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Original Article

Morphometric analysis of the structural changes in the venous bed of the testes in cases of diabetes and portal hypertension

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Abstract

The subject of this study is the venous bed, which has been found to exhibit functional and structural changes in response to increased pressure in the portal system and metabolic disorders in diabetes mellitus in the testes. However, this area has been the subject of insufficient study. The aim of this study was to perform a morphometric analysis of the venous bed of the testes in conditions of portal hypertension and diabetes mellitus. The testes of 46 white male rats were morphologically studied and divided into four groups. The diameter of the postcapillary venules and venules of the left testis increased in portal hypertension by 8.9% ($p<0.001$) and venules by 10.7% ($p<0.01$), while in diabetes, the increase was 4.5%. ($p<0.001$) and 2.3% ($p<0.05$), and in combined lesions, respectively, increased by 9.8% ($p<0.001$) and 16.3% ($p<0.01$ The outer diameter of the venous vessels of the right testis in portal hypertension statistically significantly ($p<0.001$) increased by 5.2%, in experimental diabetes – by 2.8% ($p<0.05$), and in combined lesions – by 6.8% ($p<0.001$). Conversely, the internal diameter of the venous vessels of the right testis exhibited a marked increase of 8.1% ($p<0.001$) in the setting of portal hypertension, 4.4% ($p<0.01$) in diabetes, and a substantial 10.6% ($p<0.001$) in the presence of a combination of diseases. The height of venous vessel endothelial cells, the diameter of their nuclei, and the nuclear-cytoplasmic index of the right testis exhibited minor alterations in the modeled pathologies, thereby indicating the stability of structural cellular homeostasis. Diabetes mellitus has been observed to induce a pronounced structural alteration of the venous vascular bed of the testes, particularly when combined with portal hypertension.

Keywords: microcirculation, rats, histology, diabetes mellitus, gonads

Introduction

Infertile marriage is a significant medical and social problem that necessitates elucidation of the etiopathogenetic factors of both female and male infertility, alongside the development of new and effective medical and organizational forms of care. The social ramifications of an infertile marriage are manifest in a decline in social activity, reduced workforce participation, psychological distress, and unstable family relationships. In recent years, there has been a substantial increase in infertility among men. The principal etiological factors include but are not limited to, the deterioration of the environmental situation, hormonal disorders, inflammation of the gonads, dilated spermatic cord veins, trauma and mumps, and iatrogenic infertility [1].

Morphologists are currently interested in studying the structural and functional features of the male gonads under normal conditions and the remodeling

of their structures and vascular beds under the influence of endogenous and exogenous factors. Scientists increasingly use quantitative morphological methods (morphometry) in studying biological objects, which allow for objective quantitative morphological characterization of the structural changes in organs and body systems under various physiological and pathological processes and logical interpretation [2, 3].

In recent years, researchers have increasingly focused on the unique characteristics of remodeling structures and vascular beds of organs within the portal venous system in cases of portal hypertension. The underlying cause of portal hypertension in 80% of cases is cirrhosis of the liver, representing the culminating stage of the majority of chronic liver diseases. The prevalence of chronic liver disease is on the rise globally, with the World Health Organization (WHO) classifying it as one of the foremost causes of morbidity among the global population [4–6].

It has been established that the surgical removal of substantial portions of the liver in cases of malignant and benign tumors, metastases, and liver injuries can result in portal hypertension (PH). This condition, characterized by impaired blood flow from the hepatic portal vein, leads to a precipitous increase in pressure within the vein. Concurrently, varicose veins, bleeding from the esophagus and stomach, rectum, splenomegaly, and ascites may manifest, potentially culminating in multiple organ failure [7–9].

Diabetes mellitus (DM), a prevalent metabolic disorder, poses a significant global health concern due to its associated microvascular and macrovascular complications. Recent studies have indicated a rise in obesity, type 2 diabetes and metabolic syndrome among children, adolescents and young adults [10, 11]. The remodeling of testicular structures and vasculature in diabetes mellitus requires particular attention. The evidence for a pathogenic link between liver disease and diabetes mellitus is growing, and this link is increasing.

