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Histological Study Of The Kidney Of The White Rat At Different Ages Using Electron Microscopy

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ABSTRACT

Background: Kidney development involves morphological formation and functional maturation, beginning with embryonic nephron formation and continuing post-birth. Understanding renal structure and physiology is crucial due to the kidney's vital roles in filtration, toxin susceptibility, and hormone production. This study aimed to examine the histological development of the kidney in various age groups using light and electron microscopy for detailed structural analysis.
Materials and method: This study examined age-related renal changes in 30 white rats divided into three groups: two-month-old, four-month-old, and six-month-old rats. Kidneys were extracted after anaesthesia and dissected for analysis. Samples were preserved in 10% formalin for histological staining or prepared for electron microscopy.
Results: The study revealed significant age-related changes in renal structure. Cortex thickness increased from 245.00±2.54 µm at 30 days to 287.00±2.33 µm at 6 months, while medulla thickness rose from 299.11±2.98 µm to 335.00±2.33 µm. Mesangial cell counts decreased from 7.36±0.02 to 5.45±0.00, and glomerular diameter enlarged from 47.31±0.11 µm to 57.17±0.06 µm. Conversely, Bowman's capsule diameter shrank from 12.65±0.06 µm to 8.43±0.06 µm, and proximal tubules decreased from 27.32±0.08 µm to 22.12±0.10 µm, with distal tubules showing minimal change. Electron microscopy indicated a significant glomerular size increase (10.8-fold) between two and six months, suggesting growth continued beyond four months, unlike in humans where stabilization occurs earlier.
Conclusion: These findings highlight ongoing renal maturation in mice, critical for experimental design and data interpretation. The study underscores species developmental patterns and their implications for research and clinical applications.

Key words; Histology, White Rat, Kidney, Age. Electrom Microscopy.

INTRODUCTION

The rat is one of the most commonly used animals in research, particularly in the fields of urinary system physiology and toxicology. It has been used since the 19th century to study genetic diseases, diabetes, hypertension, and other conditions. The rat is characterized by a unilobar kidney with a single papilla, composed of the cortex and medulla. The cortex contains convoluted tubules and medullary rays, while the medulla is divided into outer and inner regions that terminate at the renal papilla. The functional unit of the kidney is the nephron, which consists of the glomerulus, proximal and distal tubules, and the loop of Henle, which empties into collecting ducts that lead to the renal pelviss[1].
The right kidney differs from the left in position and size, with the right being slightly higher and larger. Additionally, male kidneys are larger than those of females[2]. The parietal cells of Bowman's capsule

appear flattened at the vascular pole and cuboidal at the urinary pole. The proximal tubules contain cuboidal cells with microvilli, while the distal tubules lack these microvilli[3]. Specialized cells in the macula densa produce the hormone renin, which regulates blood pressure. Renal blood supply in rats is similar to that of other mammals, with the renal artery branching into arcuate arteries that deliver blood to the cortex and medulla (Morawietz et al., 2004). The kidney is covered by fat and part of the peritoneum and contains a hilum through which blood vessels and the ureter pass [4].

The mammalian urinary system consists of the kidneys, ureters, bladder, and urethra. The kidneys regulate body fluids and secrete hormones such as renin and erythropoietin, which stimulates red blood cell production[5]. The nephron is the functional unit of the kidney and consists of a glomerulus, convoluted tubules, and the loop of Henle [6].

This study aimed to examine the histological development of the kidney in various age groups using light and electron microscopy for detailed structural analysis.

MATERIALS AND METHODS

The study was conducted at the Animal House affiliated to the Faculty of Veterinary Medicine at Tikrit University in Salah al-Din governorate.

Animals used in the experiment

Thirty white rats (Albino rats) purchased from the Animal House of the Faculty of Veterinary Medicine of Tikrit University. The 30 rats were distributed into three groups according to age:
The first group: two-month-old rats.
The second Group: 4-month-old rats.
The third group: 6-month-old rats.
Plastic cages (50 × 30 × 15 cm) are prepared with sawdust with their weekly change, providing ventilation, constant heat (25°M) and light circulation (12 hours light/12 hours darkness).

