Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 08, pp.55808-55813, August, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

TO STUDY THE SEQUENCE OF MICROSCOPIC CHANGES OCCURRING DURING DEVELOPMENT OF KIDNEY IN 12WK-35WK HUMAN FETUSES

***,[1]Kirti Solanke, [2]Rajan Bhatnagar, [3]Boruah Dibyajyoti, [4]Tandon Aseem and [5]Pokhrel Rishi**

[1]Department of Anatomy, SMT. Kashibai Navale Medical College & General Hospital, Narhe (Ambegaon) Pune, 411041, Maharastra, India
[2]Department of Anatomy, Dr Ram Manohar Lohiainstitute of Medical Sciences, Utter Pradesh, India
[3]Department of Pathology, Armed Forces Medical College (AFMC), Pune – 411040, India.
[4]Professor; 168 Military Hospital APS CIRCLE
[5]Department of Anatomy, College of Medicine, Nepalese army Institute of Health Sciences, Kathmandu, Nepal

ARTICLE INFO

Article History:
Received 19th May, 2017
Received in revised form
10th June, 2017
Accepted 15th July, 2017
Published online 31st August, 2017

Key words:

Kidney, Glomeruli, Nephrogenic zone, Undifferentiated, Lobulated, Tubules.

ABSTRACT

Study the sequence of microscopic changes taking place during development of kidney in human foetuses. Aborted fetuses were studied after measuring external parameter such as CRL. After undergoing tissue processing, microscopic structure of each kidney was observed after H&E staining. For analyzing various stages of development of glomeruli different groups were made and changes observed. During 12 to 14 weeks undifferentiated mesenchyme was seen increasing in amount from outer to inner hilar aspect. Transition zone appeared between cortex and medulla at 16-18 weeks. At 22 wk tubules differentiated as proximal & distal convoluted tubules. Longitudinally running collecting ducts can be appreciated at 22-27 wk, medullary rays are seen at 29 wk. Undifferentiated mesenchyme continue to appear till 35 wk.We found that nephrogenic zone was present immediately beneath the capsule; glomeruli were seen at different stages of development. S shaped tubules were observed at 20 – 24 weeks and C shaped and crescent shaped in later weeks of development.

Citation: Kirti Solanke, Rajan Bhatnagar, Boruah Dibyajyoti, Tandon Aseem and Pokhrel Rishi, 2017. "To study the sequence of microscopic changes occurring during development of kidney in 12wk-35wk human fetuses", *International Journal of Current Research*, 9, (08), 55808-55813.

INTRODUCTION

With increased advent of prenatal investigations, genetic counselling and raised consciousness among general public about congenital malformations and diseases; there has been increased curiosity in developmental anatomy. In India every year 150,000 people are diagnosed with renal failure and require renal transplant (Sampath, 2013). In U.S. out of 118,617 people awaiting organ transplant; 96,645 are waiting for kidney transplant (2013). It is thus essential to know each step in development of kidney. Organogenesis of kidney commences at 6th week of intrauterine life with the progressive appearance of pronephros, mesonephros and lastly definitive metanephros. Development of definitive or permanent kidney is from epithelial evagination of a bud from Wolffian duct, metanephric blastema and local angiogenic component (Hamilton *et al.*, 1975). The present study aims to study the histogenesis of human foetal kidney during intrauterine period ranging from 12th to 35th week.

Corresponding author:* **Kirti Solanke,
Department of Anatomy, SMT. Kashibai Navale Medical College & General Hospital, Narhe (Ambegaon) Pune, 411041, Maharastra, India.

MATERIALS AND METHODS

Foetuses with different gestational age ranging from 12 weeks to 35 weeks which showed no abnormalities on macroscopic examination were taken. The study was cross sectional, observational study. These foetuses included spontaneous abortions and stillborn foetuses. Twins and foetuses with gross anomalies of urogenital system were omitted from our study.

Measurement of external parameters: Crown Rump length (CRL) of these foetuses was measured using digital sliding vernier calliper with accuracy of 0.01mm and an osteometric board with millimetre scale and data were tabulated (Table-1). True gestational age of foetuses was collected from the medical records. Crown-rump length was correlated with gestational age chart as per text book of Embryology by Hamilton, Boyd and Mossman (Table-2) to ensure there was no intra uterine growth retardation (IUGR). Thus selection of normal for gestational age foetuses was assured. Ten % formalin was injected in the body cavities and soft tissue of foetuses and immersed and fixed in 10% formalin for a minimum period of 3 days. The kidneys were retrieved by

dissection through anterior approach. Bilateral subcostal (rooftop) incision was taken to open the abdomen. Median umbilical ligament was cut; posterior peritoneum separated aside, to expose the kidneys which were then removed by cutting pedicle of all vessels and ureter at hilum. Kidneys were preserved in 10% formal saline. After undergoing tissue processing paraffin blocks were made. Sections were cut with the help of rotary microtome 5 to 7 micron in thickness. The sections were then stained using Haematoxylin–Eosin method.

