Original Articles

LIGHT MICROSCOPIC STUDY OF RENAL MORPHOLOGICAL ALTERATIONS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Summary

The impact of hypertension on the kidney is associated with a number of morphological changes, which gradually lead to development of end-stage kidney disease. The aim of the present study was to trace the postnatal histological changes in the morphology of nephrons and renal interstitium in spontaneously hypertensive rats. In this study, we described and compare alterations in renal histology as a consequence of hypertension in two age groups of spontaneously hypertensive rats, aged 2 and 6 months (n=3; per age group). The description of the alterations in renal morphology was made by light microscopic analysis using routine haematoxylin and eosin staining, periodic acid Schiff (PAS) reaction and Mallory's trichrome staining. We did not observe significant changes in renal histology in 2-month-old rats: renal corpuscles were relatively well preserved, proximal and distal tubules were clearly demarcated, and no pathological changes in the larger intrarenal blood vessels were found. There was evidence of glomerular and tubular basement membranes thickening and focal interstitial fibrosis. In 6-month-old rats, we noted pronounced glomerulosclerosis, periglomerular and periarteriolar fibrosis and expansion of interstitial fibrosis. The vascular alterations depended on the size of the blood vessels and included hyaline arteriosclerosis, fibrinoid necrosis and myointimal thickening of interlobular arteries. Untreated hypertensive nephrosclerosis is associated with progressive renal alterations, which cause impaired renal function – a lifelong limiting factor.

Key words: spontaneously hypertensive rats, kidney, renal alterations, hypertension

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Introduction

Hypertension is a worldwide disease, which is recognized as an important risk factor for premature death [1]. It is divided into two main types: essential – 90-95% of cases, the etiology of which is unknown and secondary – over 5-10% cases, as a result of comorbid conditions [2]. The consequences of hypertension include higher frequency of cardiovascular and cerebrovascular accidents, as well as structural and functional alterations in target organs such as left ventricular hypertrophy, retinopathy and hypertensive nephrosclerosis [3-5]. In 1836, Richard Bright was the

first to suggest the existence of an association between high blood pressure and renal damage [6]. In addition, hypertensive nephrosclerosis is considered as the second most frequent cause of end-stage renal disease [7]. Depending on the severity and duration of hypertension, two main forms of nephrosclerosis are observed: benign and malignant [8]. The renal alterations are characterized by an increased proportion of sclerotic glomeruli, periglomerular and periarteriolar fibrosis, thickening of glomerular and tubular basement membranes, hyaline arteriosclerosis, myointimal proliferation and tubulointerstitial fibrosis [9]. None of the described changes are specific, however, and can be a result of diabetes or features of the aging kidney [10, 11].

The spontaneously hypertensive rat (SHR) strain was established by Okamoto and colleagues during the 1960s and is an experimental model of human essential hypertension [12]. The elevation of blood pressure in this strain begins at 4 weeks of age and morphological changes in target organs due to long term presence of hypertension can be observed a few months later [13, 14].

Materials and Methods

Male spontaneously hypertensive rats, available at the Medical University of Sofia, aged 2 and 6 months (n=3; per age group) were used for this study with the approval of the University Committee on Animal Resources. The animals were placed on a standard diet and allowed free cage activities. The level of their blood pressure was monitored regularly through the tail-cuff method. The rats were anesthetized intraperitoneally with Thiopental 40 mg/kg b.w. The chest cavity was opened and transcardial perfusion was made with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. Kidneys were quickly removed and fixed in 10% neutral-buffered formalin. After routine paraffin embedding, 5 μm thick sections were cut and stained with routine haematoxylin and eosin staining, Mallory's trichrome staining and periodic acid Schiff (PAS) reaction. Haematoxylin and eosin staining was conducted in the following way: after removal of the paraffin with xylol, the slides were washed with water and stained with haematoxylin for 5 minutes. The slides were then stained with eosin solution for 10 minutes, washed again with water and embedded in entellan. For the Mallory's trichrome stain, paraffin was removed, after which the slides were placed in 0.1% fuchsin for 1-2 minutes, washed and placed in 1% solution of phosphomolybdic acid for 3-5 minutes. After thorough rinsing, the slides were placed in a mixture of aniline blue, orange G and oxalic acid for 2 minutes, washed again and embedded in entellan. The PAS reaction involved removal of the paraffin, followed by periodic acid immersion for 5-10 minutes and thorough rinsing with distilled water. The slides were then placed in Schiff's reagent for 10-20 minutes. After that, they were rinsed for 5 minutes with lukewarm tap water. Finally, the slides were contrastained in haematoxylin for 1 minute, washed again and embedded in entellan. All reagents used in the standard histological and histochemical procedures were purchased from "Merck". We analyzed and compared morphological changes in nephrons and renal interstitium between the two age groups of animals. All animals received humane care in compliance with the "Principles of laboratory animal care" formulated by the National Society for Medical Research and the "Guide for the care and use of laboratory animals" prepared by the National Institute of Health (NIH publication No. 86-23, revised 1996).

