Histological study of tubular system and collecting tubules inkidney of rats in prenatal stages (Rattus rattus norvegicus Albinus)

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Abstract

The aim of our study to provide a more complete quantitive description of the histomorphology of the tubular system and collecting tubules in kidney of rat during prenatal development. The developmental study of tubular system and collecting tubules has been done in the rat fetuses, which including detection the timing of appearance of metanephros collecting tubules. The study revealed that the differentiation and development of the collecting tubules in metanephros began in the rat at 14 day from pregnancy. Also the histological examination showed at the end of pregnancy the tubular system and collecting tubules visible in medulla and less degree in cortex of kidney with clear renal pyramids and well developed long sharp pointed papillae.

Key word: histology , tubules , rat , prenatal

دراسة نسيجية على الجهاز النبيبي والنبيبات الجامعة في كلية الجرذان في مرحلة قبل الولادة (Rattus rattus norvegicus Albinus)

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الخلاصة

اجريت الدراسة على مراحل التطور للنبيبات الجامعة والجهاز القنوى في الكلية البعدية, في الجرذان وقد تضمنت تحديد زمن ظهور الجهاز القنوي في الكلية البعدية ,اظهرت الدراسة ان نمو وتمايز القنوات الجامعة يتم في عمر 14 يوم في الكلية البعدية. كذلك الفحص النسيجي اظهر في نهاية الحمل الجهاز القنوي واضح في اللب قليل في القشرة مع وجود هرم كلوى وحليمة كلوية مدببة. الهدف من الدراسة لتزويد وصف نسيجي شكلي للجهاز النبيبي والجامع في كلية الجرذان في مرحلة التطور قبل الولادة.

مفتاح الكلمات: نسيجي , جرذان ,انابيب , , قبل الولادة .

Materials and Methods

The study was performed on forty five rats fetuses collected from uteri of the pregnant femals in estimated ages, five fetuses prepared for every stage beginning from (14,15, 16, 17, 19, 21) days by which gestation occurred. All fetuses ages were estimated according to the days assumed to have elapsed from copulation (11). The crown-rump length (CRL) will measure for corrections. CRL (is the measurement from the vertex of the skull to the midpoint between the apices of the buttocks for prenatal only. The CRLs at each stage are summarized in the table (1). The body weight was recorded for each prenatal fetuses by using sensitive balance. The body weight was recorded before the fetuses were sacrificed. The mean weight at each stage is summarized in the table (1).

Procedure of samples preparation as following: A-the samples were dissected and washed with normal saline solution (0.9%) NaCl and fixed immediately in 10% formalin at room temperature. B-Dehydration: this step was done to remove water from the histological specimens, by using a graded series of ethanol (50%, 70%, 80%, 90%, and 100%), two changes for each one, and 2 hours for each concentration. Clearing and embedded: the free water specimens were transferred from 100%alchohol to xylene. The penetration of xylene was indicated by the clearing effect which accompanies it. Then the specimen transferred to melted paraffin wax (M.P. 58-60 c), and put into the oven where it must remain until it has become completely infiltrated with paraffin. D-Cutting and staining: Sections measured 5-

7micrometer thickness were cut by using the rotary microtome and stained routinely with hematoxylin and eosin (10).

Introduction

The formation of epithelial tubules in the developing mammalian kidney involves both the aggregation of mesenchymal cells and their conversion into polarized epithelium, as well as the growth and branching morphogenesis of existing epithelium, (3).

In domestic animals, the S-shape tubules will later be the glomerular end of the tubule and the other end form distal convoluted, while middle part form proximal convoluted tubule and later, differentiation of the renal tubule progresses from proximal convoluted tubule to distal convoluted tubule lead to formation of Helene's loop between them (1).

In the other hand The ureteric bud, elaborating the network of the collecting duct system for urine transport and induction of a mesenchymal-to epithelial transition within the mesenchyme, establishing the renal vesicles, the precursors of the renal tubule (4).

(5) also reported the similar type of epithelium of distal convoluted tubules in the embryos of rabbit and goat, respectively. Some of the tubules had more than one layers of epithelium at 21.5 cm CVRL, which indicated the process of canalization at this stage that completed afterwards.

A characteristic of vertebrate kidney organogenesis is the development of a pronephric duct in association with each pronephros, These ducts are the first or primary ureters of vertebrate kidney systems. They form the collecting duct system of the meso and metanephroi, and their derivates, are the key players in the induction of the nephrogenic mesenchyme, which forms these latter kidney generations. (6).

(7) also said from the caudal end of the mesonephric duct develop the ureter, renal pelvis and collecting duct, while from the nephrogenic cord stems the tubular system. The union between collecting duct and one end of the tubule, and the formation of a glomerulus

within a blind indentation of the other end of the tubule, result in the nephron of the metanephric or permanent kidney. (8).

In human the initial branching is bifid or dipodial. The subsequent branch is a single or monopodial which is derived from the cortical side of the initial branches. This branch then repeats bifid branching. Thus there is an alternation of dipodial and monopodial branching which is necessary because one of the two bifid branches will always fuse with a nephron and be removed from subsequent branching morphogenesis this reiterative pattern occurs 15 times in the human kidney, producing many branches with nephrons. (9).

