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RESEARCH ARTICLE

HISTO-MORPHOMETRICAL STUDY ON KIDNEY GLOMERULI AND CORTICAL TUBULE IN LATE PREGNANCY.

Asawer K.M. Al- fatlawee[1], May F.M. Al-Habib[2] and Mohammad Oda Selman[1].
1. High institution for infertility diagnosis and ART, Al Nahrin University.
2. Collage of Medicine, Al Nahrin University.

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Key words:-
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Abstract

Background: Maternal accommodation to normal pregnancy begins shortly after conception,during pregnancy the anatomical and histological changes occur in the kidneyas a maternal adaptation for physiological changes.
Aim: Evaluation morphological changes in the kidney glomeruli and tubules during pregnancy, Histomorphometrical assessment of glomeruli and tubular changes with pregnancy.
Material and Method: This study carried on 40 mature mice divided into two groups, 20 pregnant mice served as experimental group and 20 non-pregnant mice as a control group, kidneys were removed; the weight is measured and then fixed, dehydrated in ascending grades of alcohol, cleared in xylene and infiltrated with paraffin. The paraffin blocks were made and 5µm thin sections were obtained using a rotary microtome. The sections were stained with H&E stain, PAS examined under light microscope.
Result: Under the light microscope, the kidney in experimental group show the many anatomical changes as increase in Wight, elongated and increase in width, and showed many histological changes as a glomerular enlargement with decrease of urinary space and dilation in proximal and distal tubule. A statistically significant differences have been shown in the diameter of renal corpuscle, glomerular tuft and Bowman's space, and also in the renal tubules proximal and distal convoluted tubules ($p<0.001$). And in Wight, elongation and width as ($p<0.001$).
Conclusion: The results of the present study indicated during pregnancy kidney undergo to anatomic change as increase in size and Wight, and histologic change as elongation in glomeruli, and dilation in diameter of tubule.

Introduction:-

Pregnancy causes definite and marked changes in urinary tract. The most important changes as dilation of the ureter and kidney pelvis, this phenomena is common and might well be said normal concomitant of pregnant state[(1).] All mammalian kidney show a morphohistological feature which describe as a cortex enclosing a pyramid-shaped medulla, the tip of which protrudes into the renal pelvis, the medulla is divided into an outer and an inner medulla;

Corresponding Author:- Asawer K.M. Al- fatlawee.
Address:- High institution for infertility diagnosis and ART, Al Nahrin University.

the outer medulla is further subdivided into an outer and an inner stripe [2, 3].Each kidneys have about one million of tiny filters called nephrons. The components of the nephron include the renal corpuscle (glomerulus and Bowman's capsule), the proximal and distal tubules, and collecting tubule. [4]

The glomerulus is a spherical structure have a capillary network lined by a thin layer of endothelial cells, in the central region mesangial cells, and the visceral region of epithelial cells podocytes and the parietal epithelial cells of Bowman's capsule and associates basement membrane. This two epithelial layers are separated by a narrow cavity identified as Bowman's space or urinary space. [5,6]Proximal and Distal tubule there is a part of nephron, proximal tubuleis the portion of tubule start at bowman corpuscle which is highly convoluted and located in cortex, then descending in medulla to become continuous with anther tubule.[7] Distal tubule is a less convoluted and shorter tubules than proximal and have smaller diameter.[8]morphometry is Stereological method have been used to study physiological function of organ or tissue from the morphological point of view, the word of morphometry include quantification of three dimensional properties of object from two dimensional plans.

Material and Method:-

The study is performed on the Albino Mice (*Mus Musculus*). Healthy males and females were obtained from the animal housed of High Institute of Infertility Diagnosis and Assisted Reproduction Technologies /Al-Nahrain University. 40 healthy mature female mice were divided into two groups equally, GroupI non-pregnant served as control group, and Group II pregnant mice in between (15-21) used as experimental group the kidneys of control and experimental group were removed and washed by normal saline then the weight of the kidneys measured, then fixed in formaldehyde solution for (24-48) hours, dehydrated in ascending grades of ethanol alcohol (70%, 90%, 100%), cleared in xylene and infiltrated with filtered paraffin. The paraffin blocks were made and 5µm thin sections were obtained using a rotary microtome. The sections were stained with (H&E) and (PAS) stain; then sections were examined under light microscope at magnification 10X, 20X, 40X, 100X.