RESULTS

Morphological observations

12 weeks of gestation (Figure I) - At 12 weeks of gestation kidney was lobulated. Cortex and medulla could not be differentiated. Pointer number 3 depicts fibrous capsule of kidney. Towards outer aspect just underneath the capsule, various stages of development of glomeruli can be seen as showed by unnumbered arrows. Large amount of undifferentiated mesenchymal tissue showed by 1 can be seen. Number 2 depicts tubule.

14 weeks of gestation (Figure II(A, B, C, D)) - At 14 weeks of gestation kidney was still in lobulated form. Amount of undifferentiated mesenchyme was seen increasing in amount from outer towards hilar aspect. Cortex and medulla could not be established (fig.A). Outer darkly stained area (fig.B) showed increased activity of cells forming various stages of tubules and developing glomeruli. Various stages of development of tubules and glomeruli can be seen at low power (fig.A).These tubules were lined by single layered low columnar to cuboidal epithelium (fig.C). Tubules can be seen branching.

16 week of gestation (figure III (A,B)) - At 16 week of gestation zone of transition appeared between cortex and medulla. Outer aspect (fig.A, B) showed number of developing glomeruli and C & S shaped tubules. Size of glomeruli was seen increasing from outer to inner aspect (fig.A). Most of part of field was filled with number of sections of tubules (fig.A). At higher magnification (fig.B) developing glomeruli surrounded by nephrogenic vesicle lined by single layer of low columnar epithelium could be seen. Tubules (fig.B) cut longitudinally along their length can be seen between developing glomeruli. Still large amount of undifferentiated mesenchyme was present at the cortical region.

17 weeks of gestation (figure IV C) - Cortex and medulla start differentiating. Density of glomeruli was large towards cortex than towards medulla, size of glomeruli number(figureC)1, 2 were seen increasing from cortex to medulla as appreciated on low power. Medulla was full of number of sections of tubules as showed by 4(figure C) and blood vessels with RBCs. Number 4 showed tubules. Large amount of undifferentiated mesenchyme showed by 5 can be seen towards medulla.

19 week of gestation (figure V D) - Lobules appeared fused with each other in section. Corticomedullary junction was well appreciated as compared to previous stages. The blood vessels showed by 4 containing RBCs were seen scattered in medulla. Nephrogenic zone was seen reduced in thickness. But mesenchymatous tissue showed by 3 was still there. Field was full of glomeruli showed by 1, 2, cut sections of tubules and undifferentiated mesenchymatous tissue

21 week of gestation (figure VI A) - Lobes can be seen separated by connective tissue septae. Cortex & medulla was clearly demarcated. Pointer 1 depicts ureter with very well developed multi-layered urothelium. Star shaped classical lumen and muscular layer comprising of pink colour smooth muscle cells. Serosa can be differentiated. Number 3 depicted muscular artery having tunica intima, media and tunica externa. Pointer 2 and 4 shows two veins. Larger sized developing glomeruli can be seen at pointer 5, 6.

22 weeks of gestation (figure VII (B,C,D))- At 22 weeks in fig. B cortex showed developing glomeruli; S shaped stage (fig. C) of tubule bended on itself with two poles and Bowman's space, with outer aspect lined by simple squamous epithelium and tubule lined by columnar epithelium. Longitudinally running collecting ducts (fig. B) can be seen. In fig. D two types of tubules were differentiated at oil emulsion .one which is more basophilic with smaller lumen pointed by 1 and with more number of sections were probably the proximal convoluted tubule (PCT). Other with larger lumen and more eosinophilic and less number of sections pointed by 2 were probably (DCT) distal convoluted tubule (fig.D).

27 weeks of gestation (figure VIII (A, B, C))- Kidney still showed lobulations and various forms of developing glomeruli (fig.A). Staining characteristics were same as mentioned earlier. Though tubules and glomeruli were well formed, undifferentiated mesenchyme was still present (fig. B).In fig. C number of sections of proximal and distal convoluted tubules can be seen with PCT lined by cuboidal epithelium with brush border and characteristically only fewer nuclei of cells and DCT with simple cuboidal with cleaner and sharper luminal surface with all nuclei of cells in section well appreciated.