Result

At 14 days gestation period Bud surrounded by a condense cells which aggraded around the bud as a cup like (fig.1), these cells differentiated later as nephron progenitors of the metanephric blastema, increased the size of the ureteric bud compartment during development in vitro, The metanephric blastema differentiates into all tubular structures of the adult nephron with the exception of the collecting system, which derives from ureteric bud, This earliest mesenchyme- derived epithelium (metanephric blastema) follows a structurally well-defined morphogenetic pathway to generate most of the nephron and tubules, Also in this stage, there is no renal vesicle, S-shaped structures, elongate and comma shapes bodies and we notice all these formers at future stages of fetal life rat embryo.

At 15 days gestation period more cranially, uretric bud rebranching to form more tip of ampulla which also surrounded by metanephric mesenchyme, the cross section of embryo shows the double metanephric kidney development left and right, with mesonephric degenerated (fig.2).

At 16 days gestation period The development and differentiation of tubular system and collecting tubules occur when the enlargement of the ureteric anlage, the primitive renal pelvis grows in dorsal and cephalic direction and elongates in an antero-posterior direction and show central ureteric stalls and peripheral branching ampulla surrounded by condensing mesenchyme and was composed of undifferentiating cells (fig. 3).

At 17 days gestation period The limb near the collecting tubule elongated also to form the distal convoluted tubule and loop of Henley later, while middle segment developed later to form proximal convoluted tubule, from this farther development another stage of glomerular stand out, the double coma – shape represented.

The primitive renal pelvis underwent division into anterior and posterior portions which represent the two major calyces of metanephric kidney in this stage giving rise to two or three primary tubule that are the future subdivide and form secondary tubules and each secondary tubules sequentially continue to subdivide until develop large number of tubules and collecting duct (Fig. 4).

At 19 days gestation period The current study appeared that the larger tubules situated in developing medulla of metanephros and those nearer of the renal pelvis, the epithelium is columner in epithelium type (fig. 5),while those nearer the periphery, the tubules lined by simple cuboidal epithelium. Also there is no demarcation line between cortex and medulla .

At 21 days gestation period The current study showed considerable increasing in length and thickness of proximal tubules led to an enlargement of proximal convoluted tubule to generate of proximal straight tubule continuously in differentiation and development during the fetal life (fig.6).

When the secondary tubules enlarge absorbs the third and fourth generation tubules and develops into the minor calyces which give arise to papillary duct. The papillary ducts divides into numerous division giving anlagen to collecting tubules which drainage to minor calyces at papillary pores hereafter, the collecting tubules that converge on the papillary duct forming the renal pyramid and because the kidney of rat consist of a single pyramidal structure, they are referred to as unilobar kidneys (Fig.7).

The collecting tubules nearest of cortex lined by simple cuboidal epithelium tissue and change gradually until become tall columnar epithelium tissue in medulla and adjacent area of renal pelvis hereafter when papillary duct open at the tip of a renal papillae become lining by pseudo stratified epithelium tissue(fig.8).

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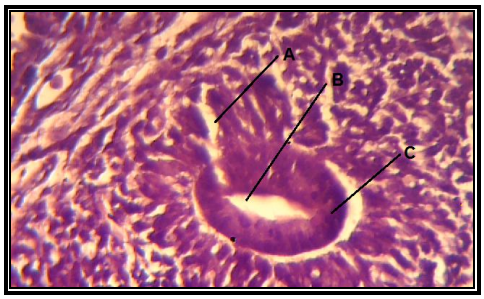

Figure (1) Parasagittal-section at 14th day of gestation in rat showing the uretric bud stalk and the cells composing the tip (ampulla)

A-stalk

B- uretric bud

C- ampulla **(H&E.X40)**

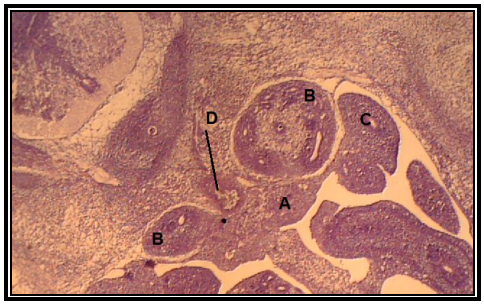

Figure (2) Cross-section at 16th day of gestation in rat showing continues degeneration of Mesonephros

A- Degeneration of Mesonephros tubules

B- Metanephros

C- Gonadal ridge

D- dorsal aorta **(H&E.X40)**

Figure (3) Parasagittal-section showing at 16th day of gestation in rat Metanephros and diferentition of Collecting tubule. A-Nephron primordium

B-Collecting tubule primordium C-Renal pelvis

D-Nephrogenic zone

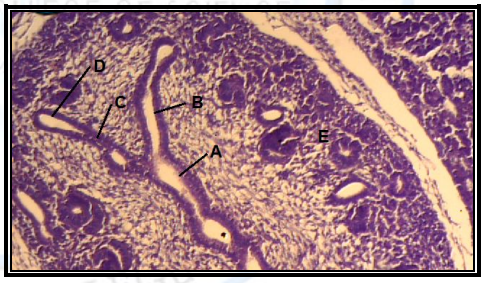

Figure (4) Parasagittal-section at 17th day of gestation in rat showing Metanephros & differentiation of collecting tubule.

