Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 25 (1-2)
Sofia ● 2018

The Aging Kidney - a Quantitative Study on Superficial and Juxtamedullary Nephrons in Wistar Rats

Stancho Stanchev, Alexandar Iliev, Lina Malinova, Boycho Landzhov*

Department of Anatomy, Histology and Embryology, Medical University of Sofia, Bulgaria

* Corresponding author e-mail: stanchev_1989@abv.bg

Renal senescence is characterized by numerous morphological alterations, which are associated with reduction in the renal functional capacity. Glomerulosclerosis is a nonspecific feature observed in the normal process of aging, as well as in pathological conditions such as hypertension and diabetes. In literature, two types of sclerotic glomeruli have been described, based on their light microscopic appearance. However, the qualitative evaluation of the glomerular changes is sometimes not sufficient for distinguishing the etiology of the kidney damage. The present study represents a detailed morphometric study on the postnatal changes in the areas of the renal corpuscles and glomerular capillary tufts of the superficial and juxtamedullary nephrons in Wistar rats.

Key words: glomerulosclerosis, renal corpuscles, morphometry, Wistar rat

Introduction

Aging is a biologically inevitable process, which is characterized by numerous alterations in many organs and systems, including the kidney. The features of renal senescence comprise both macroscopic and histological changes. During the postnatal development, the kidney weight progressively increases and reaches its maximum at four decades of life, followed by gradual reduction [5, 19]. There are convincing data, which represent a positive correlation between aging and the formation of parenchymal calcifications and simple renal cysts [8]. Furthermore, the histological examination of the aging kidney reveals morphological changes in both cortex and medulla leading to declining renal function – an increased number of sclerotic glomeruli, periglomerular and intraglomerular fibrosis, myointimal thickening of the blood vessels and hyaline arteriosclerosis. The tubulointerstitial changes include significant expansion of extracellular collagen fibers, as well as tubular atrophy along the proximal and distal tubular segments – flattening of the covering epithelium, resulting in dilation of the luminal diameter and formation of diverticula [12].

On light microscopy level, two types of glomerulosclerosis can be described - shrinkage of glomerular capillary tuft and increased space between the parietal and visceral layers of the Bowman's capsule or mesangial proliferation accompanied with glomerular enlargement [6]. However, the pathogenesis of age-related renal alterations

is still misunderstood and factors such as gender, race and genetic background have been discussed [1]. Indeed, all described features of the morphological changes in the senescent kidney are not specific and can be observed under pathological conditions such as hypertensive nephrosclerosis and diabetic nephropathy [2, 4].

The aim of the present study was to analyze and compare the areas of the renal corpuscles and glomerular capillary tufts of the superficial and juxtamedullary nephrons traced among three age groups (4-month-old, 6-month-old and 12-month-old) male Wistar rats.

Materials and Methods

In the present study, we used histological material from the kidneys of male Wistar rats. The total number of Wistar rats was 9, distributed in three age groups, each containing three animals: four months old; six months old and twelve months old. All experiments were conducted with the approval of the University Committee on Animal Resources (№ 4866). 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). The rats were anaesthetised intraperitoneally with Thiopental 40 mg/kg body weight. 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 embedding, serial coronal 5 μm thick sections were cut and stained routinely with haematoxylin and eosin. Haematoxylin and eosin staining was conducted in the following way: after removal of the paraffin with xylol, we washed the slides with water and stained them with haematoxylin for 5 minutes. They were then stained with an eosin solution for 10 minutes, washed again with water and embedded in entellan.

The morphometric analysis was performed on five slides from the kidney of each animal. Quantitative data were obtained with a computerised system for image analysis NIS Elements Advanced Research (Ver. 2.30). The areas of interest in each slide were first found on low magnifications (x100, x200), taking into account the respective age group. Results were obtained through assessment of randomly selected zones of the renal cortex with no significant ruptures resulting from the processing technique of the histological material that could compromise the proper data analysis. The standardization of the zone where the areas of renal corpuscles and glomeruli were measured was performed in line with the following criteria in order to certify the authenticity of the results: 1. only renal corpuscles with clearly demarcated vascular and tubular poles were included in this study; 2. the selected renal corpuscles of the superficial nephrons were situated in the periphery of the cortex; 3. the analyzed renal corpuscles of the juxtamedullary nephrons were located adjacent to the medulla. The following morphometric parameters of the superficial and juxtamedullary nephrons during the postnatal development of Wistar rats were analyzed: area of the renal corpuscles of the superficial nephrons (μm^2), glomerular area of the superficial nephrons (μm^2), area of the renal corpuscles of the juxtamedullary nephrons (μm^2), glomerular area of the juxtamedullary nephrons (μm^2). The obtained quantitative data were demonstrated with Bar Chart/Bar Plot diagrams and were statistically evaluated through a Student-T-test. Statistically significant differences were read in the case of $p<0.05$. Microsoft Office Excel 2010 was used to process the data and to demonstrate the obtained results in an adequate way.