Experiment design

The study included macroscopic (such as the thickness of the Shell and pulp of the kidneys) and microscopic (such as the diameter of the tubules and the number of renal cells) measurements. using special dyes such as PAS and Masson's Trichrome, as well as light and electron microscopy.

Aesthesia and anatomy

The rats were anesthetized using chloroform, and then dissected for kidney extraction. The samples were washed with 0.9% saline solution and kept in 10% formalin.

Macroscopic and microscopic description

The weight and dimensions of the kidneys were measured; histological slides were brought across:
Fixation: with 10% formalin.
Drying: with an ascending alcohol chain.
Anatomy and colouring: with haematoxylin and eosin.
Examination: light and electron microscopy.

Special tinctures

Pas tincture: for the detection of glycogen and glycoproteins.
Masson's Trichrome dye: to distinguish connective tissue.

Microscopic examination

The samples were examined with an optical microscope equipped with a digital camera and an electron

microscope after being fixed with glutaraldehyde and coated with gold.

Electron Microscopy
SEM Processing: Tissues were post-fixed in 1% osmium tetroxide, dehydrated, critical-point dried, and sputter-coated with gold.
Imaging: A JEOL JSM-IT500 SEM at 15 kV was used. Glomerular dimensions (length, width, depth) and podocyte morphology were analyzed using ImageJ.

Morphometric Analysis
Glomerular Volume: Calculated using the ellipsoid formula:
$V=43\pi\times L2\times W2\times D2 V=34\pi\times 2L\times 2W\times 2D$
Statistical Analysis: One-way ANOVA with Tukey's post-hoc test ($p<0.05$).

RESULTS
The results showed that the kidneys consist of two main layers:
Cortex: Contains renal corpuscles (glomeruli), proximal/distal convoluted tubules, connective tissue, and blood vessels.
Medulla: Comprises renal pyramids, the loop of Henle, and collecting ducts.

Key Observations
Also, the results revealed that cortical thickness increased with age (from 245 µm at 60 days to 287 µm at 180 days), while the renal capsule thickness decreased.
Development of Renal Corpuscles (Glomeruli):
Glomerular size significantly increased with age (from 47.3 µm at 60 days to 57.2 µm at 180 days), along with a rise in supporting cell count (from 5.45 to 7.36).
Scanning electron microscopy revealed structural progression: glomeruli transitioned from a smooth surface at 60 days to a rough, deposit-laden surface by 180 days, indicating early fibrotic changes.

Group	Length(µm)	Width(µm)	Depth(µm)	volume(µm³)
60	93.9±2.98	43.8±3.98	34.425±2.980	148266.2385
120	191±3.54	198±1.86	97.25±2.87	3851383.677
180	130±2.33	207±3.86	84.25±22.87	2374172.254

Table 1: Shows the measurements of the dimensions of the glomeruli, length, width and depth, in rats aged (60, 120, 180 days).

Renal Tubules
The diameter of proximal convoluted tubules increased from 22.12 µm (60 days) to 27.92 µm (180 days).
The loop of Henle became clearly distinguishable by 120 days, with variations in epithelial thickness between its descending (thin) and ascending (thick) limbs.

Functional Changes:
Filtration efficiency improved with age, particularly at 180 days, where the Macula Densa became distinctly visible.
Microscopic blood vessels (e.g., Vasa Recta) expanded with age, supporting enhanced filtration capacity.

Morphometric Results:
Overall kidney size increased, along with renal pyramid diameter and endothelial cell count.
Glomerular volume expanded by 10.8-fold between 60 and 180 days, reflecting exponential growth

during this period. Rat kidneys undergo significant structural and functional changes with age, continuing to develop until 180 days. Findings align with prior rodent kidney studies, emphasizing age as a determinant of histological and functional characteristics. Glomerular and tubular changes highlight maturation and adaptation phases, useful for comparative studies on kidney aging or external factor impacts.