Hyperglycaemia is considered by many to be the primary catalyst for the dysregulation of various anabolic and catabolic pathways within cells, consequently resulting in cellular damage and the subsequent development of various diabetes complications [12, 13]. Metabolic alterations, encompassing disturbances in carbohydrate, lipid, protein, and electrolyte metabolism, are recognized as significant contributors to the pathogenesis of chronic liver disease and cirrhosis, which frequently culminates in chronic or acute liver failure, accompanied by manifestations of portal hypertension.

In diabetes, liver cells significantly reduce the transport and utilisation of glucose. The basis of diabetic hepatopathy is the dysfunction of small liver vessels (microangiopathy). This results in the disruption of microcirculation and the development of morphological and functional changes in hepatocytes, which are prerequisites for the development of hepatitis and liver cirrhosis [13, 14].

An increase in vascular resistance in the hepatic portal vein in various chronic liver diseases and liver resection leads to an increase in venous pressure in the systemic circulation, which occurs in prolonged PH. It is worth noting that changes in the structural elements of the venous bed of the testes in case of prolonged PH in combination with DM have not been studied sufficiently.

The aim of this study was to conduct a morphometric analysis of the structural changes in the venous bed of the testes under conditions of DM and PG.

Material and methods

A set of morphological methods was used to study the testes of 46 white male rats, which were divided into four groups: a control group of 10 intact animals, a main group with a modeled PH of 12 rats, a main group with modeled DM of 12 experimental animals, and a main group with a combination of both pathologies, PH and DM of 12 rats. The PH in the experimental animals was modeled by removing the left and right lateral lobes of the liver (58.1% of its parenchyma). The model of experimental diabetes was reproduced by means of intraperitoneal injection of rats with a dose of 50 mg/kg of streptozotocin dissolved in 0.1 M citrate buffer solution (pH 4.5) [15]. The animals were euthanized by bleeding under thiopental anesthesia 28 days after the commencement of the experiment. The excised pieces from the testis were fixed in a 10% neutral formalin solution and, after passing through ethyl alcohols of increasing concentration, were placed in paraffin blocks according to the conventional method. Following deparaffinization, 5–7 μm thick microtome sections were stained with hematoxylin-eosin and Weigert's toluidine blue. In the left and right testes, the following parameters were measured: the diameter of the postcapillary venules (DPV) and diameter of the venules (DV), the outer and inner diameters of the venous vessel (ODVV, IDVV), and their wall thickness (WT). In addition, the height of endothelial cells (HEC), the diameter of their nuclei (DNEC), the nuclear-cytoplasmic ratio

in endothelial cells (NCREC), and the relative volume of damaged endothelial cells (RVDEC) were measured [16]. Quantitative indicators were subjected to statistical analysis. The results were processed at the Department of Systematic Statistical Research of the I. Horbachevsky Ternopil National Medical University using the STATISTIKA software package. The difference between comparative values was determined by the Student's t-test [17]. The experiments and euthanasia of experimental animals were carried out in compliance with the "General Ethical Principles for Animal Experiments" (Kyiv, 2001), as approved by the First National Congress on Bioethics, in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes", as well as the Law of Ukraine "On Protection of Animals from Cruelty" (21.02.2006).

Results

The veins of the left and right testes of mature white male rats were examined morphometrically under the following experimental conditions. The results of the study are presented in Table 1.

The investigation of the data presented in the above table has revealed a significant structural reorganization of the venous bed of the testes. Substantial changes in the studied morphometric parameters have confirmed this finding. Consequently, the left testicular DPV in PH demonstrated a statistically significant ($p<0.001$) increase of 8.9%, and in DM and combined lesions (DM and PH), this morphometric index increased by 4.5% ($p<0.01$) and 9.8% ($p<0.001$), respectively.