Results

The comparative analysis of the parameter area of the renal corpuscles among the young group of 4-month-old Wistar rats showed similar values in the outer and inner cortex with quite higher prevalence in the superficial nephrons. As aging advanced, in 6-month-old rats, we noted a slight increase in the parameter, which was more pronounced in juxtamedullary nephrons. That trend was also observed in the group of senescent 12-month-old Wistar rats, where the areas of the renal corpuscles of the nephrons in the outer and inner cortex reached the highest value. We should note that the analyzed parameter among the juxtamedullary nephrons in 6-month-old rats was comparable with the results obtained from the superficial nephrons in 12-month-old rats **(Fig. 1, Table 1)**.

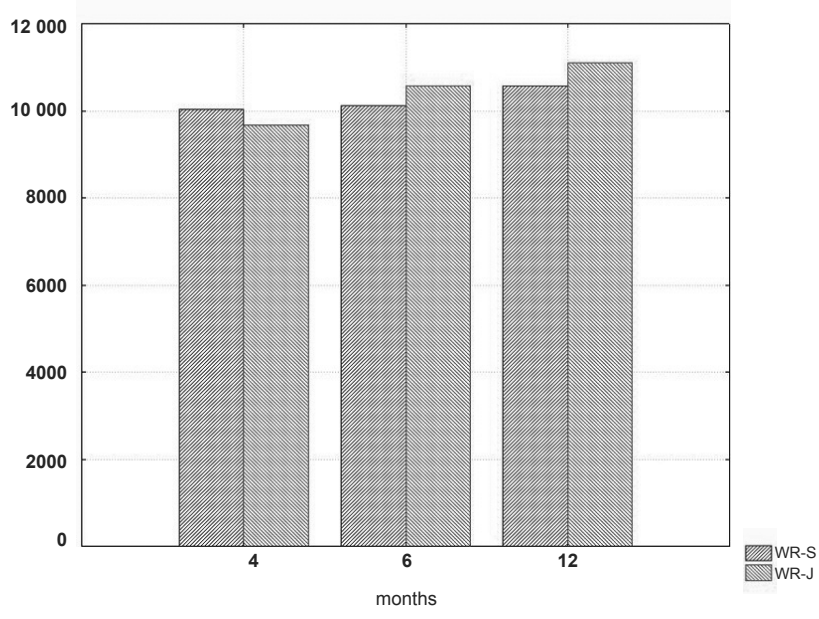

Fig. 1. Graphical representation of the comparative analysis of the morphometric parameter area of renal corpuscles of superficial (WR-S) and juxtamedullary nephrons (WR-J) in Wistar rats. Y-axis: area of renal corpuscles (μm^2)

Table 1. Numerical representation of the comparative analysis of the morphometric parameter area of renal corpuscles in Wistar rats (SD - standard deviation)

WR	Superficial nephrons		Juxtamedullary nephrons		TTEST
	Area of renal corpuscles		Area of renal corpuscles		
Age	Mean value	SD	Mean value	SD	
4 months	10035.7	660.5	9664.4	487.9	p< 0.0001
6 months	10120.3	375.3	10561.1	377.1	p< 0.000001
12 months	10560.8	617.4	11092.8	563.7	p< 0.000001

The representation of the comparative analysis of the area of renal corpuscles of superficial and juxtamedullary nephrons by a Box Plot diagram indicated that in 4-month-old rats the average value of the parameter obtained from the outer cortex was higher compared to the inner cortex. In the groups of 6- and 12-month-old rats, the increase of the area of renal corpuscles was more pronounced in juxtamedullary nephrons **(Fig. 2).**