Results

On histological specimens from the kidneys of rats, we described changes that occurred as a result of hypertension in two age groups SHR – 2 and 6 months, because this period is associated with developing histological alterations in target organs. We noted the alterations in the morphology of the nephrons – the renal corpuscles and the tubules, as well as the alterations in the interstitium and the blood vessels.

By the haematoxylin and eosin staining method, we described the histological structure of the kidney in the age group of 2-month-old SHR. The renal corpuscles were relatively well preserved (Figures 1a, 1b). Based on their location, we distinguished renal corpuscles of superficial, midcortical and juxtamedullary nephrons. No significant alterations in the

glomeruli were found – no pronounced thickening of glomerular basement membrane was observed (Figures 1c, 1d); the Bowman's space between the two layers was well seen – no cellular or fibrous proliferation was noted. Proximal and distal tubular segments were observed – the lumens of the proximal tubules were not clearly demarcated due to the covering cuboidal epithelium, which was characterized by prominent microvilli on the apical surface of the cellular membrane. The juxtaglomerular apparatus was demonstrated on some specimens and its morphological structure was normal. We noted an initial thickening of the glomerular and tubular basement membranes on PAS reaction, and focal interstitial fibrosis on Mallory's trichrome staining (Figures 1e, 1f). Intrarenal vascular changes in the observed afferent, efferent arterioles and larger blood vessels were not found. No significant histological alterations were established in the morphology of nephrons and renal interstitium in two-month-old SHR.

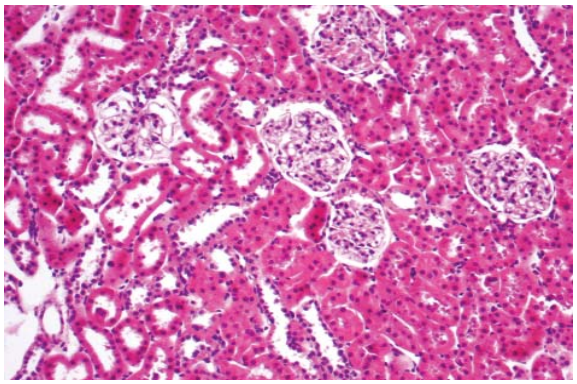

Figure 1. a. Photomicrograph of kidney, age – 2 months, haematoxylin and eosin (H&E). Magnification – x200

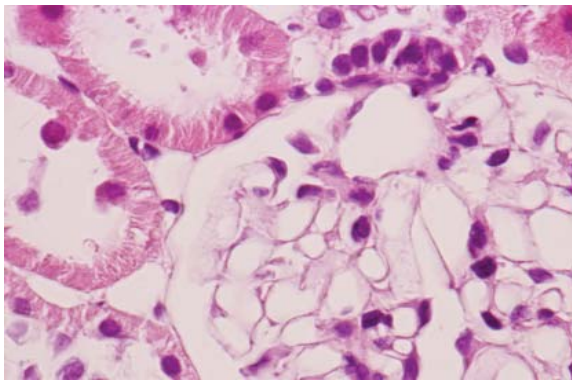

Figure 1. b. Photomicrograph of kidney, age – 2 months, H&E. Magnification – x1000

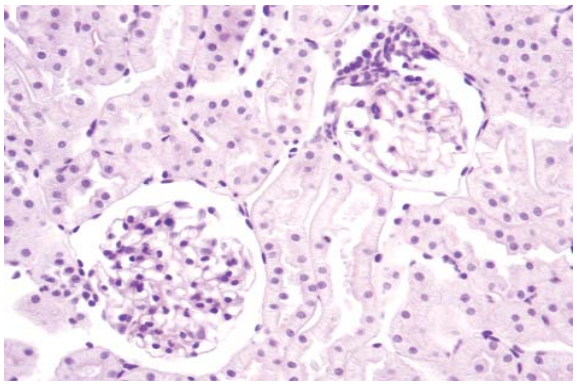

Figure 1. c. Photomicrograph of PAS reaction in the kidney, age – 2 months. Magnification – x400