29 weeks of gestation (figure IX (A, B, C, D, E, F, G, H))- Medullary rays and pyramids were formed and glomeruli were arranged in rows of 2 to 4 along with collecting duct. Field was full of differentiated tubules, glomeruli and connective tissue but some amount of undifferentiated tissue was there. Various stages of developing tubules were present in all weeks of gestation but well seen at 29 weeks of gestation i.e. comma shaped, C and crescent shaped glomeruli and mature or fully formed glomerulus as discussed below-

Stage I- S shaped structure already explained above at 22 we eks (fig. 4C)

Stage II- comma shaped structure lined with single layer of epithelium (fig. C).

Stage III- C shaped structure with space. Two layers, outer layer lined with cuboidal and inner with columnar epithelium (fig. D).

Stage IV-crescent shaped lined by columnar epithelium changing to low columnar to cuboidal then squamous epithelium (fig. E).

Stage V-glomerular capillaries had invaginated in crescent (fig. F).

Stage VI- glomerular capillaries showed well marked lobulations (fig. G)

34 weeks (figure X A)- At 34 weeks nephrogenic zone had been reduced in size but various stages still visible. Most of glomeruli in subcortical region were fully matured.

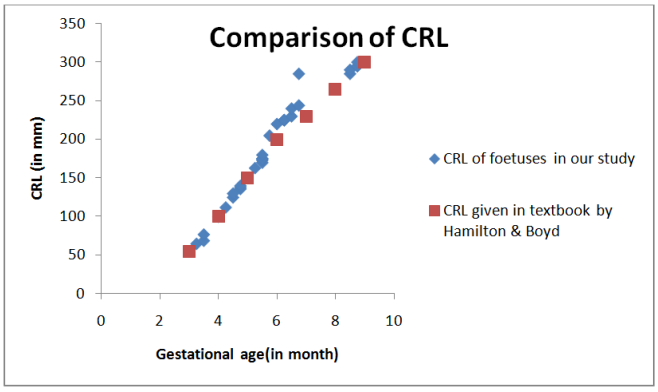
PHYSICAL DIMENSIONS-The CRL was correlated with the gestational age of the foetuses and is shown in table 1

Table I.

Serial number	Crown rump length(mm)	Gestational age(weeks)
1	55	12
2	65	13
3	69	14
4	77	14
5	100	16
6	112	17
7	125	18
8	130	18
9	136	19
10	138	19
11	140	19
12	163	21
13	170	22
14	173	22
15	175	22
16	175	22
17	180	23
18	205	24
19	220	27
20	225	27
21	225	27
22	225	28
23	230	28
24	240	29
25	244	29
26	285	34
27	285	34
28	290	35
29	295	35
30	295	35
31	300	35

Table II. From Human embryology by Hamilton, Boyd and Mossman

Age (in lunar months)	Crown rump length(in mm)
3	55
4	100
5	150
6	200
7	230
8	265
9	300
10	335



Graph-1

35 weeks (figure X (B, C))-At 35 weeks well-formed collagen fibres forming capsule of kidney was present (fig. C). Rows of glomeruli could be seen. Various stages of developing tubules were reduced. Medullary rays with collecting tubules could be seen (fig. B, C). Still some amount of undifferentiated mesenchyme was present.

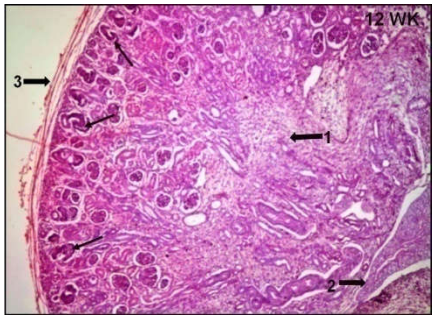


Fig. 1. Haematoxylin and Eosin stained section of a kidney at 12 weeks of gestation (40X). Key : 1= Undifferentiated mesenchyme, 2=Tubule, 3= Fibrous capsule, Unnumbered arrows =various stages of developing tubules

