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# Morphometric and Immunohistochemical Study of CK18 in the Mice Kidney at Late Pregnancy

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**Abstract**
Many changes occur during pregnancyin the kidney because the kidney considered a central player in the evolving hormonal milieu of pregnancy.The immunohistochemical reaction showed a CK18 antibody in cortical region that was highly significantin non-pregnant group compared to pregnant group, whiletheimmunohistochemical reaction showed that CK18 antibody in medullary region that was highly significant in non-pregnant group compared to pregnant group.Thedeviation of the CK18 immunohistochemical expression in renal tissues during pregnancy may be related functional phases of the kidney occurring during pregnancy,and the variability of the Ck18 expression in renal tissues of pregnant mice revealed mainly diminished CK18 expression in the tubular epithelia (both convoluted tubules and thin loop of Henle). This conclusion suggesting that the altered tubular function is one of traits of renal physiologic changes characteristic for normal pregnancy.
**Keywords**: Cytokeratins, CK18 in mice kidney, immunohistochemistry, pregnant kidney.

## Introduction

Pregnancy involves remarkable orchestration of physiologic changes. The kidneys are central players in the evolving hormonal milieu of pregnancy, responding and contributing to the changes in the environment for the pregnant woman and fetus. The functional impact of pregnancy on kidney physiology is widespread, involving practically all aspects of kidney function. The glomerular filtration rate increases 50% with subsequent decrease in serum creatine, urea, and uric acid values. Blood pressure drops approximately 10 mmHg by the second trimester despite a gain in intravascular volume of 30% to 50% (Lopes van Balen*et al*., 2019).Cytokeratins are intermediate filamentsplaying a crucial role in the integrity and mechanical stability of single epithelial cells and epithelial tissues, these filaments are organized into a complex supra-molecular network that extends from surface of the nucleus to the peripheral most portion of the cell. The genesis and maintenance of such a network involves numerous accessory proteins(Yoon*et al*., 2019).Generally there are 4 major renal keratins (CK7, CK8, CK18, and CK19) many studies had established the presence of these 4 major isoforms in the human kidney. Healthy mouse glomerular parietal epithelial cells stained positive for CK8 and CK18 only, while all 4 keratins were abundant in cortical and medullary epithelial cells of collecting duct (Toivola*et al.*, 2015).The keratins CK18 are co- expressed and constitutethe primary keratin pair of simple epithelial cells, including various parenchymatous epithelia,they arethe first keratins to appear in embryogenesis, as early as in pre-implantation embryos, and also seem to be the oldest keratins during progress process. In some epithelial cell types, CK18 are the sole keratins present. Ultrastructurall, keratin filaments are loosely distributed within the cytoplasm and show little bundling. CK18 are widely distributed among normal epithelial tissues although they are absent in differentiating keratinocytes. In regard to malignant tumors and CK18 are expressed in most carcinomas except for some differentiated squamous cell carcinomas. Therefore, CK18 antibodies strongly stain most adenocarcinomas and hepatocellular carcinomas. Another clinical application of CK18 is the detection of these

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fragments in the serum of cancer patients. These are used to monitor tumor load and disease progression (Menz*et al*., 2020) (Djudjaj*et al.*, 2016).

**Materialsandmethods**

The present study was performed on 50 adult female albino mice (*Musmusculus*). The animals aged 10-12 weeks; the animals weredivided in to 2 groups each with 25 animals: Group A (pregnant mice), Group B (non-pregnant mice).The animals were scarified by injection of chemical anesthetics (pentobarbital) intra-peritoneal (Laferriereand Pang, 2020). Then after, the two kidneys of each mice were extractedin the lab ofanimal house in biotechnology research center of Al-Nahren University.The kidney tissuewashistological prepared for paraffin sections accordingto (Bancroft *et al*., 2018). Immunohistochemical study was performed using basic fibroblast growth factor (ab668) abcamand procedure was followed according to manufacture procedure. Light microscope in Histology Department, Medical College, University of Al-Nahrain with its camera were used to capture the fields and by usingAperio Image Scope V12 count algorithms program to quantify the amount of a specific color in a tissue section.

**Result**

The renal cortex of the kidney of non-pregnant mice showed positive anti-Cytokeratins 18 immunohistochemical reactivity in both the proximal & distal convoluted tubules, and also in the outer parietal layer of urine space. Both the visceral layer of Bowman capsule and the mesangial cells showed negative immunohistochemical reactivity (Figure 1-a). Cells of the macula densa showed positive immunohistochemical reactivity (Figure 1-b).