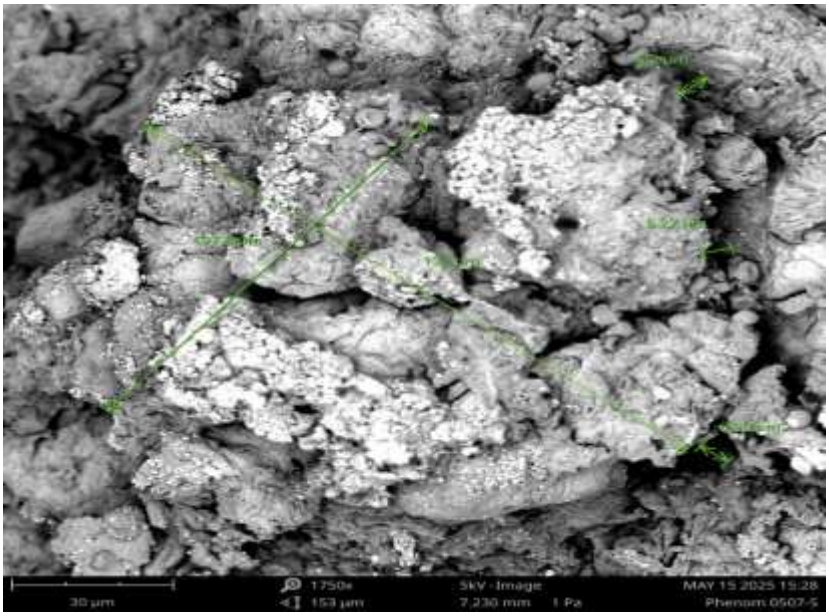

Figure 1: Glomeruli at the age of 60 days, showing glomerular measurements in addition to Bowman's area.

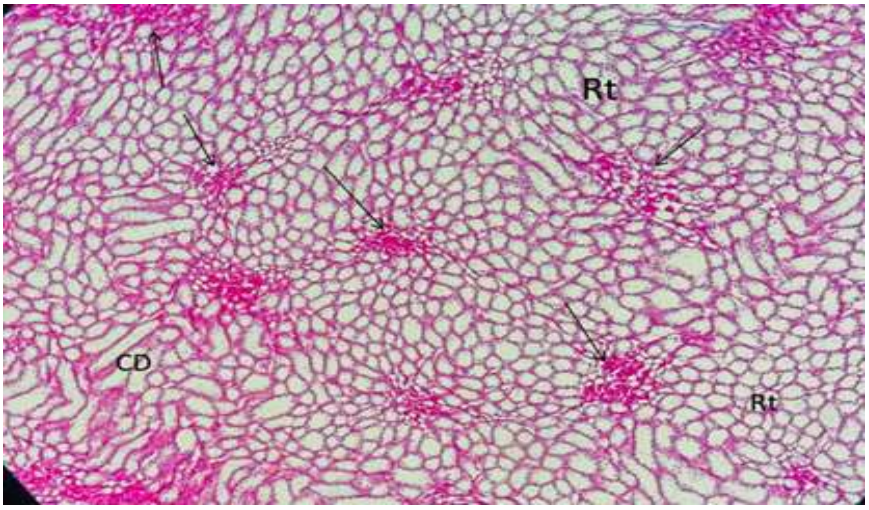

Figure 2: histological section (under light microscope) of rat kidney marrow at the age of 60 days showing renal pulp (RM) renal tubules (RT) collecting ducts (CD) rectal vessels (Black Arrow) pas dye, magnification 100x

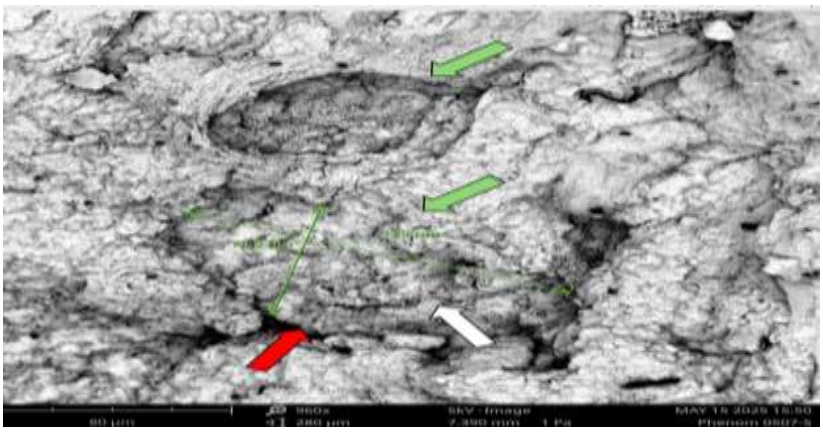

Figure 3: Glomeruli at the age of 120 days show a clear central vacuole closer to the lumen or circular fossa with a diameter of more than 100 µm, resembling in shape a section of a glandular formation or collapsed structure.

