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Glomerulogenesis and Histomorphometeric in *Mus musculus* Embryo

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Abstract

In mammals, the kidneys originate in an embryo from the mesoderm through three excretory organs, namely: Pronephros, Mesonephros, and Metanephros. After the formation of Metanephros is completed, the kidneys begin to form nephrogenesis through mesenchymal cells located at the tip of the ureteric bud, that contribute in the formation of glomerulus and Renal tubules. The stages of glomerulus formation in the embryo of albino mice at the age of 14 to 19 days of gestation were studied. It was obtained after the sacrifice of the expectant mother and the kidneys were excised from the embryos and fixed using Aqueous Bouin's solution, Microscopic slices with a thickness of 6 microns were then made in a paraffin method and were photographed by a camera for microscopic imaging. Histological measurements were performed on them using the program IMAGE J program and analyzed statistically using the SPSS program.

Results of the current study showed the presence of five stages of glomerulus formation, namely, the renal vesicle, which represents the first stage in the formation of the nephron and glomerulus. This is followed by the Comma shape stage, S-shaped stage, Capillary loop stage and finally the Mature glomerulus. This is surrounded by a capsule, known as Bowman's capsule being part of the Malpighian or Renal corpuscle. The statistical analysis showed that there were significant differences between the average diameters of the glomerular development stages, and that the mature glomerulus was larger in diameter than the rest of the stages. The study aims to determine the stages of glomerulus formation and histologically measure its diameter in the embryo of a *Mus musculus*.

Keywords: Kidney, *Mus musculus*, Embryo, Ureteric bud, Glomerulus, Histomorphometry.

1.Introduction

The urinary system plays a sensitive role in regulating the economy of the body, and this system works through intertwined processes that are intended to maintain the state of the internal homeostasis of the vertebrate bodies[1]. The main function of the kidneys is to maintain the homeostasis of fluids in the body, and to remove waste products, excess water, and electrolytes from the blood [2]. In addition, it can be considered a major gland that secretes some hormones, such as the renin hormone, which regulates blood pressure. and erythropoietin, which works to stimulate the bone marrow to produce red blood cells [3]. The kidneys in mammalian embryos consist of three excretory organs: the pronephros, the mesonephros, and the metanephros, all of which arise from the intermediate mesoderm [4,5]. The nephron consists of the renal corpuscle, which includes the glomerulus, surrounded by Bowman's capsule and the renal tubule [6]. After the formation of the Metanephros, the nephron forms, where the ureteric bud emerges from the ducts Mesonephros, then enters the Metanephros and gathers around the anterior end of the bud, Mesenchyma cell that turns into epithelial cells to form the renal vesicle From which glomeruli and renal tubules arise [7].

2. Material and methods

Sample collection

In the present study, mice were used at the last week of gestation. The embryos were anesthetized using chloroform. The embryos were then dissected and excised from their place and then fixed using the Aqueous Bouin's fluid for 12-24 hours. After the fixation period ended, the models were washed with 50% and 70% ethyl alcohol [8].

Histological study

Paraffin slices were prepared according to the method described [9], The samples were passed to dehydration with an ascending series of ethyl alcohol, starting with a concentration of 70% and ending with a concentration of 100%, for one hour for each concentration. The purpose of this procedure was to draw the water inside the sample, then using xylene to clearing the sample for 3 minutes.

Then the specimens were filtered using Paraffin wax. These were then put in a mixture of xylene and molten wax at 1:1 for 30 minute, after which the samples were transported to the pure molten wax in the electric oven for one hour. This procedure was done twice to ensure that the specimen was fully impregnated.

After that, embedding in high melting Paraffin wax. Then, the wax molds containing the samples were cut using the rotary microtome and 6 μm-thick microscopic slice were made as serial sagittal sections. The tissue sections were stained by the routine dye Hars Hematoxin-Iosin according to method [10]. The slides were loading using the Dpx (Dextrin plasticizer xylene). Finally, the glass slides were photographed after being examined with the Compound microscope using a Wifi-720B camera.

Biometric study and Statistical analysis

Metric measurements were carried out on sagittal tissue images using Image J program, to measure the diameters of the glomerulus and Malpighian formation stages under a microscope with a power of 40x. Then, the measurements were entered into the SPSS V.21 program to display the results as (Mean ± standard error) adopting the value of the least significant difference under the level of significance ($P<0.05$).

