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STUDY OF THE STRUCTURAL AND FUNCTIONAL STATE OF KIDNEYS IN CHRONIC HYPERGLYCEMIA

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Abstract

The article is presents results of the structural and functional changes in kidney at the condition of chronic hyperglycemia. It was established that in the condition of experimental streptozotocin diabetes and microvascular complications on the background of diabetes, there was an increase in urinary protein excretion and creatinine clearance, minor changes in glomerular filtration and tubular reabsorption. In both type 2 diabetes and DN, significant changes in the urinary excretion of sodium and potassium ions were observed, which is associated with a decrease in the reabsorption of these cations in the renal tubules. To confirm the DN performed a morphological study of kidney tissue. It was found that in the group of animals with DN on the background of type 2 diabetes there was a decrease in the size of renal corpuscles with dystrophic altered cells of the outer and inner walls of the glomerular capsule, focal thickening of BM capillaries, expansion of the mesangium.

All human studies were conducted in compliance with the rules of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study". Informed consent was obtained from all participants.

Keywords: *diabetes mellitus, diabetic nephropathy, hyperglycemia, kidneys*

Introduction

Diabetes is a major public health problem that is approaching epidemic proportions globally. Worldwide, the prevalence of chronic, noncommunicable diseases is increasing at an alarming rate. About 18 million people die every year from cardiovascular disease, for which diabetes and hypertension are major predisposing factors. Today, more than 1.7 billion adults worldwide are overweight, and 312 million of them are obese. In addition, at least 155 million children worldwide are overweight or obese. A diabetes epidemic is underway. According to an estimate of International Diabetes Federation comparative prevalence of Diabetes during 2007 is 8.0 % and likely to increase to 7.3 % by 2025 [1, 2, 11-13].

Almost 80 % of the total adult diabetics are in developing countries. The regions with the highest rates are the Eastern Mediterranean and Middle East, where 9.2 % of the adult population is affected, and North America (8.4%). The highest numbers, however, are found in the Western Pacific, where some 67 million people have Diabetes, followed by Europe with 53 million.

The most serious risk of diabetes is associated with complications that develop when the blood vessels are damaged. Diabetic nephropathy (DN) occupies an important place among chronic diabetic complications [3]. The problem of DN became known at the beginning of the XX century during which it gained dramatic proportions and reached its peak in the XXI century [**Errore. L'origine riferimento non è stata trovata.**].

DN is one of the leading causes of the last stage of renal disease. Until the 70s and 80s of the last centuries, among patients with diabetes, hemodialysis treatment was needed mainly by patients with type 1 diabetes (90 %) and small proportion were patients with type 2 diabetes (10 %) [5]. Today, the share in the overall structure of dialysis therapy in the world is not inferior to the share of patients with type 1 diabetes and ranges from 40 to 60 %. Given that the number of patients with type 2 diabetes is 9-10 times higher than the number of patients with type 1 diabetes, we can conclude that the scale of the "quiet" epidemic of last stage of renal disease will exceed the capabilities of dialysis service in the world [6].

The aim of our study was to investigate the structural and functional changes in kidney at the condition of chronic hyperglycemia.

Methods

Experimental studies were performed on 18 white nonlinear rats weighing 240-280 g, which were divided into 3 experimental groups (6 animals in each group): group 1 – intact control - animals kept on a standard diet of vivarium; group 2 – control pathology – animals, which after the introduction of streptozotocin and nicotinamide for reproduce type 2 diabetes; group 3 – animals, which after the introduction of streptozotocin and nicotinamide reproduced the model of DN [7, 8].

A streptozotocin model was used to reproduce type 2 diabetes. For this purpose, rats were administered a single intravenous streptozotocin ("SigmaAldrich Chemie GmbH", Germany) in dose of 65 mg/kg. After 1 week a glucose tolerance test was performed to determine fasting blood glucose levels and 30, 60, 90 and 120 minutes after intragastric administration of 40 % glucose solution at a dose of 3 g/kg and blood glucose levels from 9.0 to 14 mmol/l [7, 8].

To reproduce DN, the diet of experimental rats was based on a high-fat diet. At 35-40 weeks, the animals showed signs of DN – proteinuria, decreased glomerular filtration rate [7].

To confirm disorders of the urinary system, spontaneous diuresis was determined: experimental groups of rats were placed in individual metabolic cells, adapted to record the amount of water consumed and urine excreted. For the group of animals with simulated diabetes, the indicator was determined after 1 week; in groups of rats with DN – 40 weeks after the beginning of the experiment.