- The outer band of the outer medulla of the kidney obtained from non-pregnant mice showed positive anti-cytokeratin reactivity in straight proximal and distal tubules and also in the collecting ducts, this reactivity showed patchy pattern of distribution (Figure1-c).
- The inner band of the outer medulla of the kidney obtained from non-pregnant mice showed positive anti-cytokeratin reactivity in straight distal tubules and also in the collecting ducts and thin loop of Henle (Figure 1-d).
- The inner medulla of the kidney obtained from non-pregnant mice showed positive anti-cytokeratin reactivity in the collecting tubules and thin loop of Henle (Figure 1-e).

The renal cortex of the kidney of pregnant mice showed faint anti-Cytokeratins 18 immunohistochemical reactivity in all parts of the renal cortex if matched to the reactivity in the cortex of the kidney obtained from the non-pregnant mice. Many of the proximal & distal convoluted tubules showed negative anti-Cytokeratins 18 immunohistochemical reactivity, other tubular cells showed irresolute positive reactivity. The mesangial cells also showed negative immunohistochemical reactivity. The cells of macula densa showed negative immunohistochemical reactivity (Figure 2-a).

- The outer band of the outer medulla of the kidney obtained from pregnant mice showed positive anti-cytokeratin reactivity which was of similar pattern to that seen in sections of kidney obtained from the non-pregnant mice (Figure 2-b).
- The inner band of the outer medulla of the kidney obtained from pregnant mice showed positive anti-cytokeratin reactivity in straight distal tubules and also in the collecting ducts, however the thin loop of Henle showed negative immunohistochemical reactivity (Figure 2-c).
- The inner medulla of the kidney obtained from pregnant mice showed positive anti-cytokeratin reactivity in the collecting tubules, but thin loop of Henle showed negative immunohistochemical reactivity (Figure 2-d).

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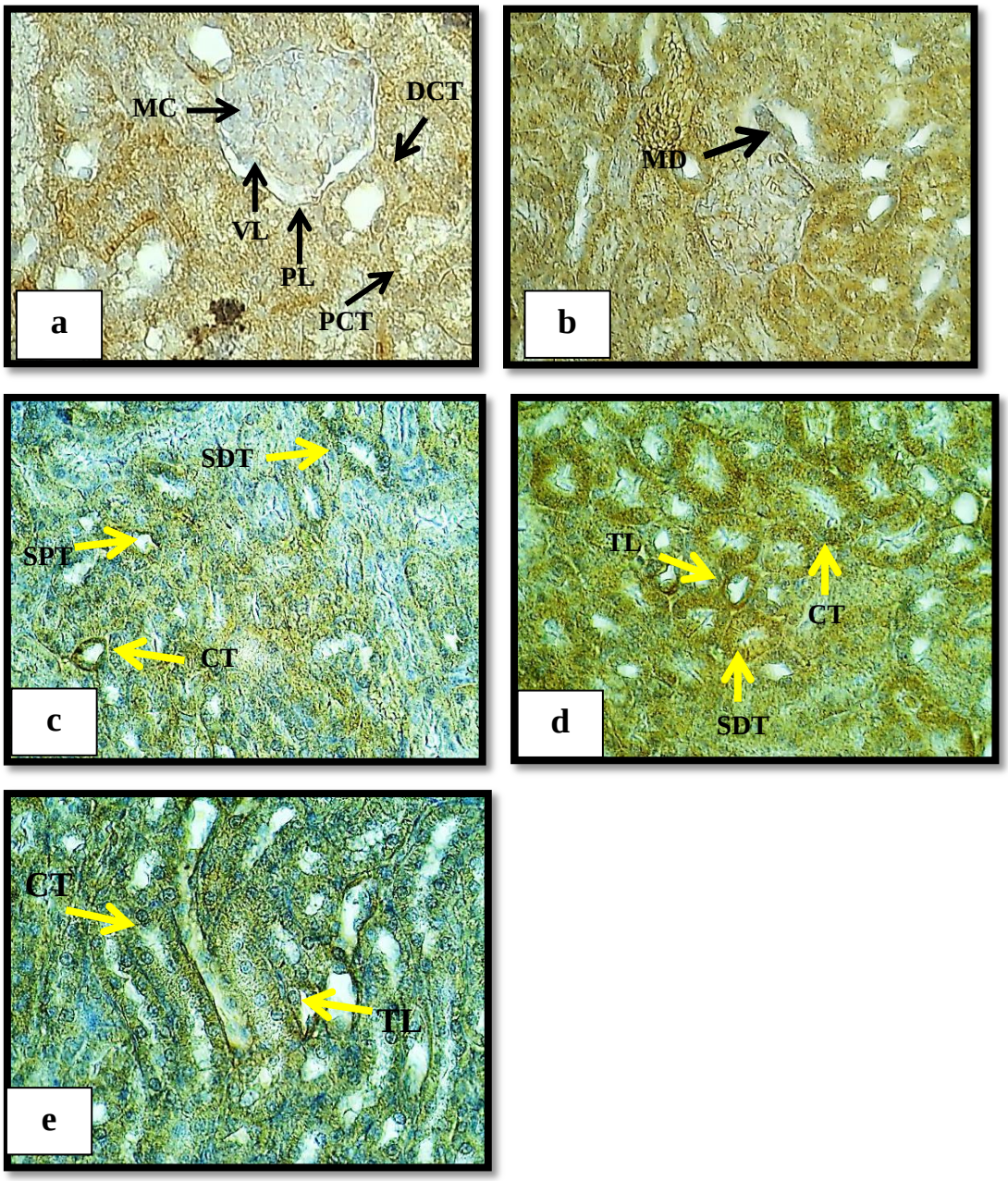