In the group with PH in the left testis, the DV increased statistically significantly ($p<0.01$) by 10.7%, and in DM and combined lesions, it increased by 2.3% ($p<0.05$) and 16.3% ($p<0.01$), respectively. The ODVV and IDVV underwent significant changes under these experimental conditions; in the case of PH, it increased statistically significantly ($p<0.001$) by 5.7%, and in DM and combined pathology of the studied animals, it increased by 3.1% ($p<0.05$) and 7.0% ($p<0.001$), respectively.

The left testicular IDVV increased from (28.30±0.21) to (30.80±0.15) μm in PH, with the above morphometric parameters displaying statistically significant ($p<0.001$) variation from each other. The final digital value was 8.8% higher than the previous one. In the intact left testis, the internal diameter of the studied vessels was (28.30±0.21) μm, and in diabetes and combined lesions, it was (29.70±0.21) and (31.40±0.24) μm, respectively. A statistically significant difference ($p<0.05$) and ($p<0.001$) was found between the above morphometric parameters. Meanwhile, the lumen of the venous vessels of the left testis in diabetes mellitus was found to be 4.9% larger than in the control group, and in the combined lesion, this morphometric parameter increased by 11%.

The thickness of the media of these vessels was found to decrease slightly. In the group with hypertension, this morphometric parameter decreased by 1.9% with a high degree of confidence ($p<0.01$). In the group with diabetes mellitus, it decreased by 1.3% ($p<0.05$). The combination of nosologies decreased by 2.4% ($p<0.01$).

In the left testis, the HEC venous vessels and the diameter of their nuclei exhibited minimal alterations in PH, while the NCREC of the studied cells underwent a slight change, indicative of the stability of structural cellular homeostasis. Under PH conditions, the height of endothelial cells decreased by 1.5% and the diameter of their nuclei by 0.28%, resulting in unexpressed changes in the nuclear-cytoplasmic ratio in these cells. Conversely, under these experimental conditions, the nuclear-cytoplasmic ratio exhibited an increase of 0.55% ($p<0.01$), suggesting that structural cellular homeostasis remained uncompromised. In the context of PH, RVDEC in the studied vessels exhibited a significant increase of 3.9 times ($p<0.001$). Furthermore, in cases of DM and the combination of pathologies, the height of endothelial cells in the studied vessels of the left testis underwent minor alterations. These quantitative morphological parameters demonstrated a reduction of 1.1% ($p<0.05$) and 1.9% ($p<0.01$), respectively. The nuclear-cytoplasmic ratio in the endothelial cells of the venous vessels of the left testis in diabetes exceeded the same control value by 0.36% ($p<0.01$) and in the combined lesion by 0.55% ($p<0.01$), which did not indicate a violation of structural cellular homeostasis. Furthermore, RVDEC in the studied vessels of the left testis in diabetes and combined pathologies increased by 2.9 and 5.4 times ($p<0.001$). Consequently, damage to a significant number of endothelial cells could lead to endothelial dysfunction, which significantly affects the degree of remodeling of the studied vessels.

The morphometric parameters of the venous vessels of the right testis underwent changes that were similar but less pronounced to those previously observed. Consequently, the DPV of mature white male rats in PH exhibited a statistically significant ($p<0.001$) increase of 7.0%, while in cases of diabetes and combined lesions, this morphometric index increased by 3.6% ($p<0.05$)

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Table 1: Morphometric characteristics of the venous bed of the testes in different experimental groups of animals (M±m).