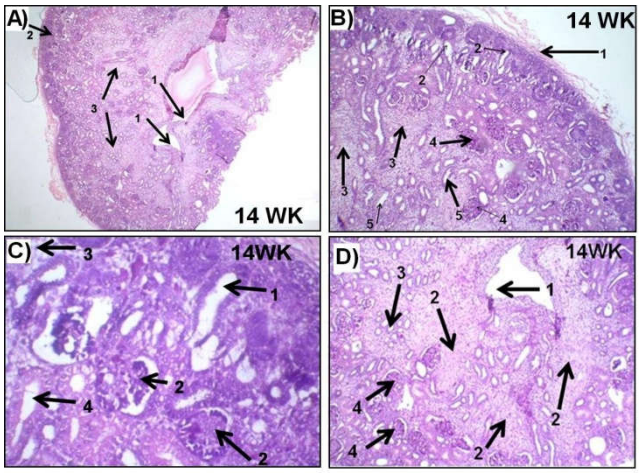


Fig. II(A,B,C,D). Haematoxyline and Eosin stained section of kidney at 14 weeks of gestation. A) (40X), Key: 1=Branching tubule, 2=Outer darkly stained region, 3= Undifferentiated mesenchymal tissue; B) (100X), Key: 1=Fibrous capsule, 2=Longitudinally cut tubule, 3=Undifferentiated mesenchyme, 4=Developing glomeruli, 5=Number of sections of tubules cut in various directions; C) (400X), Key: 1=Longitudinally cut tubule, 2=Developing glomeruli, 3=Undifferentiated mesenchyme, 4=Low columnar epithelium lined tubule ; D) (100X), Key: 1= Branching tubule, 2=Large amount of undifferentiated mesenchyme, 3=Number of sections of tubule, 4=Developing glomerulus

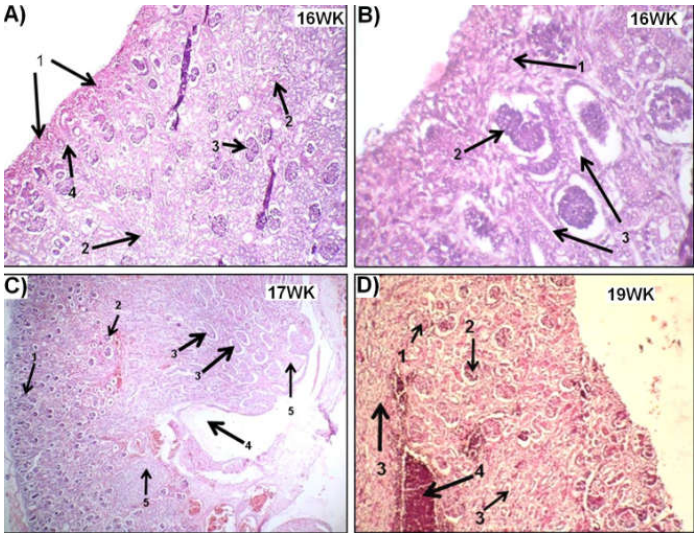


Fig. III(A,B);IV(C);V(D). Haematoxylin and Eosin stained section of a kidney: A) 16 weeks of gestation (40X); Key: 1= Actively dividing cells and tubules, 2= Sections of tubule, 3= Developed glomerulus with large size, 4= Undifferentiated mesenchyme; B)16 weeks of gestation (400X) Key: 1=Undifferentiated mesenchyme, 2=Developing glomerulus, 3=Longitudinally cut sections of tubules; C) 17 weeks of gestation (40X), Key: 1=Developing glomeruli, 2=Larger sized glomeruli, 3=Sections of tubule, 4=Tubule, 5=Undifferentiated mesenchyme; D) 19 weeks of gestation (100X), Key: 1, 2=Developed glomeruli, 3=Undifferentiated mesenchyme, 4=Blood vessel with RBCs within it.