The level of protein excretion was determined by colorimetric method using bromophenol blue "Vital Diagnostics" (Russia), serum creatinine (followed by calculation of creatinine clearance) was determined by the Jaffa method using the kit "Pliva-Lachema" (Czech Republic) [9].

In order to analyze the mechanism of changes in the water and electrolytes excretion in diabetes mellitus and DN on the background of type 2 diabetes, the processes of glomerular filtration and tubular reabsorption were studied. Glomerular filtration was determined by the clearance of

endogenous creatinine, its purification factor is determined by the ratio of the concentration of creatinine in the urine to the concentration in blood plasma [9].

For morphological examination, the material obtained from the kidneys was fixed for 24 hours in 10 % solution of neutral formalin (pH 7.4), dehydrated and embedded in paraffin according to conventional methods. Sections 3-5 μm thick were made on a rotary microtome, which were then stained with Hematoxylin-eosin according to Masson [10].

During the work with animals we complied with the International Code of Medical Ethics (Venice, 1983), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and the General Ethical Principles for Animal Experiments adopted by the First National Congress of Bioethics (Kyiv, 2001), Directive 2010/63/EU of the European Parliament and Council on the protection of animals used for scientific purposes.

Statistical processing of the obtained results was performed using the program "Statistica 8.0". The probability of differences between the indicators of the control and experimental groups was determined by the criteria of Student and Fisher. The level of reliability was taken at $p<0,05$.

Results

At the first stage of our study of the model pathology of late complication on the background of type 2 diabetes, we registered the excretion of protein (proteinuria) in the urine and creatinine clearance (Fig. 1, Fig. 2).

It was established significant increase of protein in the urine in 3.2 times ($p<0.05$) after 3 weeks of the experiment in animals with type 2 diabetes compared with the intact control group; in rats with DN on the background of type 2 diabetes, this indicator increased in 3.3 times ($p<0.05$) relative to the intact group of rats. After 6 weeks of the experiment, there was a tendency to increase the excretion of protein in the urine: in the group of animals with type 2 diabetes in 2.7 times ($p<0.05$) relative to the group of intact animals and rats with DN – in 3.4 times ($p<0.05$), respectively. The obtained data indicate the loss of negative charge of the glomerular membrane due to disruption of the synthesis of proteoglycans, which are part of the structure of BM capillaries.

Creatinine clearance in rats with type 2 diabetes increased in 2.5 times ($p<0.05$) after 3 weeks of the experiment beginning compared with the group of intact animals; in rats with DN on the background of type 2 diabetes – in 3.0 times ($p<0.05$), respectively. The tendency to increase creatinine clearance in experimental groups of animals with reproductive pathology persisted for 6 weeks of the experiment, which may occur as a result of creatinine hyperfiltration and indicates the development of early-stage diabetic nephropathy.

It is known that under the chronic hyperglycemia, glucose penetrates into the endothelial cells of blood vessels, causing pathological changes. The endothelium is affecting to the effects of both systemic and intraorganic hypertension, which develops in the microcirculatory tract on the background of diabetes.

Disorders of renal hemodynamics and microcirculation in the nephron lead to decrease in water and electrolyte function of the kidneys.

Therefore, the next stage of our study was to establish the mechanism of damage to the renal apparatus on the background of experimental type 2 diabetes: the study of indicators characterizing glomerular filtration and tubular reabsorption (the main processes of urination).

It was investigated that in type 2 diabetes decreased glomerular filtration and tubular reabsorption, while in rats with experimental DN glomerular filtration did not differ from the intact group, and tubular reabsorption decreased in comparison with intact rats and animals with diabetes mellitus 2 type (Fig. 3).

It is known that increased diuresis is accompanied by increased urinary excretion of sodium (Fig. 4). Because sodium is freely filtered in the glomeruli and almost 99 % of it is reabsorbed in the renal tubules, it is likely that increased sodium excretion may be the result of increased filtration load on the nephron or decreased tubular reabsorption.

The analysis of the obtained results indicates an increase in urinary sodium excretion in both studied groups of animals (with type 2 diabetes and DN) (Fig. 4). At the same time, we found an increase in potassium excretion in the urine: in the group of animals with type 2 diabetes, this figure exceeded

similar data from intact animals by 1.4 times; in animals with DN in 1.9 times ($p<0.05$).