A-Major calyx

B-Minor calyx

C- Primordium of papillary duct

(H&E.X10)

D-Collecting tubule primordium

E-Nephron primordium

F-Nephrogenic zone **(H&E.X10)**

Figure (5) Cross-section in metanephric kidney at 19th day of gestation in rat showing large collecting duct

A- pelvis

B-Bowman's space

C-collecting duct

D- embryonic connective tissue **(H&E.X40)**

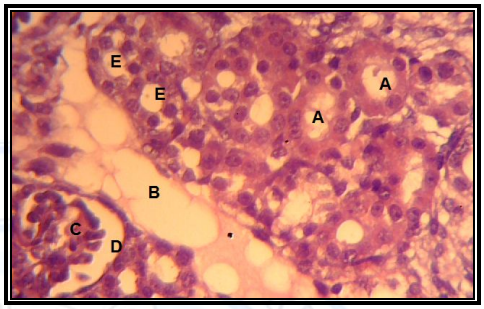

Figure (6) Parasagittal-section at 21th day of gestation in rat showing tubules develop

A-proximal convoluted tubule

B-blood vessels

C-glomerular

D-bwoman space

E-collecting tubule **(H&E.X40)**

Figure (7) Parasagittal-section at 21 day of gestation in rat showing

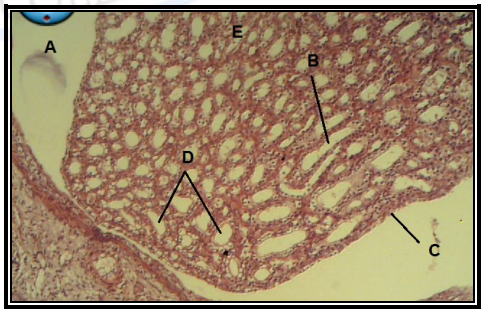

Figure (8) Parasagittal-section at 3ed day of gestation in rat showing Collecting

Collecting tubule and duct.

A- Renal pelvis

B-collecting duct

C- Renal papilla

D- Papillary duct

E- Medulla **(H&E.X10)**

tubule and duct.

A- Renal pelvis

B-collecting duct

C- Renal papilla

D- Papillary duct

E- Medulla **(H&E.X10)**

Table (1) show the body weight (BW) and crown rump length (CRL)

Age	B.W Mg	CRL mm
14 days	0.124 ± 0.0043	3.7 ± 0.3354
15 days	0.282 ± 0.0082	9.8 ± 0.2236
16 days	0.452 ± 0.0065	17.4 ± 0.570
17 days	1.18 ± 0.0086	25.2 ± 0.7416
19 days	2.6 ± 0.0035	32.6 ± 1.3964
21 days	4.41 ± 0.075	42.2 ± 1.5165

Discussion

The observation of present study in 14 days gestation period the same with (13), (14) which said The formation of the metanephric kidney begins with the protrusion of the ureteric bud from the caudal region of the nephric duct and its outgrowth into a loose group of nearby cells termed the metanephric mesenchyme.

The result of 16 days age not in agreement with the results found previously by (15) who they observed that the kidney of spinay mouse has taken on a more definitive shape,

while the first branch of the ureteric bud is still clearly visible, multiple branching events have taken place, while (16) said that first differentiation and formation of the tubular system and developmental collective tubules at 12 days of mouse embryo.

In 17 days in this study the result not in agreement with the results found previously by (15) whom they observed that the kidney of spinay mouse has taken on a more definitive shape, while the first branch of the ureteric bud is still clearly visible, multiple branching events have taken place, while (16) noticed that first differentiation and formation of the tubular system and developmental collective tubules at 12 days of mouse embryo, in other hand, our result in variance with (17) who mentioned that the first appearance of collecting tubules at sixth week of pregnancy in human.

The collecting tubules nearest of cortex lined by simple cuboidal epithelium tissue and change gradually until become tall columnar epithelium tissue in medulla and adjacent area of renal pelvis hereafter when papillary duct open at the tip of a renal papillae become lining by pseudo stratified epithelium tissue. This agreement with results of (18) in domestic animals who mentioned that the epithelial cells of the collecting tubules are pale and vary from cuboidal near the distal tubules to columnar close to the papilla. Cell boundaries are normally clearly defined compared with the cells of the proximal and distal convoluted tubules. As they progress toward the renal papilla, the collecting tubules become wider. The terminal portion of these tubules is lined by a columnar or pseudostratified epithelium and is called the papillary duct.

Conclusion: As development proceeds, the first anlage of development of tubular system and collecting tubules occur at 14th day of gestation period as uretric bud , and continouse divided and develop until birth .

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