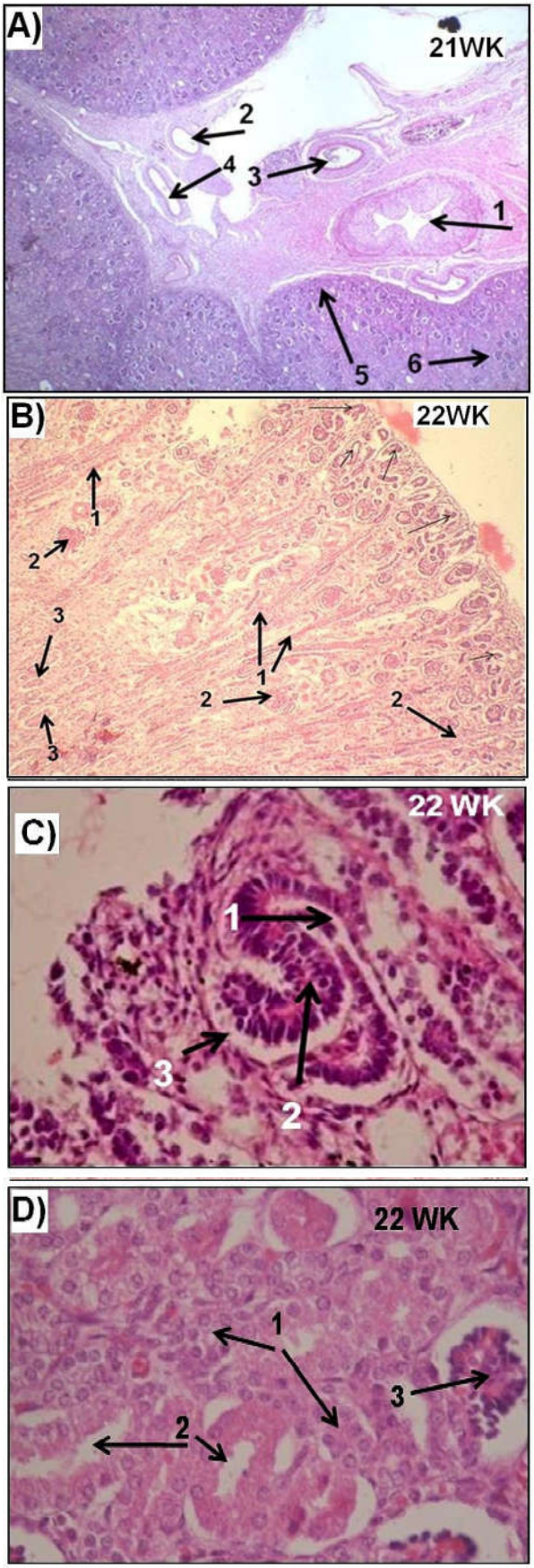


Fig.VI(A); VII(B,C,D): Haematoxylin and Eosin stained section of a kidney: A) 21 weeks of gestation (40X), Key: 1=Ureter, 2, 4=Veins, 3= Artery, 5=Developing tubules in different stages, 6=Sections of tubules; B) 22 weeks of gestation (40X), Key: 1=Collecting tubules, 2= Developed glomeruli, 3=Sections of tubules, Unnumbered arrows=Various stages of developing tubules; C) 22weeks (400X), Key: 1= Distal pole of S shaped tubule, 2=Intermediate area, 3=Bowman's space; D) 22weeks (400X), Key: 1=Distal convoluted tubule, 2=Proximal convoluted tubule, 3=Mature glomerulus

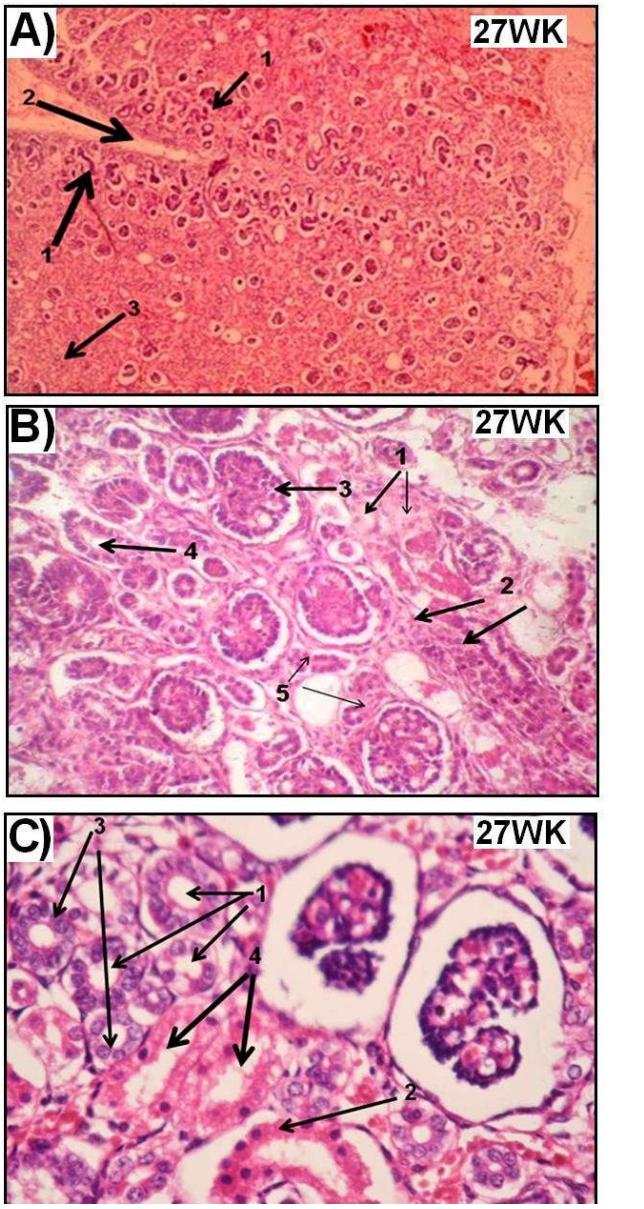


Fig. VIII(A-C): Haematoxylin and Eosin stained section of a kidney at 27 weeks of gestation: A) (40X), Key: 1=Developing tubules, 2=Septum separating two lobes, 3=Sections of tubules ; B) (200X), Key: 1-Sections of PCT, 2-Undifferentiated mesenchymal tissue, 3-Glomerulus, 4-Sections of tubules, 5-Sections of DCT; C) (1000X), Key: 1=Distal convoluted tubule, 2=Cuboidal epithelium with brush border, 3=Simple cuboidal epithelium, 4=Proximal convoluted tubules

