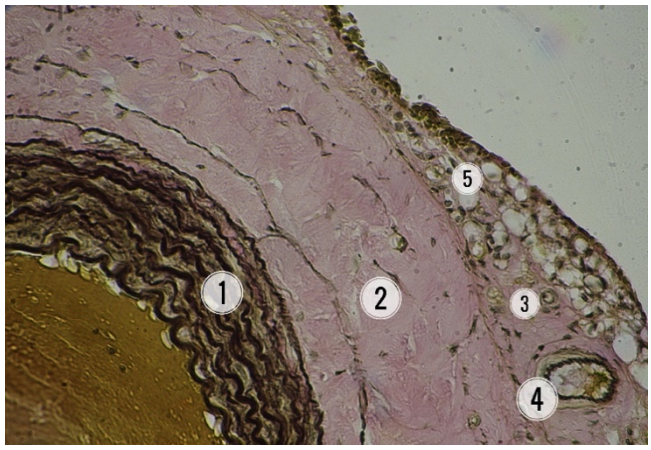

Figure 4. Increase in thickness of the internal elastic lamina of the tunica media of the aortic wall, appearance of pseudocysts filled with lipid content (1), edema of the tunica externa (2), moderate edema of the capillary wall (3), moderate edema of the arteriole wall (4),

lymphoid infiltrates peripherally (5) aortic wall 4 weeks after the onset of experimental diabetes mellitus. Photomicrograph. Staining with resorcinol-fuchsin by Weigert with addition of picrofuxin by Van Gizon. Image: 200.

After 4 weeks from the onset of experimental diabetes mellitus, signs of macroangiopathy were detected, in particular disorganization of the layers of tunica media of the aortic wall with the appearance of pseudocysts filled with lipid content, as well as the growth of loose connective tissue with small "cloud-like" lymphoid infiltrates on the periphery of the aortic wall, which were not observed in the control group (Fig. 3, Fig. 4).

The above is confirmed by the data of morphometric and statistical studies. In particular, after 4 weeks of experimental diabetes mellitus, thickness of tunica media of the aortic wall decreased and amounted to 58.68 (53.33; 65.25) μm, the difference compared with the control group was statistically significant ($p < 0.001$), thickness of the internal elastic lamina of tunica media of the aortic wall was 10.50 (8.68; 12.35) μm, the difference was statistically significant, both in comparison with the control group ($p = 0.003$) and in comparison with the previous term ($p < 0.001$).

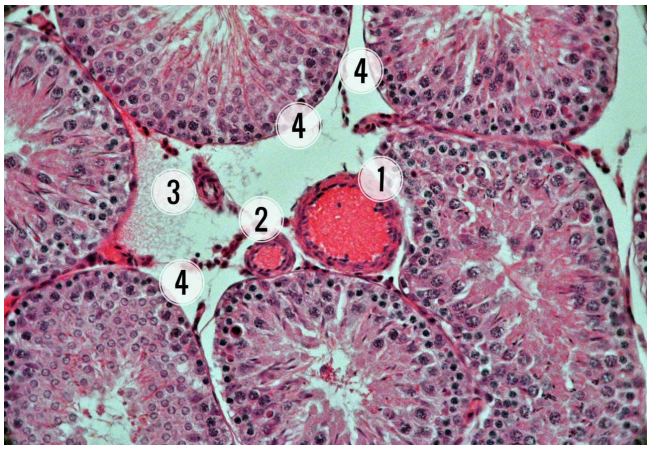

Figure 5. Plethora and formation of parietal thrombi in arterioles (1) and precapillary arterioles (2), edema of connective tissue layers (3) and destructuring of convoluted seminiferous tubules wall of the white rat (4) 6 weeks from the onset of streptozotocin-induced diabetes mellitus. Photomicrograph. Hematoxylin-eosin staining. Image: x400.