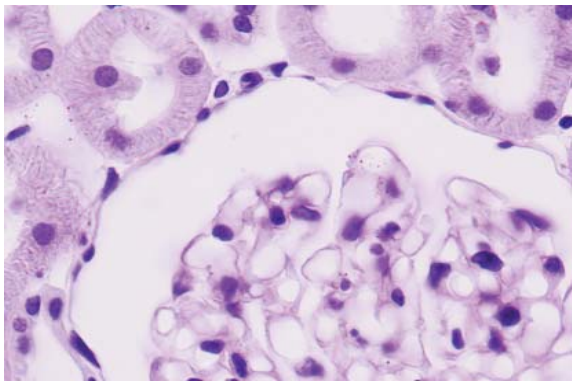

Figure 1. d. Photomicrograph of PAS reaction in the kidney, age – 2 months. Magnification – x1000

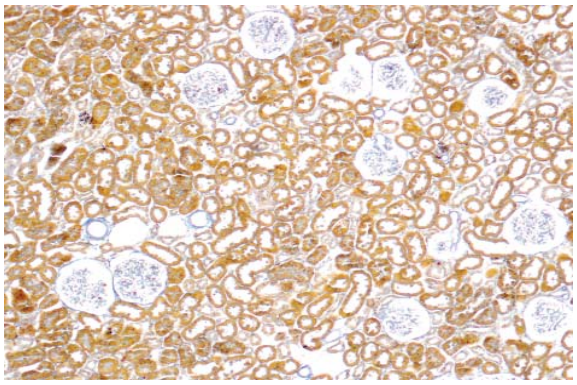

Figure 1. e. Photomicrograph of kidney stained with Mallory's trichrome method, age – 2 months. Magnification – x100

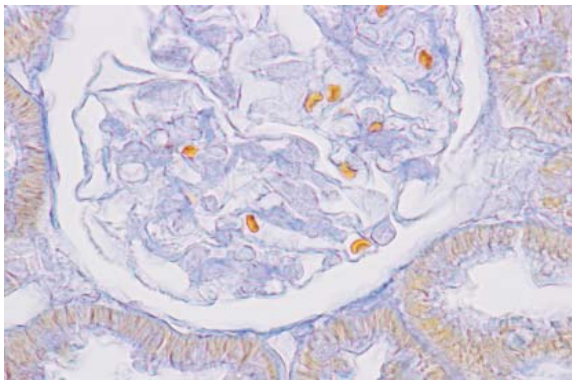

Figure 1. f. Photomicrograph of kidney stained with Mallory's trichrome method, age – 2 months. Magnification – x1000

In the 6-month-old SHR group, the morphological renal alterations, demonstrated with haematoxylin and eosin staining method, were ubiquitously pronounced. There was evidence of an increasing percentage of generally sclerotic glomeruli; glomerular tufts were characterized by wrinkling and thickening of glomerular capillary walls. We also noted a marked expansion of the mesangial matrix. Tubular changes included atrophy, flattening of the covering epithelium and dilatation of the lumen (Figures 2a, 2b). The glomerular and tubular basement membranes showed thickening, demonstrated by PAS reaction (Figures 2c, 2d). The vascular alterations were pronounced and their characteristics depended on the size of the blood vessel. Recruitment of smooth muscle cells was observed in the intimal layer of interlobular arteries, as well as fibrinoid necrosis in the small arteries. In addition, hyaline arteriosclerosis was primary observed in arcuate arteries. This finding was characterized

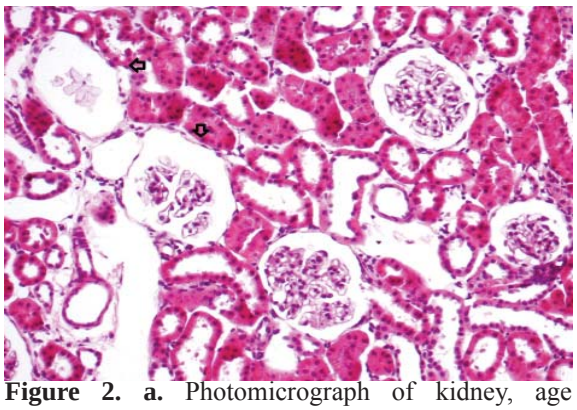

Figure 2. a. Photomicrograph of kidney, age – 6 months, haematoxylin and eosin (H&E). Magnification – x200. Arrows – glomerular alterations