Therefore, in experimental streptozotocin diabetes and in DN on the background of diabetes, there were changes in glomerular filtration and tubular reabsorption. However, in both type 2 diabetes and DN, there were significant changes in the urinary excretion of sodium and potassium ions (natriuresis and potassium uuresis), which is associated with decreased reabsorption of these cations in the renal tubules.

At the final stage of the study of model pathology of late microangiopathic complications on the background of type 2 diabetes we conducted a morphological assessment of changes in the renal apparatus (Fig. 5).

Morphological study showed that in the group of animals with DN on the background of type 2 diabetes there was decrease in the size of renal corpuscles with dystrophic altered cells of the outer and inner walls of the glomerular capsule, focal thickening of BM capillaries, expansion of the mesangium.

Conclusions

1. In the condition of experimental streptozotocin diabetes and microvascular complications on the background of diabetes, there was an increase in urinary protein excretion and creatinine clearance, minor changes in glomerular filtration and tubular reabsorption.

2. In both type 2 diabetes and DN, significant changes in the urinary excretion of sodium and potassium ions were observed, which is associated with a decrease in the reabsorption of these cations in the renal tubules.

3. To confirm the DN performed a morphological study of kidney tissue. It was found that in the group of animals with DN on the background of type 2 diabetes there was a decrease in the size of renal corpuscles with dystrophic altered cells of the outer and inner walls of the glomerular capsule, focal thickening of BM capillaries, expansion of the mesangium.

Acknowledgments

The authors declare that there are no conflicts of interest.

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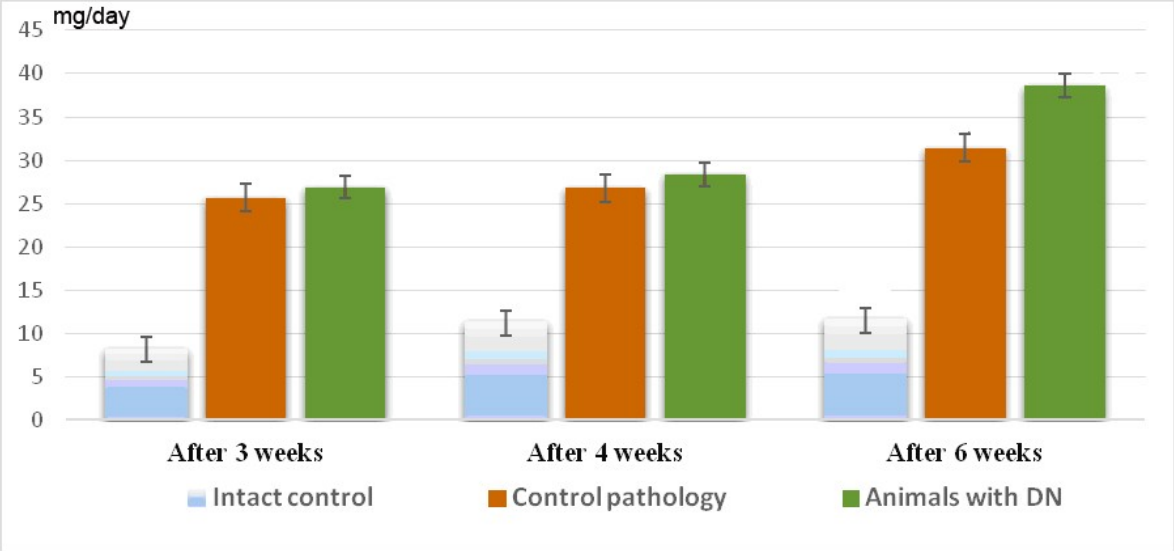

Figure 1. The protein level in the urine of rats with experimental streptozotocin diabetes and DN on the background of type 2 diabetes
Note. * - p<0.05 relative to the indicators of the intact group of animals.