In the group of 4-month-old Wistar rats, the comparative analysis of the area of the glomerular tufts in the outer and inner cortex showed comparable values, with quite higher prevalence in the juxtamedullary nephrons. The results obtained from 6-month-old rats revealed a progressive increase in the analyzed parameter and the trend for higher value of the area of glomerular tufts was preserved. In the group of 12-month-old animals there was a significant increase in the parameter in juxtamedullary nephrons, where it reached its highest value. In contrast, the increased area of the glomerular tufts of superficial nephrons was less pronounced and was comparable with the results obtained from juxtamedullary nephrons in the 6-month-old group **(Fig. 3, Table 2)**.

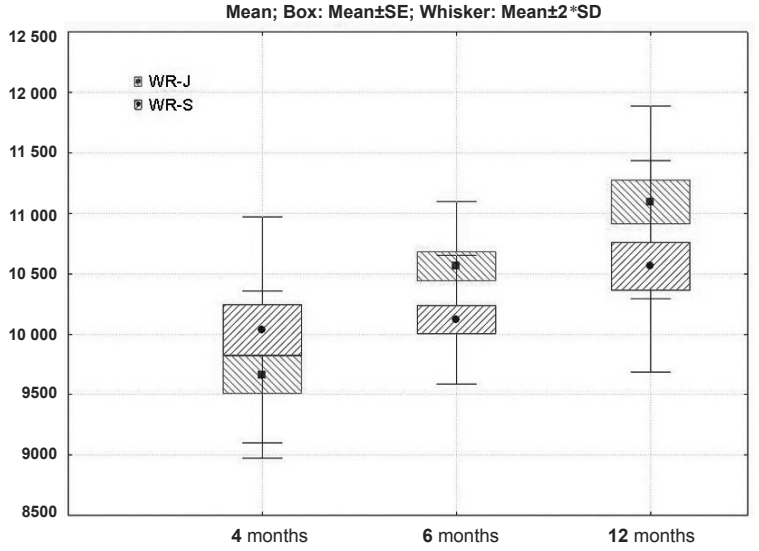

Fig. 2. Box Plot graphical representation of the comparative analysis of the morphometric parameter area of renal corpuscles of superficial (WR-S) and juxtamedullary nephrons (WR-J) in Wistar rats

Table 2. Numerical representation of the comparative analysis of the morphometric parameter glomerular area in Wistar rats (SD - standard deviation)

WR	Superficial nephrons		Juxtamedullary nephrons		TTEST
	Glomerular area		Glomerular area		
Age	Mean value	SD	Mean value	SD	
4 months	6572.4	386.7	6866.1	334.4	p< 0.00001
6 months	7287.1	261.2	7510.8	445.8	p< 0.0001
12 months	7738.1	864.0	9767.5	225.5	p< 0.000001

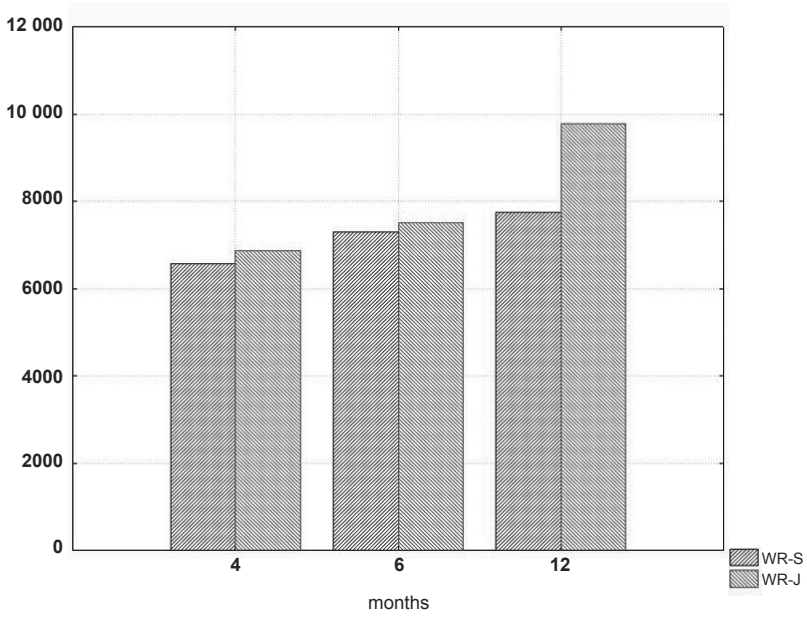

Fig. 3. Graphical representation of the comparative analysis of the morphometric parameter glomerular area of superficial (WR-S) and juxtamedullary nephrons (WR-J) in Wistar rats. Y-axis: glomerular area (μm^2)