Morphometric parameter:-

The image analysis program is used to measure the diameter of renal corpuscles, glomerular tuft and Bowman's space in corticomedullary area at magnification 40X and also measure the diameter of proximal and distal convoluted tubules at magnification 100X.

Result:-

Observation of anatomical criteria of kidney in both groups yielded the facts that both kidneys we found to be situated on the posterior abdominal wall on each sides,in control group the measurement of kidneys dimensions were as follows: Length 10mm, width 5mm, thickness 2.5mm. This was done by using vernia.While in pregnant group the dimensions showed slightly increase especially in regards to the length where 1-2mm increase was recorded.In regards to kidney weight both kidney were weighted and the mean weight of kidney in control group was 0.19±0.003gram, while pregnant group kidneys showed marked increase in the weight 0.28± 0.006 gram.This increase is statically significant increase was recorded as shown in table (1).

Table 1:- Showing the statically significant increase in length, width and weight of kidney during pregnancy.

Parameter	Control Mean ±SE	Pregnant Mean ±SE	P-Value
Kidney length(um)	778±3.1	888±7.6	*P≤0.05
Kidney width (um)	417±3.1	443±4.4	*P≤0.05
Kidney weight (gm)	0.19±0.003	0.28±0.006	*P≤0.05

The histological aspect the glomeruli:-

The glomeruli is tuft of blood vessels located inside the renal corpuscle Examination of the corpuscle under light microscope reviled the following:Group A: showed normal arrangement and Size of renal corpuscle and glomeruli with well identified wide space. That continuous to form proximal convoluted tubule.

Group B: pregnant group showed number of changes both histologically and morphometecally:The glomeruli in pregnant group were enlarged in size, this enlargement was assessed morphometically using ImageJ software. The glomerular area was calcuted and statistically analysis.

Control group showed a mean glomeruli area of 658±19 um2. While in pregnant group the mean area of glomeruli was 710±13um2. A significant P value was estimated in between these two group in regards to glomerular area. Figure (2) & Table (2) Bowman's Space (urinary space) showed a marked decrease in pregnant group, Figure (1). Morphometric analysis of this result showed that urinary space area changed from (156±1 um2) in control group to (72±1.5um2) in pregnant group, table (2).

Table (2): showing the statically significant increase in glomerular area and urinary space.

parameter	Control Mean ±SE	Pregnant Mean ±SE	P- Value
Glomerular area(um^2)	658±19	710±13	*P<0.05
Urinary space (um^2)	156±1	72±1	*P<0.05

Histological observation of proximal convoluted tubule (PCT):-

As a part of nephron, proximal tubule poses two different portion as a convoluted part and straight part.Examination of PCT in pregnant group reviewed the following criteria (Figure 3, 4, 5, 6): The size of the tubule where more than seen in group A, The cells forming PCT where long cuboidal, eosinophilic, with large prominent nucleus and densely stained cytoplasm, Brush border of PCT cells was dens, thick, occupies the lumen totally and was densly stained by both H&E and PAS stain, A stricking Feature of vaculation was noticed in these cell apical surface.Pregnant group showed a high mean area for PCT (545±14um2) while the control group PCT area was (424±24um2). A statistical analysis of these result showed a significant P value of (*P<0.05) between these two group.