After 6 weeks of experimentation, lumens of the arterioles and precapillary arterioles of the wall of convoluted seminiferous tubules are filled with blood elements. There are destructive changes of all layers of the wall of convoluted seminiferous tubules, degeneration of sperm, thickening of collagen fiber layer in non-cellular parts of convoluted seminiferous tubular walls (Fig.5)

After 6-8 weeks of diabetes, the hemato-testicular barrier between the hemocapillaries and convoluted seminiferous tubules is disorganized, dilated, fluffy, discontinuous (Fig. 6). The cytoplasm of sustentocytes is transparent, nuclei of irregular shape, with sparse chromatin in the nucleoplasm. In mitochondria we can observe homogenization of cristae. Accumulations of abortive electron-dense and abortive electron-transparent spermatid heads contained in the system of disorganized cytoplasm of sustentocytes were revealed. The amount of collagen fibers in the connective tissue adjacent to the basement membrane of the wall of the convoluted seminiferous tubules increases.

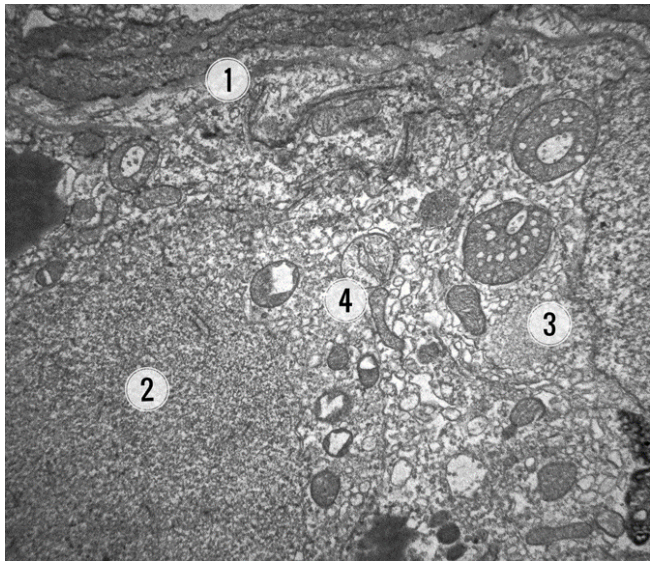

Figure 6. Destructured hematotesticular barrier (1) and deformed sustentocyte (2), mitochondria with homogenized cristae (3) of the convoluted seminiferous tubules (4) of the white rat testis after 6 weeks of streptozotocin-induced diabetes mellitus. Electronic microphotography. Image: x4000.

After 6 and 8 weeks of experimental diabetes mellitus, there have developed some visible destructive changes in the submandibular

gland, in particular the parenchyma of the gland is thickened (acinuses are dense), the shape of the acinus is changed, mostly they are formed by sericites with basal location of nuclei and cells of interparticle ducts as well as epitheliocytes of interparticle and intraparticle ducts are thinned, layers of connective tissue thickened due to edema, showed desquamation of the epithelium into the lumen of the ducts and stagnation of secretion, many granular ducts have no secretory granules (Fig. 7).

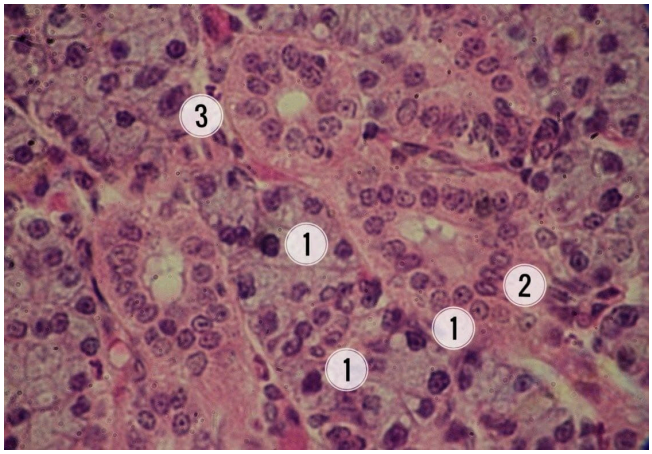

Figure 7. Epitheliocytes of acinus with hyperchromic nuclei with signs of apoptosis (1), striated duct filled with secretory masses (2) and granular excretory ducts (3) of the submandible gland after 6 weeks of experimental diabetes mellitus. Photomicrograph. Staining with hemotoxylin and eosin. Image: x400.

Discussion

The issues of development of vascular complications, their early diagnosis, effective therapy and prevention are of primary importance for lots of researchers and practitioners nowadays[23].