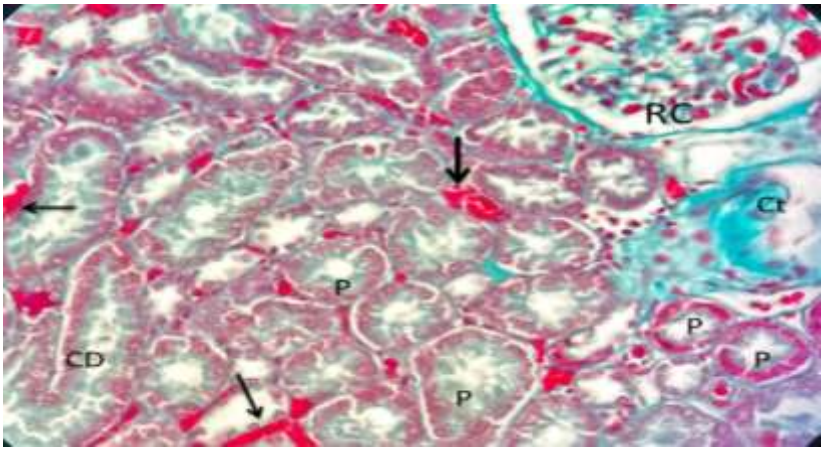

Figure 4: histological section of the renal cortex of a 120-day-old Rat shows, renal glomerulus (RC), proximal convoluted tubules (P), collecting duct (CD), straight vessels (Black Arrow), Mason's tricolor dye, magnification 400, (under light microscope)

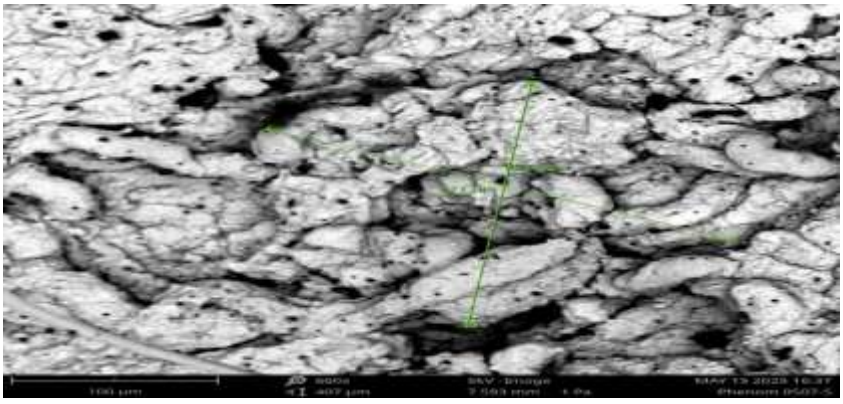

Figure 5: Glomeruli at the age of 180 days. Glomeruli are clearly visible, indicating subcellular aggregates or perhaps deposited cell units or interconnected tissue cells.