Histological observation of distal convoluted tubule (DCT):-

Distal convoluted tubule were found to be located in between glomeruli and proximal convoluted tubule. They were Small, Wide, Pale type of Tubule with wide clear lumen. The cells form in this type of tubule were low type of cuboidal cell, with fain eosinophilic cytoplasm and small round centrally located nuclei. The Number of DCT in compered to PCT were less in the cortex. Compression in regards to morphological point of view between pregnant and non-pregnant group showed dilation in DCT in pregnant group seen in H&E (Fig 7)& (Fig 8). A morphometrical measurement for the size of DCT was performed using Image J software. A total tubular area was chosen as a criteria to detect the include in size of DCT recorded by visual examination. Pregnant group showed a high mean area for DCT (369±9um^2) while the control group DCT area was (263±4.5um^2) .A statistical analysis of these result showed a significant P value of (*P<0.05) between these two group.

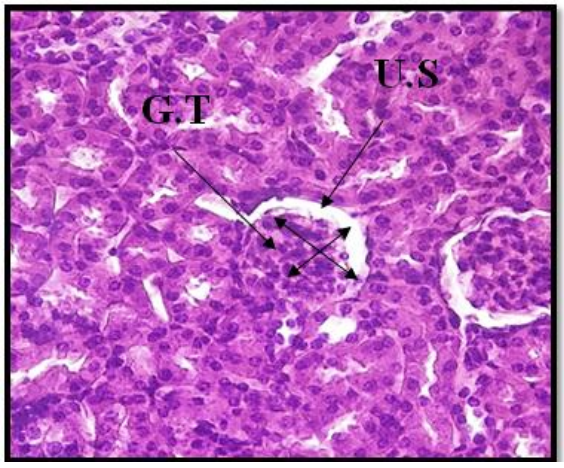

Figure 1:- shows the glomeruli tuft (G.T) and glomeruli with urinary space (U.S) in control group. (H&E, control group, 400 X).

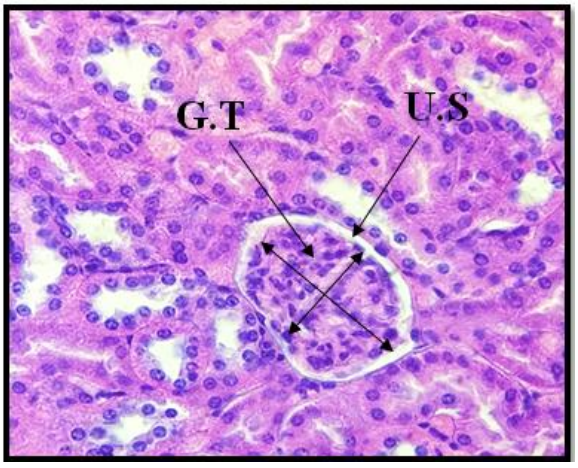

Figure 2:- shows the glomeruli tuft (G.T) and narrow urinary space (U.S) in pregnant group. (H&E, pregnant group, 400X).

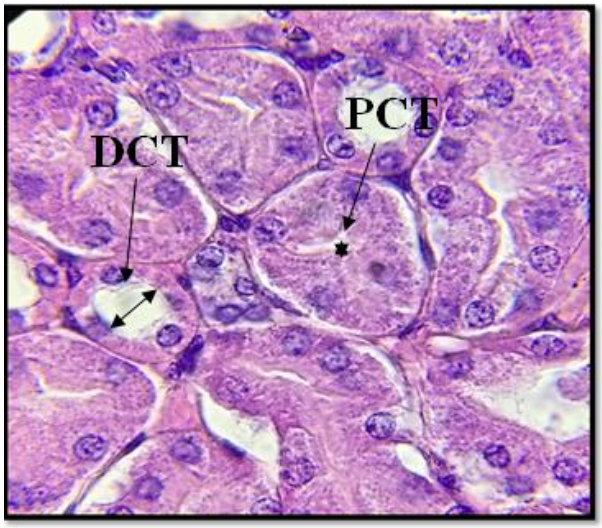

Figure 3:- Showing the PCT diameter in control group, H&E, 1000X.