**Figure (1): Sagittal section of the kidney, a: showed cortical region with the anti-Cytokeratins 18 immunohistochemical reactivity (non-pregnant group, 40X), PCT: proximal convoluted tubule, DCT: distal convoluted tubule, MC: mesangial cell, VL: visceral layer, PL: paritale layer, MD: macula densa, b: showed medullary region outer band with the anti-Cytokeratins 18 immunohistochemical reactivity (non-pregnant group, 40X), SPT: straight proximal tubule, SDT: straight distal tubule, CT: collecting tubule, c: showed inner band of the outer medulla region with the anti-Cytokeratins 18 immunohistochemical reactivity (non-pregnant group, 40X),SDT: straight distal tubule, CT: collecting tubule, TL: thin loop of henle, d: showed inner medulla region with the anti-Cytokeratins 18 immunohistochemical reactivity (non-pregnant group, 40X), CT: collecting tubule, TL: thin tubule of henle.**

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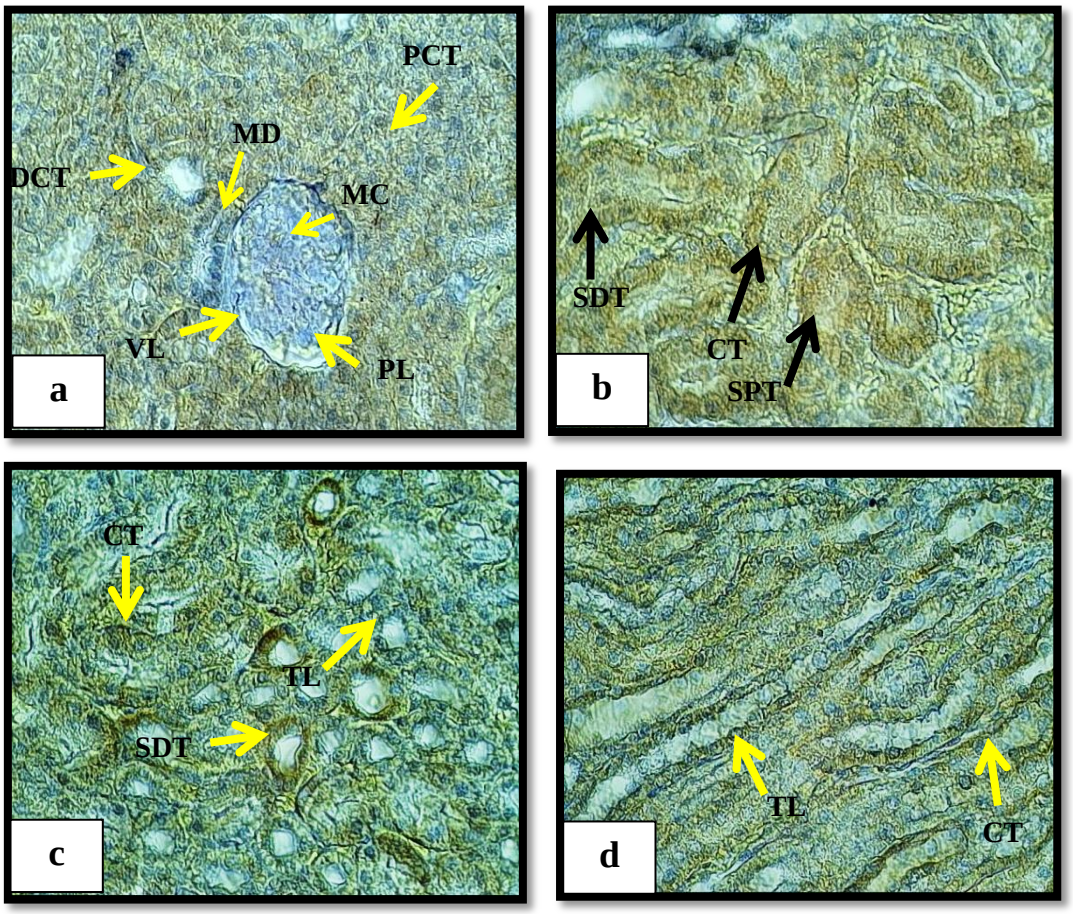