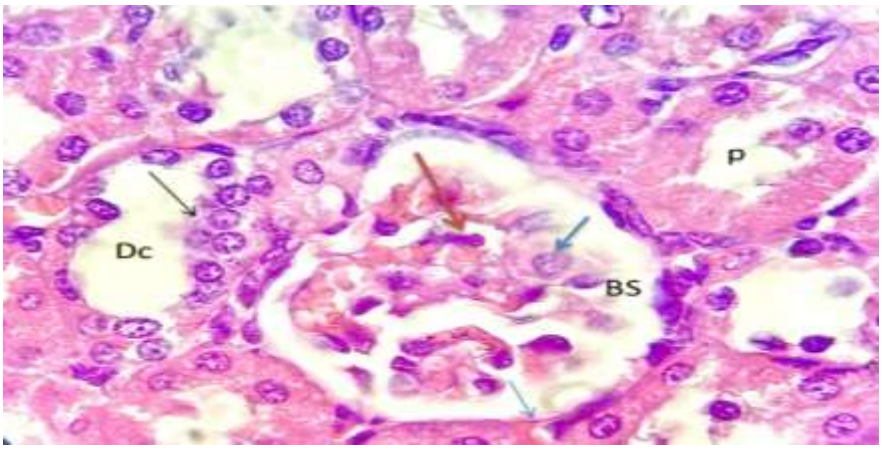

Figure 6: histological section of the renal cortex of a 180-day-old rat showing the renal glomerulus (renal corpuscle), distal convoluted tube (DC), proximal convoluted tube (P)Bowman's space (BS) parietal layer (Blue Arrow) mesangial cells (thick blue arrow) apical cells (red arrow) capillaries (orange arrow) dense spot (Black Arrow), H&E pigment, enlarged, (under light microscope)

DISCUSSION

The current study examined rat kidneys at different ages. The microscopic study demonstrated the histological morphology of the renal tissue sections at these ages, which included that the kidney consists of the cortex and medulla. The cortex at all ages covered by the study consists of renal corpuscles, including the glomerulus and Bowman's membrane, in addition to other renal structures such as adipose tissue, blood vessels, renal pyramids, and the renal pelvis, which collects urine after filtering it from the renal nephrons toward the ureter. The study demonstrated in the histological section that the renal papillae are an important tissue structure in the kidney, playing a significant role in filtering fluids and toxins through the epithelial membrane, which is represented by the epithelium, as well as the connective tissue and blood vessels. The important part is the renal tubules, which branch off from these papillae and play an important role in filtration. These results are consistent with many previous studies showing that the newborn kidney is not merely a miniature version of the adult kidney with identical renal functional mechanisms [7]. Rats, mice, guinea pigs, and rabbits, among the least complex laboratory animal species from a phylogenetic perspective, are valuable models for exploring fundamental aspects of development and clinical phenomena. [8] The developing kidney is susceptible to morphological and functional abnormalities during postnatal growth and differentiation. Kidney development involves anatomical and functional features that unfold over predictable timescales, identifying critical time periods in the anatomical and functional development of the kidney. Other studies, [10, 12] demonstrated the presence of a cortical tubular population of stem cells, suggesting an alternative environment within the kidney. [11] also reported that mesenchymal cells, presumably including mesenchymal stem cells, are located primarily in the cortico-medullary region. Research has also demonstrated their ability to motility and direct their migration toward the renal parenchyma. In this study, microscopic examination of the renal capsule, surrounded by thin connective tissue, revealed a predominant collagen fiber composition. The thickness of the capsule gradually decreases with age, particularly in the interstitial tissue adjacent to the subcapsular connective tissue. This phenomenon may be related to the process of kidney formation, as a large number of nephrons are believed to arise from the renal capsule or subcapsular tissue during the early postnatal stages, specifically in pups at one and ten days of age. These results are consistent with those reported in a mouse study[12].

This study documents progressive glomerular hypertrophy followed by degenerative changes in aging rats. The 120-day group exhibited peak glomerular volume, aligning with physiological hypertrophy [13]. By 180 days, glomerular collapse and podocyte injury mirrored human aging phenotypes [14]. Notably,

SEM revealed podocyte effacement and mesangial expansion, hallmarks of glomerulosclerosis [15]. The decline in glomerular volume at 180 days suggests capillary rarefaction, consistent with senescence-associated attrition [16].

CONCLUSION

In conclusion, this study revealed significant age-related structural changes in rat kidneys. As rats matured, both cortical and medullary regions increased in thickness. Glomeruli enlarged, yet Bowman's capsule and mesangial cell counts decreased. Proximal tubules also shrank, while distal tubules remained stable. Electron microscopy confirmed sustained glomerular growth in rats, contrasting with earlier stabilization seen in human kidney maturation. This highlights valuable species-specific developmental patterns.

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NA

CONFLICT OF INTERESTS

All authors declare that they have no any conflict of interests.

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