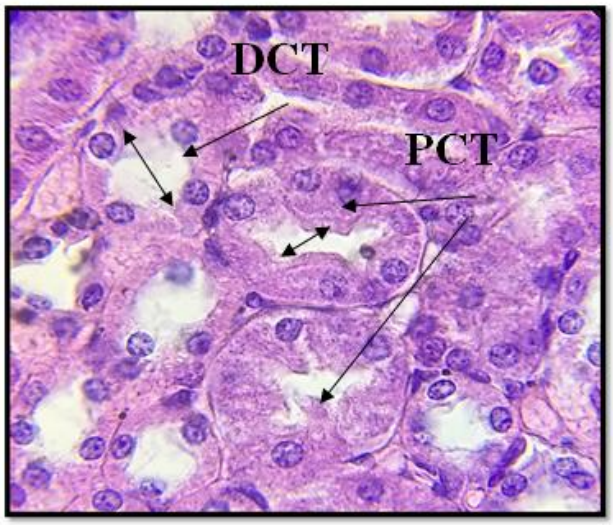

Figure 4:- Showing the PCT diameter in pregnant group, (pregnant group, H&E, 1000X)

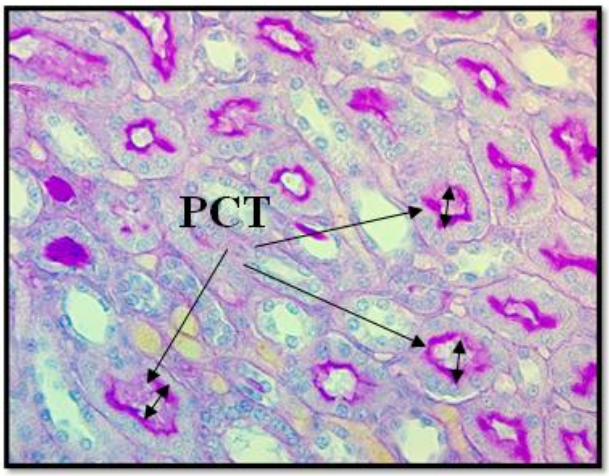

Figure 5:- Showing the PCT in pregnant group and a thin basement membrane (PAS stain, pregnant group 400X.

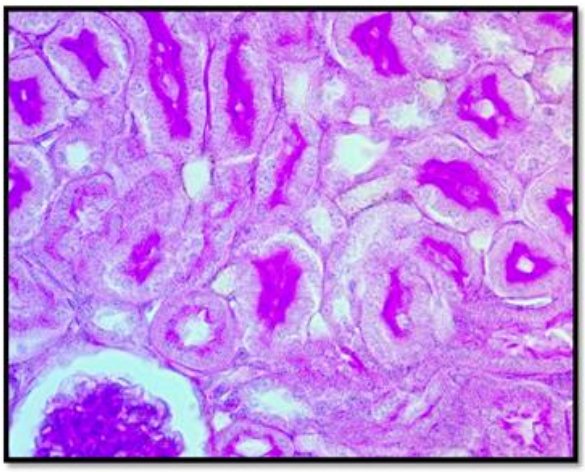

Figure 6:- Showing proximal convoluted tubule PCT with it brush border, thicking in basement membrane (B.M) in control group (PAS stain, control group 400X).

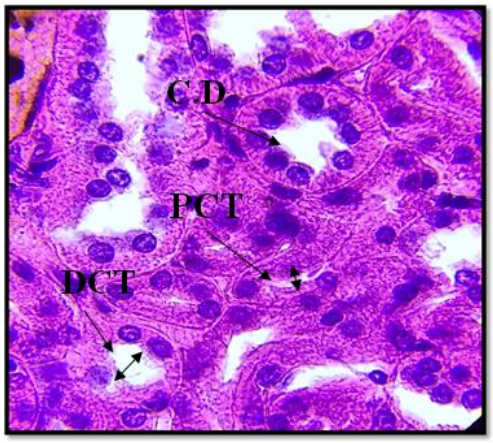

Figure 7:- showing DCT with clear lumen, no brush border and thicken in basement membrane, collecting duct (C.D) & PCT. (H&E, control