DISCUSSION

Histogenesis

On panoramic view kidney was seen to be lobulated in accordance with previous authors (Osathanondh & Potter, 1963; Mezzogiorno *et al.*, 2002; Ellis, 2010; Sutherland *et al.*, 2011; Patil *et al.*, 2012). In the current study, S shaped tubules were observed at 20 to 24 weeks; C shaped and crescent shaped tubules were observed in later weeks of development. Takano K *et al*. found S stage highest at 20-24 weeks and C stage proportion was maximum at 25-29 weeks; these findings are in agreement with present study (Takano *et al*., 2007; Rao & Padmini, 2012). Development of tubules varying from comma and S shaped bodies was observed, as described by various workers (Hamilton *et al.*, 1975; Almeida & Mandarim-de-Lacerda, 2002). Though these stages were seen more or less in all weeks of the gestational period, they were well appreciated

in later weeks of development. In early weeks the number of mature glomeruli was less. The number increased with age as was observed by Tank *et al* (Tank *et al.*, 2012). Present study had similar findings. Darkly stained highly dividing nephrogenic cell zone was seen beneath the capsule. They were more marked at 16 weeks of gestation in the present study; Sudha Patil *et al*. in 2012 noted this nephrogenic zone decreasing with increasing foetal age and disappeared at 38th week of gestation (Patil *et al.*, 2012). Such disappearance was not found in present study. In the present study, undifferentiated mesenchyme was found till 35 weeks. This finding is in accordance with Sadiqali Syed *et al*. Sabita Mishra *et al*. who found it till 28 weeks. Vladislava Zohdi *et al*. 2012 said that no new nephrons are formed after birth during lifetime and nephrogenesis is complete by 32-36 weeks (Zohdi *et al.*, 2012). Morag MacDonald and John Emery described nephrogenic phase in which new glomeruli were formed may continue until 44 weeks after conception, but was usually over by 36th week of gestation (Macdonald & Emery, 1959).