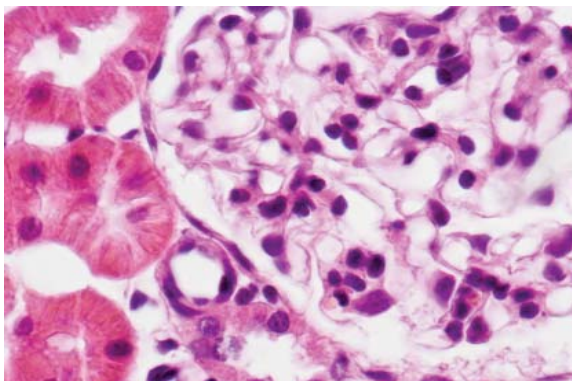

Figure 2. b. Photomicrograph of kidney, age – 6 months, H&E. Magnification – x1000

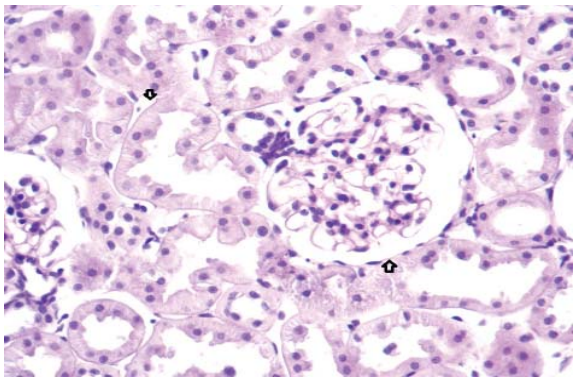

Figure 2. c. Photomicrograph of PAS reaction in the kidney, age – 6 months. Magnification – x400. Arrows – alterations in basement membranes

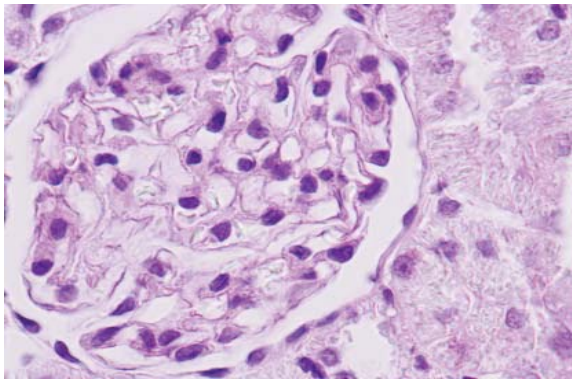

Figure 2. d. Photomicrograph of PAS reaction in the kidney, age – 6 months. Magnification – x1000

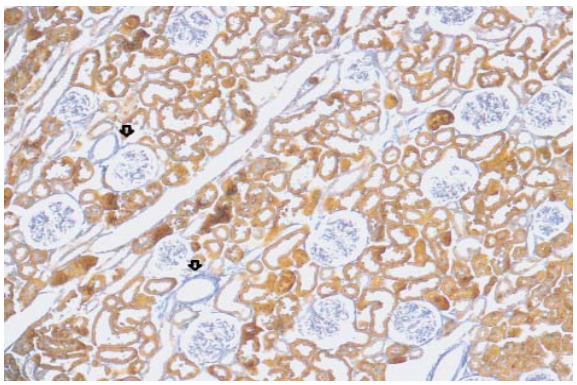

Figure 2. e. Photomicrograph of kidney stained with Mallory's trichrome method, age – 6 months. Magnification – x100. Arrows – periglomerular and perivascular fibrosis