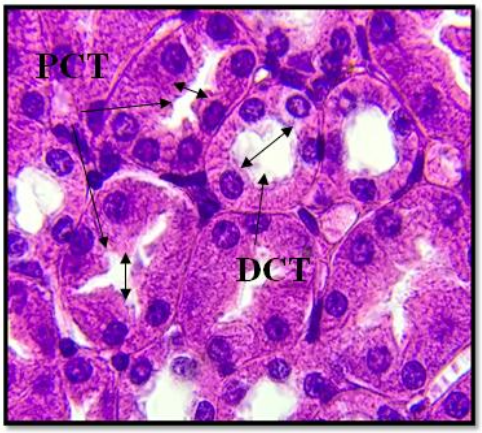

Figure 8:- showing DCT with clear lumen, no brush border, enlarged diameter and less thicken in basement membrane& PCT. (H&E, pregnant group, 1000X).

Discussion:-

It was reported that the volume of right kidney in normal individuals was significantly smaller than that of the left because the spleen is smaller than the liver so the left kidney has more space for growth. [9]

The volume of the kidney is greatly affected by the method of fixation, we used the immersion method in this study but actually the perfusion method was more reliable for calculating the absolute weight and volume of the kidney before processing. [10]

From an anatomical view we demonstrated an increase in the weight and size of the kidneys in pregnant group due to increase in two dimensions of three (length & width). These results agree with some previous studies which reported normal increase in size of the kidney up to 30% with 1–1.5cm increase in length in pregnant females mainly due to changes in the vascular and interstitial spaces or hydronephrosis.[11, 12]

There were another possible reasons explain the increase in the renal size during pregnancy for example the renal plasma flow might be increased by 25% and the glomerular filtration rate increases up to 50% above normal. The increase of renal function had been attributed to the growth, hormone-like metabolic effects of placental lactogen and to increased free cortisol. Hormone secretion by the fetus might be responsible for the hypertrophy of the maternal organs. From the available evidence the kidneys might take several months to return to normal form of the non-pregnant state. On other hand the change in the renal dimensions might be an indicator of presence or progression of disease. [13, 14].

Glomerular size selectivity appeared to be altered in pregnant women and oncotic pressure was decreased because of expansion of the plasma volume, thus contributing to a rise in GFR. There may also be modest changes to glomerular ultrafiltration coefficient due to changes in the surface area for filtration and the hydraulic permeability. [13] Even severely hydronephrotic kidney had the normal number of glomeruli, this agrees with our results where it often realized that the glomeruli may be surprisingly well preserved in cases of marked tubular and interstitial changes. [15]

In normal adult non pregnant mice the means diameters of the lumen of the proximal convoluted tubules, the distal convoluted tubules, thick, and thin loop of Henle were 11.5±4.183µm, 16.5±2.850µm, 12.6±1.596µm, 18.6±1.294µm respectively. These results disagree with ours because we demonstrated larger cross sectional area in proximal convoluted tubule than distal convoluted tubule in control and pregnant groups. [16]

This study agree with Bentley et al, who reported that the proximal tubules had large diameters and opaque walls, whereas distal tubules, connecting tubules, and collecting ducts had smaller diameters and less opaque walls. [17, 18]

This study reported an increase in the surface area (diameter) of proximal and distal convoluted tubule in pregnant group with respect to control group. The previous studies reported 20% increase in length of the proximal tubule within 5-6 days after mating without further significant changes to term. The increase in length would represent an increased area for reabsorption. While the proximal tubular diameter remains constant during pregnancy. The proximal tubular elongation was not detected at 2-3 days of pregnancy but it occurs later when both the absolute and fractional amounts of salt and water reabsorbed were significantly greater than control values during the high rate of infusion. [19, 20]

The pregnancy caused slight changes in shape of the proximal convoluted tubules and over 10% height reduction of the cuboidal epithelium lining the proximal convoluted tubules were observed in the kidney. The changes in the convoluted tubules proceed in a heterogeneous way and lead to a mixture of atrophic and hypertrophic units. The dilatation of the tubules due to accumulation of fluid tends to shortening of the tubules. The increase in the tubular diameter will reduce the number of the tubules profiles counted per section of tissue. [20, 21]

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