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**Original research article**

# Assessment of the chronology of nephrogenic events in staged aborted human embryos

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## Abstract

**Aim:** to evaluate chronology of nephrogenic events in staged aborted human embryos and foetuses.

**Materials and methods:** The present study was the conducted in Department of Anatomy. Total 50 aborted embryos and dead foetuses of 5 weeks gestational age to full term were include in this study. The study was done in Anugrah Narayan Magadh Medical College and Hospital, Gaya, Bihar, India, for 1 year. The entire specimens were preserved in formalin after recording the weight, CR length and CH length. Kidneys were removed from the foetuses of more than 8 weeks gestational age by opening the abdominal cavity. The specimens were subjected to routine tissue processing and H&E staining. 10 embryos of less than 8 weeks gestational age were processed as a whole and were serially sectioned. The histological sections were observed for the time of appearance of various nephrogenic components and photographed.

**Results:** Less than 12 weeks GA group: In this group a total of 8 embryos (less than 8 wks GA) and two fetuses were observed for renal histogenesis. 5 wks. Embryo: The youngest embryo observed in this group was that of 5 weeks GA with a CRL of 0.8 cms. In this embryo urogenital mesentery, degenerating pronephros and pronephric tubule could be identified. 6 wks. Embryo: In the 6 weeks GA (1.3 cms CRL) embryo urogenital mesentery containing developing mesonephric kidney and gonad were identified. 8 weeks embryo: At 8 weeks GA (1.6 cms CRL) differentiating renal corpuscles, proximal and distal convoluted tubules and collecting ducts could be identified. 12 weeks foetus: At 12 week (2.1 cms CRL, male foetus) mesonephric components could not be identified. 13-24 wks GA group: In this group a total of nine foetuses were observed for renal histogenesis. Sections from a specimen of 16 weeks and that of 24 weeks were compared for the developmental progression. During this period cortico-medullary differentiation was observed.

**Conclusion:** Detailed findings of this study could aid the embryologists, neonatologists and nephrologists to understand the chronology of nephrogenic events and related consequences of developmental abnormalities.

**Key words:** Nephrogenic Events, Human embryos, Foetal Kidney, Pronephric, Mesonephric, Metanephric, Phylogeny

## Introduction

It is important to know the normal developmental anatomy and histogenesis of urinary system for better understanding of various congenital renal conditions. Kidney plays a vital role in development of fetuses. Accurate assessment of gestational age of fetuses is essential for both clinical and medicolegal point of view. Prenatal development is a very crucial period for human development Embryologically, Kidneys develop from intermediate mesoderm of human embryos in cranio-caudal direction. These are pronephric kidney, mesonephric kidney and metanephric kidney. Metanephros appears in lumbosacral segments, develop in 5th week. It develops from Ureteric bud which forms collecting part and metanephrogenic blastema forms secretory part. Ureteric bud its distal end dilates and invades the caudal part of nephrogenic cord dorsal to mesonephric ridge. It repeatedly divides. It is capped with a metanephric blastema on further sub-division some parts of the blastema separate from the main mass and form clusters of cells on each side of the tubule forms pear shaped hollow renal vesicles. First vesicle is formed at the end of 7th week. Cells at the proximal pole of the vesicles organize to form C-shaped or comma shaped body followed by cellular reorganization of tubular cells at distal end to form an 'S' shaped body or S-body. In the cleft of the S-body at the distal pole, formation of extra cellular matrix and penetration by capillaries targets at formation of future mesangial region. The proximal limb of the S-body organizes to form distal convoluted tubule while the intermediate limb enlarges to form loop of Henle and the proximal convoluted tubule resulting in entire development of a nephron.[1-4] Many such nephrons are present in the fetal kidney due to multiple branching of the ampullary bud and induction of various mesenchymatous condensates to form nephron arcades. The permanent kidneys become functional in intrauterine life and urine produced by them is added to amniotic fluid from 10th week of gestation.