Discussion

The current study represents a detailed comparative morphometric analysis of the changes in the areas of the renal corpuscles and glomerular capillary tufts of the superficial and juxtamedullary nephrons during the postnatal development in Wistar rats. The change in the areas of renal corpuscles was not so pronounced, but the trend for higher value of the analysed parameter in juxtamedullary nephrons was preserved in 6- and 12-month-old rats. Comparing 4-month-old with 6-month-old animals, we noted a gradual increase in the glomerular tuft areas of the nephrons in the outer and inner cortex, as well as a well-expressed increase in the parameter in juxtamedullary nephrons of 12-month-old rats, where it reached its highest value.

In the process of aging, the remaining functional capacity of the kidneys depends on the number of intact glomeruli. Numerous studies have shown a positive correlation between age and development of glomerulosclerosis [13]. Furthermore, a formula for estimation of the percentage of the senescent sclerosed glomeruli was suggested by Smith et al. [15]. On the other hand, the evaluation of glomerulosclerosis is based not only on qualitative criteria. In fact, the development of glomerulosclerosis includes initial enlargement of the glomerular capillary tuft described by the term glomerular hypertrophy, which is followed by collapsing of the glomerulus in the late stages [17]. Therefore, the glomerular size changes in the different stages of glomerulosclerosis and the morphometric analysis of the renal corpuscles can contribute to the complex evaluation of renal senescence. In the present study, both superficial and juxtamedullary nephrons were characterized by progressive increase in the areas of glomerular capillary tufts, which was most pronounced in the juxtamedullary nephrons

in the group of 12-month-old rats. Our results indicate that glomerular hypertrophy affected mostly nephrons in the inner rather than the outer cortex in the process of aging. There is evidence that the aged rat kidney is characterized by hypertrophic podocytes, which undergo apoptosis [18]. In contrast, some studies have shown a prevalence of sclerotic glomeruli in the outer cortical zone, as well as inversely proportional correlation between age and glomerular size [10, 11]. Moreover, Kasiske demonstrated a positive correlation between globally sclerotic glomeruli, intrarenal vascular alterations and age [7]. The reason for the opposite results obtained can be explained by the use of different experimental models. Under pathological conditions such as hypertension, glomerulosclerosis is more severe in the inner cortex [3]. This was confirmed by our previous comparative morphometric study on spontaneously hypertensive rats, where juxtamedullary nephrons were characterized by significant decrease in glomerular tuft area – a feature of the late stages of glomerulosclerosis [16]. The role of hemodynamic changes leading to glomerular hyperperfusion has also been discussed in the genesis of age-related glomerulosclerosis [14]. Some authors have commented on the presence of atubular glomeruli, which are non-functional and on the possibility for sclerosed glomeruli to disappear with age [9, 17]. Therefore, these circumstances should be taken into account in the evaluation of the severity of renal morphological alterations.

Conclusion

The current study is a detailed morphometric study on the postnatal changes in the areas of renal corpuscles and glomerular capillary tufts of the superficial and juxtamedullary nephrons in Wistar rats. Such quantitative information can be used in the evaluation of the severity of kidney damage under pathological conditions.

R e f e r e n c e s:

1. **Bolignano, D., F. Mattace-Raso, E. J. Sijbrands, C. Zoccali.** The aging kidney revisited: a systematic review. - *Ageing Res. Rev.,* **14,** 2014, 65-80.
2. **Caetano, E. R., R. Zatz, L. B. Saldanha, J. N. Praxedes.** Hypertensive nephrosclerosis as a relevant cause of chronic renal failure. - *Hypertension,* **38**(2), 2001, 171-176.
3. **Feld, L. G., J. B. Van Liew, R. G. Galaske, J. W. Boylan.** Selectivity of renal injury and proteinuria in the spontaneously hypertensive rat. - *Kidney Int.,* **12**(5), 1977, 332-343.
4. **Fioretto, P., M. Mauer.** Histopathology of diabetic nephropathy. - *Semin. Nephrol.,* **27**(2), 2007, 195-207.
5. **Gourtsoyiannis, N., P. Prassopoulos, D. Cavouras, N. Pantelidis.** The thickness of the renal parenchyma decreases with age: a CT study of 360 patients. - *Am. J. Roentgenol.,* **155**(3), 1990, 541-544.
6. **Hughson, M. D., V. G. Puelles, W.E. Hoy, R. N. Douglas-Denton, S. A. Mott, J. F. Bertram.** Hypertension, glomerular hypertrophy and nephrosclerosis: the effect of race. - *Nephrol. Dial. Transplant.,* **29**(7), 2014, 1399-1409.
7. **Kasiske, B. L.** Relationship between vascular disease and age-associated changes in the human kidney. - *Kidney Int.,* **31**(5), 1987, 1153-1159.
8. **Lorenz, E. C., J. C. Lieske, T. J. Vrtiska, A. E. Krambeck, X. Li, E. J. Bergstralh, L. J. Melton 3rd, A. D. Rule.** Clinical characteristics of potential kidney donors with asymptomatic kidney stones. - *Nephrol. Dial. Transplant.,* **26**(8) 2011, 2695-2700.
9. **Marcussen, N.** Tubulointerstitial damage leads to atubular glomeruli: significance and possible role in progression. - *Nephrol. Dial. Transplant.,* **15**(6), 2000, 74-75.
10. **Newbold, K. M., A. Sandison, A. J. Howie.** Comparison of size of juxtamedullary and outer cortical glomeruli in normal adult kidney. - *Virchows Arch. Pathol. Anat. Histopathol.,* **420**(2), 1992, 127-129.

11. **Nyengaard, J. R., T. F. Bendtsen.** Glomerular number and size in relation to age, kidney weight, and body surface in normal man. - *Anat. Rec.,* **232**(2), 1992, 194-201.
12. **Pannarale, G., R. Carbone, G. Del Mastro, C. Gallo, V. Gattullo, L. Natalicchio, A. Navarra, A. Tedesco.** The aging kidney: structural changes. - *J. Nephrol.,* **23**(15), 2010, 37-40.
13. **Rule, A. D., H. Amer, L. D. Cornell, S. J. Taler, F. G. Cosio, W. K. Kremers, S. C. Textor, M. D. Stegall.** The association between age and nephrosclerosis on renal biopsy among healthy adults. - *Ann. Intern. Med.,* **152**(9), 2010, 561-567.
14. **Silvia, F. G.** The aging kidney: a review - part I. - *Int. Urol. Nephrol.,* **37,** 2005, 419-443.
15. **Smith, S. M., W. E. Hoy, L. Cobb.** Low incidence of glomerulosclerosis in normal kidneys. - *Arch. Pathol. Lab. Med.,* **113**(11), 1989, 1253-1255.
16. **Stanchev, S., A. Iliev, G. Kotov, L. Malinova, B. Landzhov.** A comparative morphometric study of the superficial and juxtamedullary nephrons during the postnatal development in spontaneously hypertensive rats. - *Arch. Anat. Physiol.,* **3**(1), 2018, 001-004.
17. **Stojanović, V. R., I. D. Jovanović, S. Z. Ugrenović, L. P. Vasović, V. S. Živković, M. V. Jocić, B. K. Kundalić, M. N. Pavlović.** Morphometric analysis of nonsclerosed Glomeruli size and connective tissue content during the aging process. - *Sci. World J.,* **2012,** 2012, 845046.
18. **Wharram, B. L., M. Goyal, J. E. Wiggins, S. K. Sanden, S. Hussain, W. E. Filipiak, T. L. Saunders, R. C. Dysko, K. Kohno, L. B. Holzman, R. C. Wiggins.** Podocyte depletion causes glomerulosclerosis: diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. - *J. Am. Soc. Nephrol.,* **16**(10), 2005, 2941-2952.
19. **Zhou, X. J., D. Rakheja, X. Yu, R. Saxena, N. D. Vaziri, F. G. Silva.** The aging kidney. - *Kidney Int.,* **74,** 2008, 710-720.