Indicator	Observation groups							
	Control group		The main group with portal hypertension		The main group with diabetes mellitus		The main group with diabetes mellitus and portal hypertension	
	Left testis	Right testis	Left testis	Right testis	Left testis	Right testis	Left testis	Right testis
Diameter of the postcapillary venules, µm	12.82±0.09	12.82±0.09	13.96±0.12***	13.72±0.12***	13.40±0.08**	13.28±0.12*	14.08±0.12***	13.91±0.12***
Diameter of the venules, µm	26.96±0.18	26.96±0.18	29.84±0.21**	29.48±0.21**	27.58±0.15*	27.55±0.12*	31.36±0.21**	31.15±0.21**
Outer diameter of the venous vessel, µm	40.32±0.42	40.30±0.42	42.60±0.36***	42.40±0.36***	41.56±0.39*	41.44±0.30*	43.14±0.24***	43.06±0.24***
Internal diameter of the venous vessel, µm	28.30±0.24	28.32±0.24	30.80±0.15***	30.61±0.15***	29.70±0.21*	29.58±0.18**	31.40±0.24***	31.32±0.18**
Thickness of the venous vessel wall, µm	12.02±0.09	11.98±0.06	11.8±0.06**	11.79±0.06**	11.86±0.06*	11.86±0.06**	11.74±0.06**	11.74±0.06**
Height of endothelial cells, µm	4.80±0.03	4.80±0.03	4.72±0.03**	4.73±0.04**	4.75±0.04*	4.76±0.04*	4.71±0.03**	4.71±0.03**
Diameter of endothelial cell nuclei, µm	3.56±0.02	3.56±0.02	3.55±0.02**	3.52±0.02**	3.56±0.03	3.55±0.03	3.54±0.03**	3.51±0.02**
Nuclear-cytoplasmic ratio of endothelial cells	0.550±0.003	0.550±0.003	0.553±0.003**	0.553±0.003**	0.552±0.003**	0.552±0.003	0.553±0.003**	0.553±0.004**
Relative volume of damaged endothelial cells, %	2.20±0.03	0.250±0.003	8.60±0.04***	8.50±0.04***	6.38±0.04***	6.12±0.04**	11.78±0.03***	11.53±0.03***

Note: * – p<0.05; **– p<0.01; *** – p<0.001.

and 8.5% ($p<0.001$), respectively. In the group with PH, the DV of the right testis demonstrated a statistically significant ($p<0.01$) increase of 9.3%. In diabetes, the DV increased by 2.2% ($p<0.05$). In a combination of pathologies, the DV increased by 15.5% ($p<0.01$). The ODVV right testis demonstrated a statistically significant ($p<0.001$) increase of 5.2% in PH, 2.8% in diabetes ($p<0.05$), and 6.8% in combined lesions ($p<0.001$). In the intact right testis, the internal diameter of the studied vessels was (28.32±0.24) μm, and in PH, it was (30.61±0.15) μm. A statistically significant difference ($p<0.001$) was found between the above morphometric parameters. Meanwhile, the lumen of the venous vessels in the right testis in PH was observed to be 8.1% larger than in the control group. The IDVV right testis demonstrated an increase of 4.4% in diabetes mellitus and 10.6% in combined lesions. The observed morphometric parameters were found to be statistically significantly different ($p<0.01$) from each other.

The thickness of the media of these vessels also underwent changes. In the group with PH, this morphometric parameter decreased by 1.6% with a high degree of confidence ($p<0.01$). In the diabetes and the combination groups, respectively, this parameter decreased by 1.0% ($p<0.01$) and 2.0% ($p<0.01$).

The data obtained indicate that the remodeling of the venous vessels of the testis in PH and combined lesions (DM and PH) was more pronounced compared to the group with DM alone and dominated in the left testis, which can be attributed to the peculiarities of blood outflow from this organ.

Discussion

It has been established that the venous bed of the testes originates from the venous link of the haemomicrocirculatory bed, comprising the capillaries and venules. It is noteworthy that the structure of intra-organic veins differs considerably from that of arteries. In veins, delineating the boundaries between the inner, middle, and outer layers of the venous vascular wall can be challenging. In smaller veins, endothelial cells are located on the basement membrane, while smooth muscle cells' elastic and collagen fibers are present in the vessel wall. The presence of these smooth muscle cells and fibers indicates that the primary function of these vessels is not metabolic but rather the drainage of venous blood.