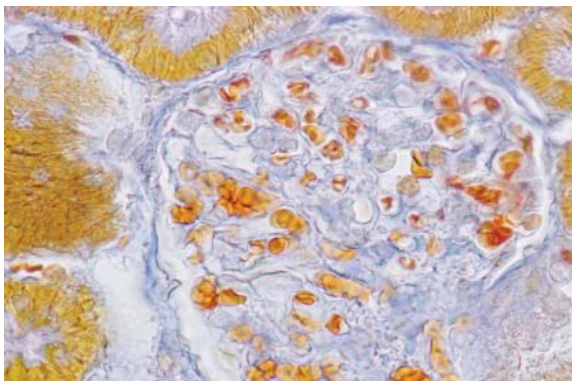

Figure 2. f. Photomicrograph of kidney stained with Mallory's trichrome method, age – 6 months. Magnification – x1000

by narrowing of the vascular lumen, which was a result of the deposition of protein in the subendothelial space. Hyaline stained in bright magenta by PAS reaction. There was evidence of reduplication of the internal elastic lamina in arcuate and interlobular arteries. The expansion of interstitial fibrosis was demonstrated with Mallory's trichrome staining method. It was well pronounced both in the renal cortex and medulla with periglomerular, peritubular and periarteriolar fibrosis. Collagen fibers were stained in blue colour by this method (Figures 2e, 2f).

Discussion

The relationship between high blood pressure and renal damage has been studied in different experimental models of rats. Given that essential hypertension represents 95% of all cases in human population, the SHR strain seems to be an accurate model for studies of the alterations in kidneys as a result of this type of hypertension. A review of the pertinent literature shows that hypertensive glomerulosclerosis and tubular atrophy can be observed in SHR [15]. These alterations were also noted in the present study. Some studies have suggested that glomerulosclerosis and tubular alterations in hypertension are more pronounced in the inner as opposed to the outer cortex [16-18]. Comparing 2-month-old and 6-month-old SHR, we found an increasing prevalence of globally sclerotic glomeruli – predominantly in juxtamedullary nephrons. In contrast to some data, we established segmental thickening of glomerular basement membrane and glomerulosclerosis in the outer cortex. Expansion of the mesangial matrix in the older group was found. Ischemia due to narrowing or obstruction of preglomerular vessels has been discussed as the underlying factor in the development of the observed changes [19]. Glomerulosclerosis can also be a result of renal aging. The percentage of age-related sclerotic glomeruli in human population can be estimated by the following formula: age/2-10, when the age is above 40 years [20]. Vascular changes were primarily observed in the small arteries: hyaline arteriosclerosis of the afferent arterioles, which caused narrowing of the vascular lumen, ischemia and glomerular sclerosis; fibrinoid necrosis of arcuate arteries and myointimal thickening of interlobular arteries. In the literature, hyperplastic arteriosclerosis is characterized by concentric proliferation of the smooth muscle layer – an appearance described as "onion skin" [21]. These vascular alterations have been reported in human renal biopsies [8, 22], but we established them in the SHR strain.

Hypertensive vascular damage caused renal ischemia, which was associated with alterations in the tubulointerstitium. The observed tubular atrophy observed was characterized by flattening of the covering epithelium, dilatation of the lumen, and a lack of clear demarcation of the borders between the cells. There was evidence of mononuclear cell infiltration in some slides, as well as thickening of the tubular basement membranes, which was demonstrated by PAS reaction. The relation between vascular and glomerular alterations and tubular atrophy has been discussed in literature [23]. It has been found that tubular atrophy is induced by afferent arteriolopathy and glomerular collapse, rather than by segmental sclerosis. The observed interstitial fibrosis, which was associated with expansion of extracellular matrix proteins, was characterized by periglomerular, peritubular and periarteriolar fibrosis. In the present study, these alterations were demonstrated by Mallory's trichrome staining – the collagen fibers stained in blue. In the normal kidney, collagen synthesis is associated with the function of interstitial fibroblasts. However, myofibroblasts are the major cell type responsible for renal fibrosis in hypertensive kidney disease. These cells originate from interstitial fibroblasts upon stimulation by TGF-$ß_1$ and have the characteristics of both fibroblasts and smooth muscle cells [24-26]. The role of oxidative stress in renal fibrogenesis has also been studied [27]. It has been found that oxidative stress stimulates myofibroblasts and subsequently, collagen synthesis and renal fibrosis.

Conclusions

In conclusion, the present study illustrates the changes in the morphology of nephrons and renal interstitium in two age groups, representative for two stages of the postnatal development in spontaneously hypertensive rats. The light microscopic study expands the knowledge of

characteristics of the kidney in conditions of hypertension. In our present work, we studied the dynamic development of renal morphological alterations which were characterized by an increasing number of sclerotic glomeruli, tubular and vascular damage and expansion of extracellular matrix proteins. We demonstrated these pathological changes in SHR strain, which changes have been described predominantly in human kidney.

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