**Figure (2): Sagittal section of the kidney, a: showed the cortical region with the anti-Cytokeratins 18 immunohistochemical reactivity (pregnant group, 40X), PCT: proximal convoluted tubule, DCT: distal convoluted tubule, MD: macula densa, MC: mesangial cell, VL: visceral layer, PL: paritale layer, b: showed the outer band of the outer medulla with the anti-Cytokeratins 18 immunohistochemical reactivity (pregnant group, 100X), SPT: straight proximal tubule, SDT: straight distal tubule, CT: collecting tubule, c: showed the inner band of the outer medulla with the anti-Cytokeratins 18 immunohistochemical reactivity (pregnant group, 40X),SDT: straight distal tubule, CT: collecting tubule, TL: thin loop of henle, d: showed the inner medulla region with the anti-Cytokeratins 18 immunohistochemical reactivity (pregnant group, 40X), CT: collecting tubule, TL: thin loop of henle.The analysis of mean value of immunohistochemical reaction intensity for CK18 antibody in kidney tissue showed the mean value of the cortical region in non-pregnant mice kidney (0.77±0.01) pixel/micron$^2$ was highly significant difference in the cortical region of the renal tissues obtained from pregnant mice (0.18±0.01) pixel/micron$^2$. Also the mean value of IHC reaction in the medullary region obtained from non-pregnant mice kidney (0.71±0.01) pixel/micron$^2$ was highly significant difference in the medullary region of the renal tissues obtained from pregnant mice (0.27±0.01) pixel/micron$^2$.**

The compare between the mean value of the cortical (0.18±0.01) pixel/micron$^2$ and medullary region (0.27±0.01) pixel/micron$^2$ of the renal tissue in the pregnant group showed highly significant ($p<0.001$) difference. The compare between the mean value of the cortical (0.77±0.01) pixel/micron$^2$ and medullary region (0.71±0.01) pixel/micron$^2$ of the renal tissue in the non-pregnant group showed highly significant difference (Table 1) (Figure 3).

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Table (1): Showed the positivity of CK18 between pregnant and non-pregnant kidney of mice, with their level of significance (independent t-test).

| Positivity of CK18 | Non-pregnant N=50 Mean±SE pixel/micron$^2$ | Pregnant N=50 Mean±SE pixel/micron$^2$ | P value |
|---|---|---|---|
| Cortex | 0.77±0.01 | 0.18±0.01 | <0.001 |
| Medulla | 0.71±0.01 | 0.27±0.01 | <0.001 |
| P value | 0.002 | <0.001 | |

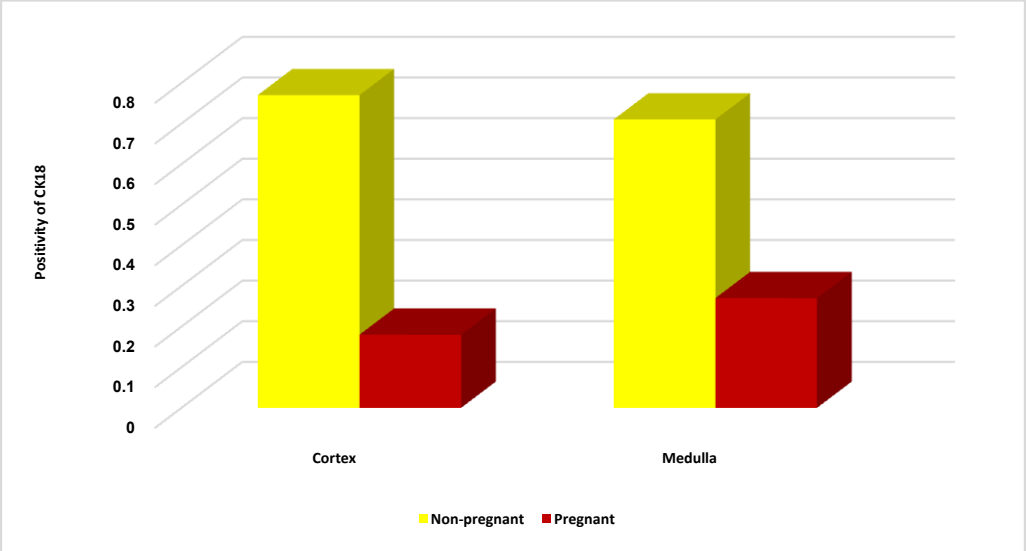


Figure (3): The diaphragm showed comparable between the pregnant and non-pregnant kidney of mice in the positivity of CK18.