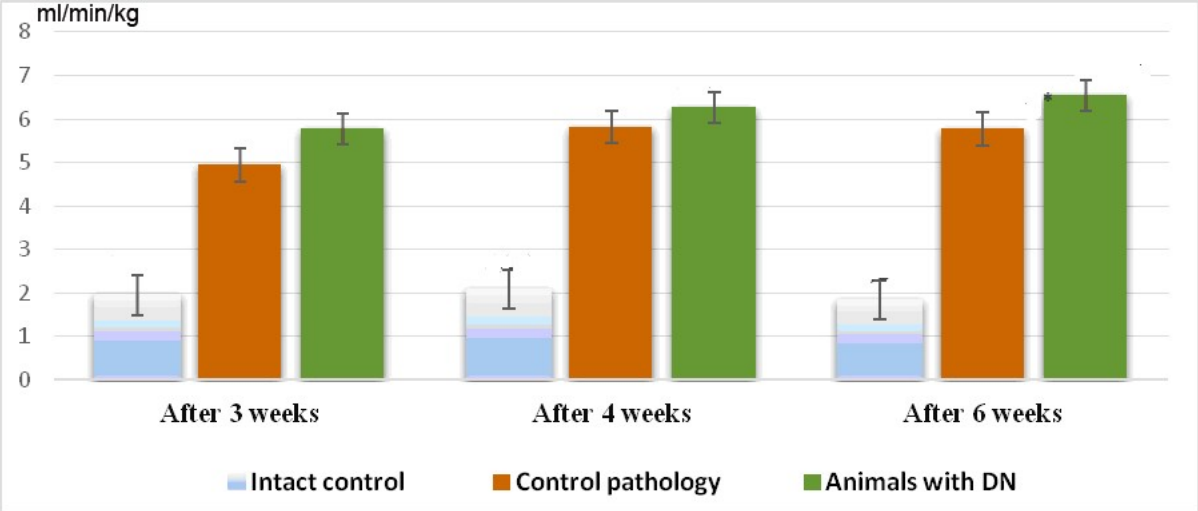

Figure 2. Creatinine clearance in rats with experimental streptozotocin diabetes and DN on type 2 diabetes
Note. * - p<0.05 relative to the indicators of the intact group of animals

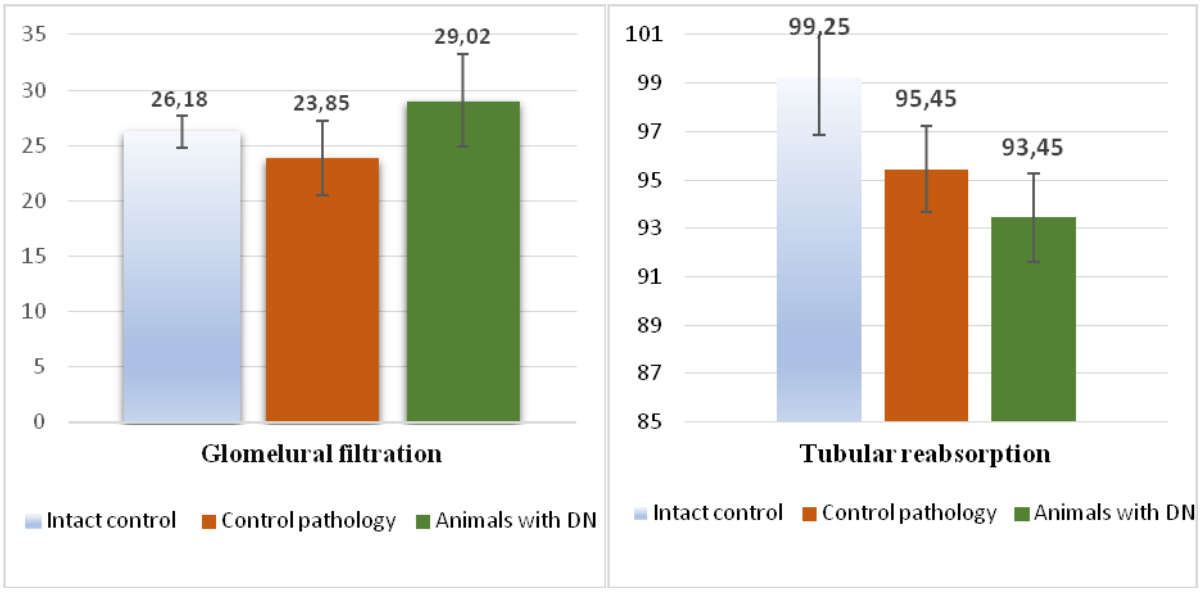

Figure 3. Changes in the main processes of urination in rats with experimental streptozotocin diabetes and DN