The predominant dilatation of the external (venous) vessels of the haemomicrohaemocirculatory bed resulted in venous hemorrhage, which induced the development of edema in the vascular wall and perivascular tissues. This process maintained and exacerbated the state of hypoxia [7]. The latter not only contributed to edema but also exacerbated it, leading to dystrophic and necrobiotic changes in cells and tissues. This was aggravated by the inflammatory process of all parts of the microhaemocirculatory system and perivascular tissues, which also led to the blockage of microvessels and their exclusion from the blood flow system and caused progressive destruction, edema and destruction of their endothelial cells. Damage to the latter led to increased endothelin-1 synthesis and decreased nitric oxide production, which exacerbated arterial vasospasm [5, 13, 17]. It is also important to note that the marked venous full-bloodedness of the postcapillary venules and venules of the testicular haemomicrohaemocirculatory bed in the modeled pathologies was combined with signs of prestasis, stasis, sluggish phenomenon and even the DIC-syndrome. The detected haemomicrocirculatory changes were accompanied by a violation of blood rheology and an increase in the permeability of microvascular walls. This led to hemorrhage of the vascular walls and surrounding stroma, perivascular edema and saturation of the perivascular stroma with blood proteins. The resultant effects included increased hypoxia, disorganization and dissociation of fibrous structures, and a significant deterioration in the diffusion of nutrients and oxygen. The sustained increase in hypoxia was characterized by heightened fibroblastic activity, manifesting in the polymerization and saturation of collagen fibrils with glycosaminoglycans. This process led to stromal sclerosis, resulting in the exacerbation of hypoxia.

The damage observed to the cells under study was primarily attributable to apoptosis, a process influenced by premedication, anesthesia, surgical trauma, and the administration of medications during the postoperative period [5].

The rising incidence of diabetes mellitus is a grave concern for medical professionals and researchers alike. Many researchers hypothesize that the vessel wall is the primary target for damage in diabetes. The development of insulin resistance and hyperinsulinemia, triggered by pathogenic factors, has been identified as a key factor in the progression of metabolic disorders, including impaired glucose metabolism, dyslipidemia (characterized by increased triglycerides and decreased high-density lipoprotein cholesterol, as well as increased low-density lipoprotein cholesterol), and hemodynamic and homeostasis disorders. These

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adverse changes, in turn, can lead to a range of serious complications, including endothelial dysfunction, hypertension, thrombosis, and macro- and microangiopathy. The development of microangiopathies is a complex process involving the thickening of the basement membrane. This is the result of many factors, including impaired blood flow, leading to hypoxia and reduced endothelial nutrition, impaired metabolism of carbohydrates or a complex of polysaccharides (glycosaminoglycans) in the basement membrane of capillaries and connective tissue, glycosylation of proteins and accumulation of glycosylation end products. Compounds, reduced ability of red blood cells to deform, leading to increased pressure in capillaries and thickening of the basement membrane, deposition of immune complexes in the basement membrane and extracellular matrix with subsequent impairment of phagocytic activity of basement membrane cells and humour-mediated gene expression of various proteins, increased permeability of the vascular wall to plasma proteins or other macromolecules, microcirculatory disorders etc. [13, 17].

The histological micrographs of the testes revealed dilatation of primarily venous vessels, particularly of the postcapillary venules and venules. These microvessels were visualized as thick-walled fibrous formations with indications of hyalinosis, and their total or incomplete obliteration of the luminae was also observed. In the context of modeled combined pathology, stromal and perivascular edema, foci of parenchymal and endothelial dystrophy, and foci of infiltration were observed, with the detected pathological changes manifesting predominantly in the left testis in cases of postresection portal hypertension and combined lesions.

Conclusion

The findings of this study indicate that portal hypertension and diabetes mellitus result in significant hemodynamic and structural alterations in the venous bed of the testes. These alterations impede the efficient drainage of venous blood from these organs, compromise their nutritional supply, and contribute to the development of lesions. The most pronounced degree of venous vessel remodeling was found in cases of portal hypertension, which leads to a significant structural restructuring of the vascular bed, characterized by vascular wall dilation, blood overflow, foci of stasis, thrombosis and diapedesis hemorrhage. The detected pathomorphological changes dominated in the left testis in cases of portal hypertension and combined lesions.

Conflict of interest

The authors declare no conflict of interest.

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