3.Results and Discussion

3.1. Histological study

The results of the current study of the kidney in the *Mus musculus* embryo showed five stages in the formation of the glomerulus, which are as follows:-

3.1.1 Renal vesicle stage

In the kidney of the *Mus musculus* embryo at 14 day of gestation, it appears that the mesenchymal cells located at the tip of the ureteric bud transformed into a closed V-shaped structure with a central cavity known as the renal vesicle. It also appears that it is composed of a row of cells with oval nuclei located at the superficial part of the renal cortex (3-1). [11] noted that the formation of a glomerulus requires the occurrence of segmentation and morphological changes in the renal vesicle, which is the first epithelial structure to arise from the mesenchymal cell. Thus, it suffers on its lower side from invagination to form a vascular cleft (Fig. 3-1). It also represents the primitive form of the glomerulus [12].

3.1.2 Comma shaped stage

In the kidney of the Albino mice embryo, a comma-like shape appeared. This represents the second stage of the formation of the glomerulus, which arises from the invagination of the renal vesicle and is divided into two regions: the proximal and distal segment, between which the vascular cleft lies. This corresponds to what was found [13]. This form consists of Parietal cells [14] Figure (3-2).

3.1.3 S-Shaped stage

At this stage, the shape of the glomerulus in the kidney of the *Mus musculus* embryo was in the form of a letter S-shape (3-3) consisting of three segment, an upper called the proximal segment, located near the capsule, lined with columnar tissue, and a lower representing the distal segment lined with cuboidal tissue between them located Median curve. [11] confirmed in his study that the three segments play an important role in the formation of the glomerulus and renal tubule. He found the parietal layer of Bowman's capsule and the visceral epithelium arise from the Proximal segment (upper segment), while the proximal tubule, the Henle loop and the distal tubule, arise from Median segment. As for the distal segment(Lower), it connects the renal tubules to the collecting tubule. At this stage, cells begin to migrate to a vascular cleft called the endothelial cell, arises from stem cells known as Angioblast, to form glomerular capillaries [15].

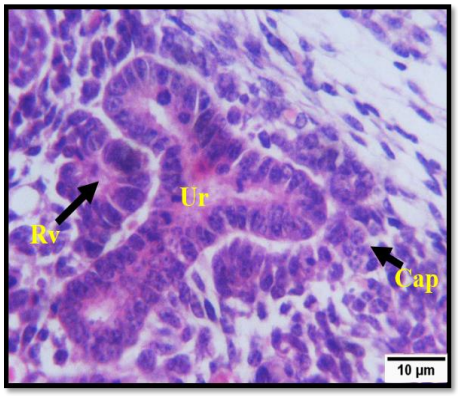
3.1.4 Capillary loop stage

In the kidney of the mice embryo, invagination appeared in a letter S shape at its lower segment while its cuboidal cells turned into flat. Then, it appeared in the shape of the letter C. Its edges are lined with flatting cells and it reaches the endothelial cell to form glomerular capillaries to settle in the previously formed vascular cleft (Figure 3-4). [16] indicated in his study that these capillaries undergo rapid changes, including the appearance of the glomerular capillary system, differentiation of the presumptive endothelium cells to Malpighian corpuscle, and the formation of renal arteries and veins.

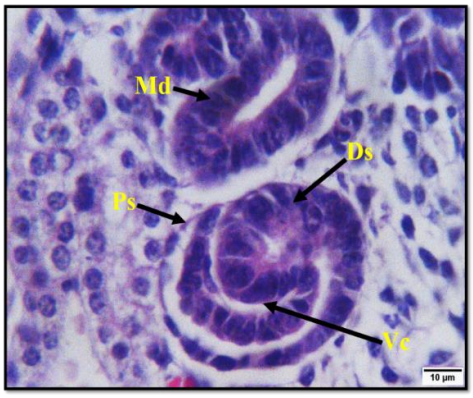
3.1.5 Mature Glomerulus

The mature glomerulus can be distinguished in the area of the cortex as being surrounded by Bowman's capsule, It is formed of the following two continuous layers of epithelium: an external or parietal layer of Bowman's capsule which is formed of simple squamous epithelium surrounded by reticular fibres and an internal or visceral layer which is adherent to the glomerular blood capillaries the visceral layer of the Bowman's capsule is formed of modified