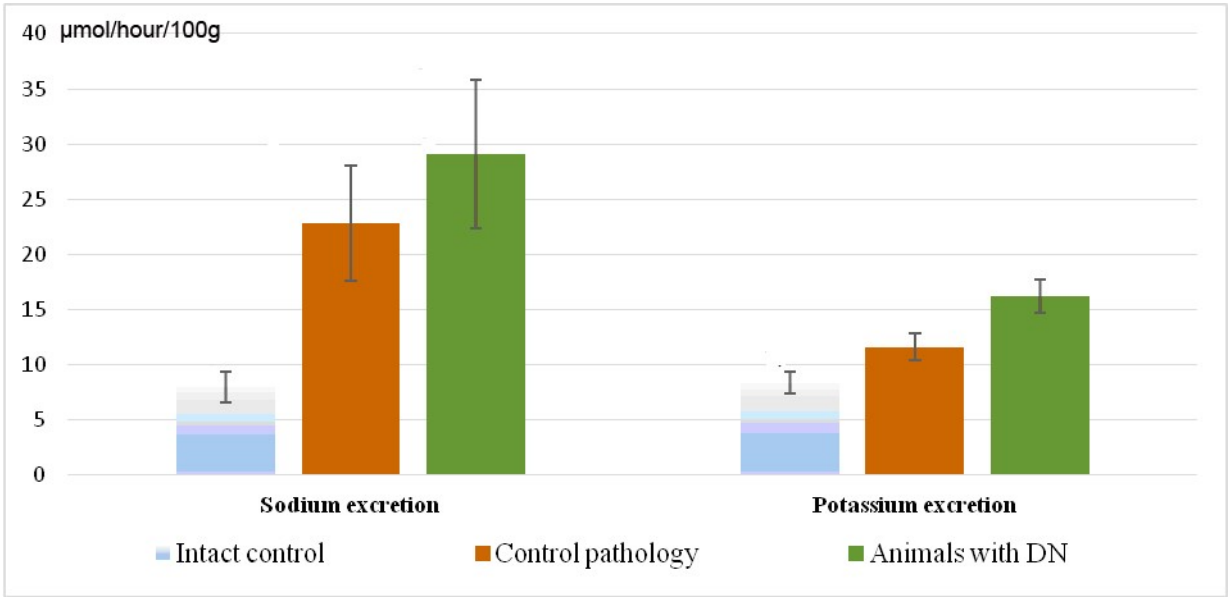

Figure 4. Changes in sodium and potassium excretion in rats with experimental streptozotocin diabetes and DN
Note. * - p<0.05 relative to the indicators of the intact group of animals

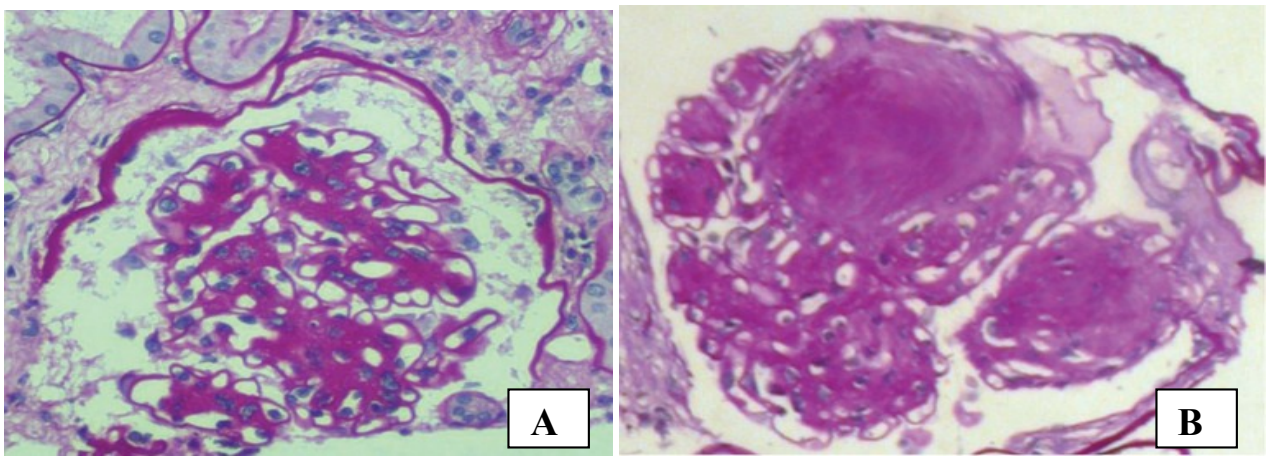

Figure 5. Morphological changes of the renal apparatus in DN on the background of type 2 diabetes: A – diffuse expansion of the mesangia with small concomitant mesangial proliferation (hematoxylin-eosin staining, × 200); B - nodular glomerulosclerosis with the formation of nodular structures (staining with hematoxylin-eosin, × 200)