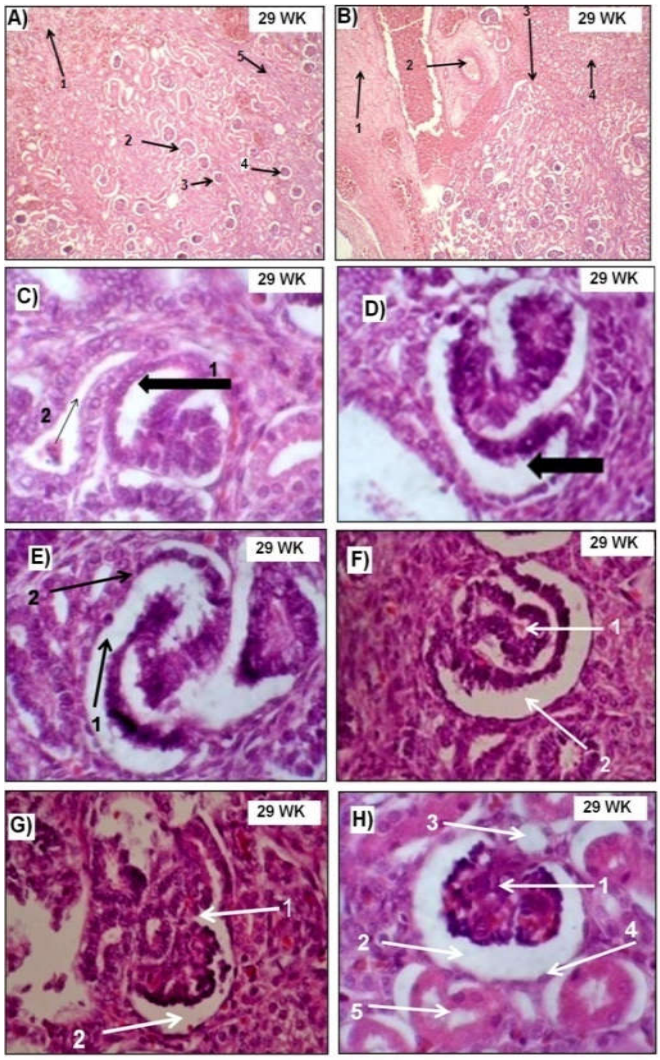


Fig.IX(A-H): Haematoxylin and Eosin stained section of a kidney at 29 weeks of gestation: A)(100X), Key: 1=Sections of tubules, 2, 3 = Rows of glomeruli, 4 = Glomeruli, 5= Collecting tubules; B) (100X), Key: 1=Connective tissue, 2=Vessel, 3=Collecting tubule, 4=Sections of tubules; C) (1000X), Key: 1=Renal vesicle, 2=Section of tubule in different direction; D) (1000X), Key: Arrow=Space between two layers inner columnar and outer cuboidal epithelium; E) (1000X), Key: 1=Two layers with space in between i.e. Bowman's space,2=Change of epithelium from cuboidal to columnar in outer layer; F) (1000X), Key: 1= Capillaries invaded into tubules, 2=Bowman's space; G) (1000X), Key: 1=Lobulations at capillary, 2=Bowman's space; H) (1000X), Key: 1= Mature glomeruli, 2=Bowman's space, 3=DCT, 4=Simple squamous epithelium, 5=PCT

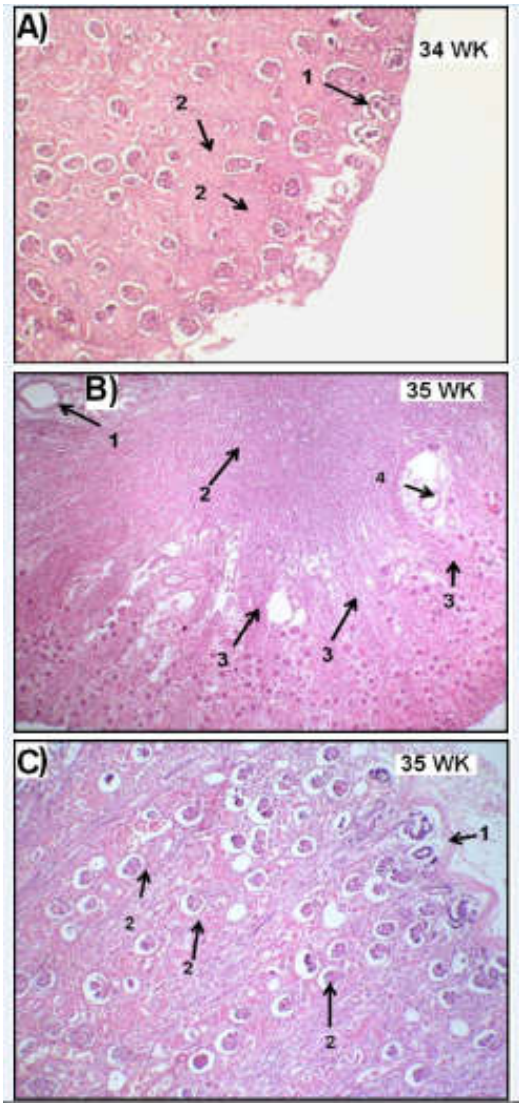


Fig.X(A,B,C): Haematoxylin and Eosin stained section: A) 34 weeks of gestation(100X), Key: 1=C shaped tubule, 2=sections of tubules; B) 35 weeks of gestation(40X), Key:1, 4=Vessel with connective tissue surrounding it, 2=Medulla with numerous sections of tubules, 3=Medullary rays; C) 35 weeks of gestation(100X), Key: 1= Fibrous capsule, 2=Differentiated glomeruli and tubules

Megan Sutherland *et al*. 2011 stated that preterm neonates' exhibit accelerated postnatal renal maturation with a reduced percentage of immature V-stage glomeruli and an increased number of glomerular generations compared with post-conceptional age matched gestational controls. Lina Gubhaju *et al*. Observed that in preterm birth, nephrogenesis is on-going in extra uterine environment and is associated with a high proportion of abnormal glomeruli in some neonatal kidneys. (Gubhaju *et al.*, 2009) This could be confirmed by the present study. In the present study, cortex and medulla differentiated at 17 weeks, tubules differentiated as two types initially at 20 weeks and clearly by 22 weeks. These findings are in accordance with Sabita Mishra *et al*. and Sadiqali Syed *et al* (Mishra *et al*., 2006; Syed *et al*., 2012). Bhattam Narasinga Rao 2012 mentioned differentiation at 30th week (Rao & Padmini, 2012). In the present study cortex and medulla started differentiating at 17 weeks and was well demarcated at 19 weeks; Sadiqali Syed *et al* mentioned it at 24 weeks of gestation. Ureter with lining urothelium was well seen at 21 weeks, proximal & distal convoluted tubules were seen at 22 weeks. Medullary rays were seen at 29 weeks in the present study.