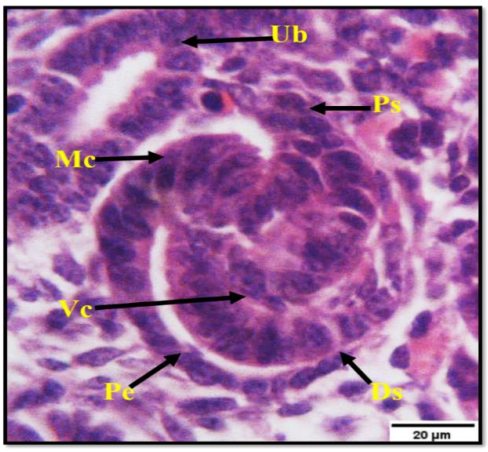
simple squamous cells known as podocytes or glomerular epithelial cells.these cells facilitate the passage of glomerular filtrate to the capsular space). It appears as being composed of two layers: a parietal layer consisting of squamous epithelium and a layer surrounding the glomerulus known as the visceral layer. While the space between the two layers is called Bowman's space Figure (3-5). In embryos 18 and 19 day of gestation, the juxtaglomerular apparatus, as well as the macula densa, were observed (Fig. 3-6). [12] indicated that the first three stages can be observed near the surface of the cortex, while the last two stages can be observed deep in the cortex area. Although the stages of Glomerulogenesis are different, they can be seen in all *Mus musculus* embryos from the age of 14 to 19 days of gestation. This was confirmed by [17], and it is also consistent with what was stated by [12]. That show the development of kidney in human fetus at 14 week gestation. The results of the statistical analysis showed that there were significant differences in the diameters of the stages of glomerulus development at the significance level of ($P \leq 0.05$). Table (3-1). Thus, the mean diameter of the glomerulus reached (45.4786 ±2.11797) and it was the largest in diameter, while the mean diameter of the Renal vesicle was (25.9578±2.50643). This was the lowest mean than the rest of the diameters of the other stages Figure (3-7).



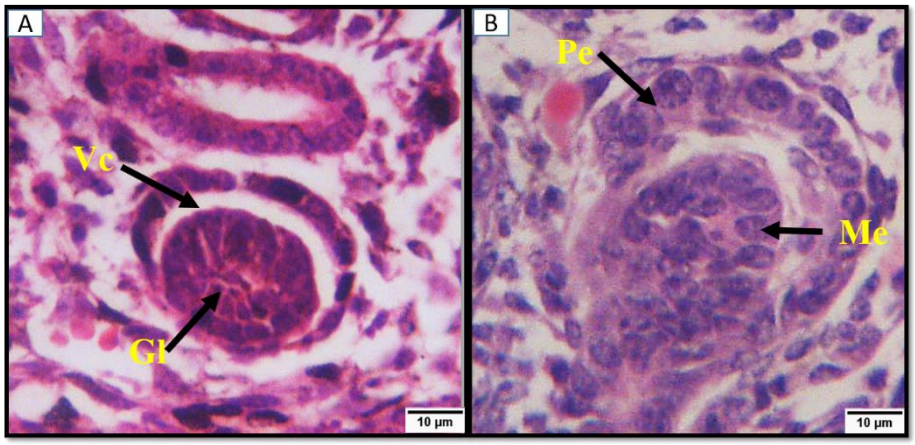
Figure(3-1):Sagittal section in the kidney of Mice embryo showing First stage of Glomerulogenesis Glomerulogenesis at 14 day gestation observe: (**Rv) Renal vesicle (Cap) Cap mesenchyme** , **(Ur) Ureteric bud** (H&E stain, 40x)



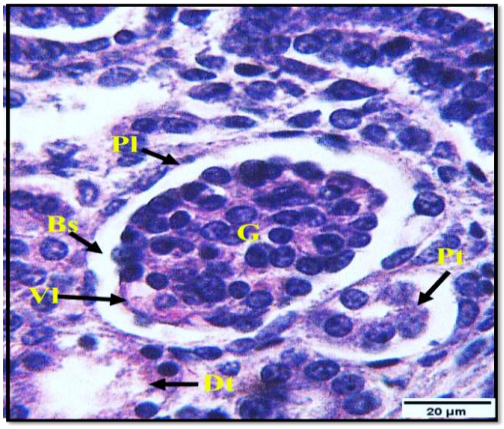
Figure(3-2):Sagittal section in the kidney(Comma shape stage) of Mice embryo at 16 day gestation observe: **(Ps) Proximal segment, (Vc)Vascular cleft, (Ds) Distal segment, (Md) Metanephric duct** (H&E stain, 40x)



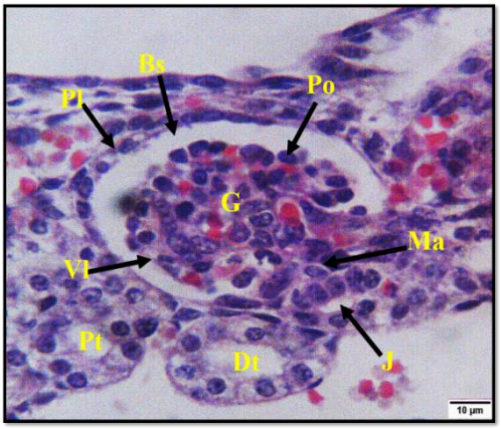
Figure(3-3):Sagittal section in the kidney(S- shape stage) of Mice embryo at 17 day gestation observe: **(Ub)Ureteric bud, (Ps)Proximal segment, (Mc) median curve, (Vc) Vascular cleft, (Pe) Parietal epithelial cell, (Ds) distal segment** (H&E stain, 40x).