**Discussion**

It was found that expression of CK 18 in renal tubules was increased during progressive renal tubular injury (Djudja*et al*., 2016) resulting in renal damage and fibrosis (Nangaku, 2004). The diminished CK18 immunohistochemical reactivity found in this study, therefore, could hypothetically indicate the non-progressive renal tubular injury in mice during pregnancy.It was also documented that detection of CK 18 in urine sample of human and mice has been considered as a marker for the established acute renal tubular injury (Djudja*et al*., 2016).The detection of Ck18 in urine sample during pregnancy should be evaluated in future researches to be correlated with the histochemical deficit CK18 seen in this study in renal tissue of pregnant mice. This recommended proposal may validate a hypothetical CK18 depletion of the renal tissue during pregnancy associated with CK18 elimination in urine as a sign for acute renal tubular alterations occurring during pregnancy. The differences of Cytokeratins immunohistochemical reactivity in various kidney epithelia were related to morphological differences of epithelial organization and regional differences of function (Werner *et al*., 2020). Therefore, the deviation of the CK18 immunohistochemical expression seen in renal tissues during pregnancy in this study may be related functional phases of the kidney occurring during pregnancy. The expression of Cytokeratins depends on the type of epithelial cells, and degree of differentiation (Jacob *et al*., 2020). The CK 18 examined in this study is an acidic type I. It was documented that cytokeratin 18 found in single-layered "simple" epithelial tissues and is localized in the cytoplasm and per nuclear region. The presence of CK18 and CK8 keratins appears to be both necessary and sufficient for the formation of keratin filaments (Menz*et al*., 2020).Adaptations in the kidney physiology during pregnancy result in changes of the kidney

"normal" laboratory values (Vinturache*et al*., 2019). The keratin investigated in this study tried to elaborate the possibility of its functional role in these physiological changes occurring during pregnancy. This trial was based on the suggestion that keratins were functionally suggested to regulate intracellular organization and transport (Ku *et al*., 1999). Many functional roles for keratins were reported different cellular and intracellular localization in physiological conditions compared to injury (Etienne-Manneville, 2018,Salas *et al*., 2016).The adaptations in the kidney physiology during pregnancy could be related to many other factors in correlation with the changes in the pattern of CK18 investigated in this study. The variability of the pattern of Ck18 expression in renal tissues of pregnant mice revealed mainly diminished CK18 expression in the tubular epithelia (both convoluted tubules and thin loop of Henle). This finding could be an explanation for the previous reports suggesting that the altered tubular functions is one of traits of renal physiologic changes characteristic for normal pregnancy (Odutayo and Hladunewich, 2012).In the mammalian kidney, par cellular permeability decreases from the proximal tubule to the collecting duct (Denker and Sabath, 2011). The proximal tubules are functioning for reabsorbing most of the macromolecules filtered by glomeruli, this function demanding high energy that is provided by a high number of mitochondria inside the tubular cells. This function is very vulnerable to ischemia, metabolic dysfunction, or external toxins (Simons, 2018). It was concluded previously that Ck18 maintains mitochondrial structures (Kumemura*et al*., 2008). Accordingly, the deficit Ck18 in the proximal tubules seen in this study may disturb mitochondrial functions resulting in disturbance of tubular re-absorption of glomerular filtration. The adaptations of kidney physiology during pregnancy were reported to be associated with swelling of podocytes, endothelial, and mesangial cells, with thickening of the glomerular basement membranes, the tubules were ultra-structurally normal (ÇELİK *et al*., 2002). The Ck18 was reported to maintain cell shape, mechanical stability, provides a flexible intracellular scaffolding to cytoplasmic structures, (Cheng *et al*., 2019) and providing resist to stresses externally applied to the cell (Coulombe and Wong, 2004). Accordingly, the diminished renal tubular Ck18 expression may be accompanied the disturbance of their cytoplasmic scaffolding of the tubular cells as a result of the external stress produced by the increased volume of the swollen renal tissue surrounding these tubules.

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