Summary

Morphological observations

Structure of foetal kidney was observed at various objectives and following results were obtained

1) At 12-14 weeks (Group-1) = Kidney was lobulated. Large amount of undifferentiated mesenchyme was seen increasing in amount from outer to inner hilar aspect.
2) At 16-18 weeks (Group-2) = Transition zone appeared between cortex and medulla. Nephrogenic zone well marked under capsule at 16 weeks. Cortex and medulla started differentiating.
3) At 19-21 weeks (Group-3) = Tubules differentiated as proximal and distal convoluted at 22 weeks. Ureter with developed urothelium was seen at 21 week.
4) At 22-27 weeks (Group-4) = Longitudinally running collecting ducts can be appreciated.
5) At 28-29 weeks (Group-5) = Medullary rays were seen at 29 weeks.
6) At 34-35 weeks (Group-6) = Undifferentiated mesenchyme was found till 35 weeks.

Conclusion

We studied 31 human foetuses from 12 to 35 weeks of gestation and found that nephrogenic zone was present beneath the capsule; glomeruli were seen at different stages of development. S shaped tubules were observed at 20 – 24 weeks and C shaped and crescent shaped in later weeks of development.

Acknowledgement

No any acknowledgment.

REFERENCES

(2013) National Kidney Foundation. Organ Donation and Transplantation Statistics.

Almeida, J.R. & Mandarim-De-Lacerda, C.A. 2002. Quantitive Study Of Comma Shaped Body, S-Shaped Body And Vascularized Glomerulus In The Second And Third Human Gestational Trimesters. *Early Human Development*, 69, 1-13.

Ellis, H. 2010. Anatomy's Dark Past. *BMJ*, 341:C6111.

Gubhaju, L., Sutherland, M.R., Yoder, B.A., Zulli, A., Bertram, J.F. & Black, M.J. 2009. Is Nephrogenesis Affected By Preterm Birth? Studies In A Non -Human Primate Model. *American Journal of Physiology*, 297, F1668-F1677.

Hamilton, W.J., Boyd, J.D. & Mossman, H.W. 1975. Growth And Development Of Embryo. In Mossman, W.H.H. (Ed) *Boyd And Mossman's Human Embryology*. W. Heffer & Sons Ltd, Cambridge, Pp. 172-177.

Macdonald, M.S. & Emery, J.L. 1959. The Late Intrauterine and Postnatal Development Of Human Renal Glomeruli. *Journal of Anatomy*, 93, 331.

Mezzogiorno, A., Mezzogiorno, V. & Esposito, V. 2002. History of the Nephron. *Am J Nephrol* 22, 213.

Mishra, S., Dinesh, A. & Kaul, J.M. 2006. Morphological and Morpho-Metrical Study Of Human Renal Development During Mid-Gestation Period. *JASI*, 55, 5-10.

Osathanondh, V. & Potter, E.L. 1963. Development of Human Kidney As Shown By Microdissection. I. Preparation of Tissue With Reasons For Possible Misinterpretations of Observations. *Arch Pathol*, 76, 271-276.

Patil, D.S., Patil, D.P. & Mane, D.A. 2012. Histogenesis of Human Fetal Kidney. *NJIRM*, 3, 122-127.

Rao, B.N. & Padmini, M.P. 2012. Prental Histogenesis of Kidney In Human Foetuses *International Journal Of Basic And Applied Medical Sciences*, 2, 144-147.

Sampath, P. 2013. World Kidney Day: Things You Should About Kidney Transplants.

Sutherland, M.R., Gubhaju, L. & Moore, L. 2011. Accelerated Maturation and Abnormal Morphology In The Preterm Neonatal Kidney. *Journal of the American Society of Nephrology*, 22, 1365-1374.

Syed, S.A., Joshi, R.A. & Herekar, N. 2012. Histogenesis of Kidney In Human Fetuses. *International Journal of Recent Trends in Science and Technology*, 3, 44-48.

Takano, K., Kawasaki, Y., Imaizumi, T., Matsuura, H., Nozawa, R., Tannji, M., Suyama, K., Isome, M., Suzuki, H. & Hosoya, M. 2007. Devlopment Of Glomerular Endothelial Cells, Podocyte And Mesangial Cells In The Human Fetus And Infant. *Tohoku Journal of Experimental Medicine*, 212, 81-90.

Tank, K., Saiyad, S., Pandya, A., Akbari, V. & Dangar, K. 2012. Histogenesis Of Human Fetal Kidney. *Int J Biol Med Res*., 3, 1315-1321.

Zohdi, V., Sutherland, M.R., Lim, K., Gubhaju, L., Zimanyi, M.A. & Black, M.J. 2012. Low Birth Weight Due To Intrauterine Growth Restriction And/Or Preterm Birth: Effects on Nephron Number and Long-Term Renal Health. *International Journal of Nephrology*, 2012.

****