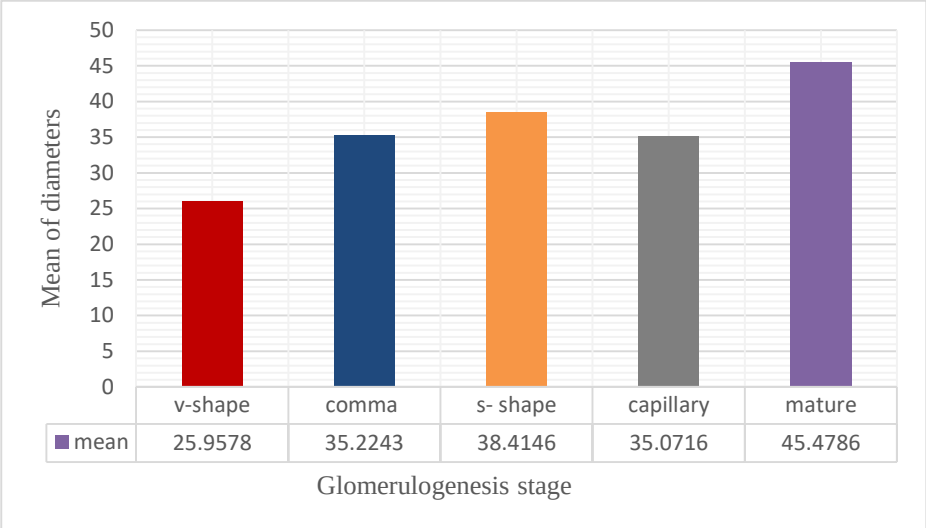
Figure(3-4):Sagittal section in the kidney (Capillary loop stage) of Mice embryo at 17 day gestation observe: A**(Vc) Vascular cleft , (Gl) Glomerular capillary loop;** B: **(Me) Mesangial cell, (Pe) Parietal epithelial cell,** (H&E stain, 40x).



Figure(3-5): Sagittal section in the kidney (Mature glomerulus) of Mice embryo at 19 day gestation observe: **(G) Glomerulus, (Pl) Parietal layer, (Bs) Bowman's spase, (Vl) Visceral layer, (Pt) proximal convoluted tubule , (Dt) Distal convoluted tubule**(H&E stain, 40x)



Figure(3-6):Sagittal section in the kidney of Mice embryo at 19 day gestation observe: **(G) Glomerulus, (Pl) Parietal layer, , (Bs) Bowman's spase, (Vl) Visceral layer, (Pt) proximal convoluted tubule , (Dt) Distal convoluted tubule, (Po) Podocyte, (Ma) Macula densa , (J) juxtaglomerular apparatus** (H&E stain, 40x)



Figure(3-7): statistical analysis to mean diameter for glomerular development stages in *M. musculus* embryo.

Table(3-1):The mean diameters the glomerular development stage in *Mus musculus* embryo

Diameter of Glomerulogenesis	
Stage	**(Mean±S.E)µm**
Renal vesicle (V-shape)	25.9578±2.50643
Comma shape	35.2243±1.05198
S- Shape stage	38.4146±1.1356
Capillary loop stage	35.0716±4.0729
Mature from	45.4786±2.11797

4. Conclusions

The glomerulogenesis considered one of the main developmental processes in the kidney of embryo ,that occur after formation of Metanephros, Mesenchymal cell is contribute in happening that ‹This study showing five stage for Development glomerulus represented by Renal vesicle, Comma shape stage, S- shape stage, Capillary loop stage, and finally the mature glomerulus, and make statistical analyze for diameters to every stage, This study explain the important role in glomerulogenesis during the life of the embryo.

Ethical Clearance

Ethics of scientific research were carried out in accordance with the international conditions followed in dealing with laboratory animals, and included animal health, husbandry and care for it, and providing appropriate conditions for it in terms of food, and appropriate methods were

adopted in dealing with it when experimenting, and this is consistent with the instructions of the Iraqi Ministry of Health and Environment.

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