The results of our study clearly showed that the basis of structural changes in organs in diabetes is primary microangiopathy. Despite the huge number of studies on the pathology of the microcirculatory tract in patients with diabetes, there is no consensus on the morphogenesis of microangiopathies, factors of pathogenesis, prediction and early/timely control of development, prevention and more. In our opinion, which is supported by other authors[24], diabetic microangiopathy is characterized by generalized lesions of all parts of the hemomicrocirculatory tract and is accompanied by degenerative changes in arterioles, capillaries and venules. The protrusions of cytoplasm in the form of microvilli into the lumen of microvassels, edema of endothelial cells, loosening (fluffiness) and discontinuity of the basement membrane, aggregation of erythrocytes and their adhesion to the endothelium complement the described in the literature characteristic of diabetic microangiopathy which corresponds to the theory of Rudolf Virchow about triad of factors typical for diabetic angiopathy: changes in vasculature, blood coagulation issues and slow blood flow, which create favourable conditions for microthrombus formation[25,26].

In most patients with diabetes mellitus, combined vascular lesions of different calibers are detected and are manifested by functional vasomotor disorders of varying severity in the form of vasodilation or vasoconstriction of vessels[27]. The results of our research provide an opportunity to resolve the controversial issue of correlation between the depth of structural changes and duration of the process and suggest that vascular lesions in diabetes correlate with the duration of the process, in particular macroangiopathy, with the example of structural chages in aorta, provided in our study, which is a consequence of early microangiopathy of the hemomicrocirculatory tract of the aortic wall[28,29].

Vascular lesions in diabetes are found in the retinal vessels, which leads to incurable blindness, in the glomeruli, where glomerulosclerosis develops side-by-side with the development of chronic renal failure, in the distal lower extremities, which contributes to the development of gangrene (“diabetic foot”)[30,31]. Our study allowed us to trace changes in the structural organization of the submandibular gland and testis in diabetes and to demonstrate that the basis for these changes is the development of microangiopathy in these organs. Ultramicroscopic studies have shown that similar to the pancreas, the mandibular salivary glands of laboratory animals contain centroacin cells in their terminal secretory units. In comparison, the percentage of cells with aldehyde fuxinophilic and glucagon-like material in the granular ducts of the salivary glands of rats is equal to the ratio between B- and A-insulinocytes of the pancreas and therefore demonstrates a close similarity between large salivary glands and pancreas, functional disorder of which is the basis for the development of diabetes[32,33].

The changes we observed in the testis in diabetes mellitus in the experiment can be interpreted as nonspecific, as we found degeneration of sperm, destruction of organelles of suspentocytes, increase in the proportion of connective tissue in non-cellular layers of the wall of convoluted seminiferous tubules- the conditions which are typical for pathologies of different genesis [34].

Conclusions

1. The first changes in the structural organization of the hemomicrocirculatory system of organs were detected after 2 weeks of streptozotocin-induced diabetes mellitus. Edema of endothelial cells, protrusions of cytoplasm in the form of microvilli into the lumen of microvessels, loosening/fluffiness and discontinuity of the basement membrane; lumens of microvessels become irregular, there occurs aggregation of erythrocytes and their adhesion to the endothelium.

2. Under experimental conditions, the first structural changes in the aortic wall occur 4 weeks after the start of the experiment: disorganization of the layers of tunica media of the aortic wall with appearance of pseudocysts filled with lipid content, growth of loose connective tissue with small "cloud-like" lymphoid infiltrates in peripheral aorta, there is a statistically significant increase in the thickness of internal elastic lamina of the tunica media of the aortic wall to 10.50 (8.68; 12.35) μm.

3. After 6-8 weeks of experimental diabetes mellitus, hematotesticular barrier of hemocapillaries and convoluted seminiferous tubules is disorganized, dilated, fluffy, discontinuous. There are destructive changes in all layers of the wall of the convoluted seminiferous tubules of the testis, in particular, sperm degeneration, nuclear shrinkage, intussusception of nucleolemma, cytoplasmic transparency, reduction of cristae of mitochondrial sustentocytes, thickening of the collagen layer in non-cellular layers of the convoluted seminiferous tubules.

4. After 6-8 weeks of streptozotocin-induced diabetes mellitus structural changes of the submandibular gland are manifested by connective tissue edema, increased number of granular excretory ducts with localization of conglomerates of secretory granules mainly in the apical part of cells. At the same time, granular excretory ducts with no secretion granules are identified.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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