Kidneys develop in the intermediate mesoderm in the cranio-caudal direction. They develop in pronephric, mesonephric and metanephric stages. The development of the kidneys illustrates the famous dictum by Haeckel that ‘ontogeny recapitulates phylogeny’. This means that the development of the three kidney types follow an evolutionary pattern. The development of kidney is a very complex process having two parts, collecting and excretory parts. The collecting part develops from ureteric bud while the excretory part develops from metanephric blastema.[5] Ureteric bud is a primordial of ureter, renal pelvis, calices and collecting tubules. Its distal end dilates and invades the caudal part of nephrogenic cord dorsal to mesonephric ridge. Ureteric bud repeatedly divides until about 13 or more generations of tubules are formed. Ureteric bud is capped with a metanephric blastema on further sub-division some parts of the blastema separate from the main mass and form clusters of cells on each side of the tubule forms pear shaped hollow renal vesicles. First vesicle is formed at the end of 7th week in relation to 6th division of ureteric bud.[6] Microscopically the kidney is composed of many tortuous closely packed uriniferous tubules bound by little connective tissue in which run blood vessels, lymphatic and nerves.[7]

## Materials and methods

The present study was the conducted in Department of Anatomy. Total 50 aborted embryos and dead foetuses of 5 weeks gestational age to full term were include in this study.The study was done in Anugrah Narayan Magadh Medical College and Hospital,gaya, Bihar, India, for 1 year, after taking the approval of the protocol review committee and institutional ethics committee.

## Methodology

The entire specimens were preserved in formalin after recording the weight, CR length and CH length. Kidneys were removed from the foetuses of more than 8 weeks gestational age by opening the abdominal cavity. The specimens were subjected to routine tissue processing and

H&E staining. 10 embryos of less than 8 weeks gestational age were processed as a whole and were serially sectioned. The histological sections were observed for the time of appearance of various nephrogenic components and photographed.

**Results**

**Less than 12 weeks GA group**: In this group a total of 8 embryos (less than 8 wks GA) and two fetuses were observed for renal histogenesis.

**5 wks. Embryo:** The youngest embryo observed in this group was that of 5 wks. GA with a CRL of 0.8 cms. In this embryo urogenital mesentery, degenerating pronephros and pronephric tubule could be identified. Pronephric tubule was opening in to the coelomic cavity. There was no glomerulus at this stage. A detailed observation at higher magnification (10x) presented renal, gonadal and supra renal primordia along with pronephric and parameson-ephric ducts. In the same slide degenerating pronephros, mesonephros, mesonephric tubules, pronephric duct and cloaca were identified.

**6 wks. Embryo:** In the 6 wks. GA (1.3 cms CRL) embryo urogenital mesentery containing developing mesonephric kidney and gonad were identified. Condensation of metanephric mesenchyme along with mesentery could be identified at this stage. Growth and differentiation of mesonephros with degenerating mesonephric tubules, mesonephric and paramesonephric ducts and renal corpuscles were seen. Degenerating mesonephric tubules, cut sections of mesonephric duct (MND), paramesonephric duct (PMND), differentiating renal corpuscle (RC) presenting vesicle and 'S' stages of mesonephric nephron showing continuity with proximal convoluted tubule (PCT) were observed at 6wks. GA. Change in the pattern of metanephrogenesis from linear to radial fashion could be recognized in the metanephric kidney. Further, interaction between the two functional components i.e. ureteric bud (ductogenesis) and metanephric mesenchyme known as mesenchymal epithelial transition (MET) and morphogenesis could be observed. The various stages in nephrogenesis i.e. interaction, cap, blastemal, vesicle, comma and renal corpuscle stages could be identified

8 wks. embryo: At 8 wks. GA (1.6 cms CRL) differentiating renal corpuscles, proximal and distal convoluted tubules and collecting ducts could be identified. Up to 8 weeks gestational age the sex of the embryo could not be identified.

**12 weeks foetus:** At 12 week (2.1 cms CRL, male foetus) mesonephric components could not be identified. There is central to peripheral progression in metanephric kidney development and there is no cortico-medullary differentiation of functional components.

**13-24 wks GA group:** In this group a total of nine foetuses were observed for renal histogenesis. Sections from a specimen of 16 weeks and that of 24 weeks were compared for the developmental progression. During this period cortico-medullary differentiation was observed. At 16 weeks there is increase in the size of the kidney and lobulation was visible. Differentiation of cortex and medulla could be identified. Growing renal pyramid, renal papilla and renal columns could be identified at 16 wks. and 24 wks. Differentiation of developmental components proceeded from deep cortex to the periphery. Cortex presented three zones. From periphery to centre they are nephrogenic zone presenting immature renal corpuscles, intermediate zone of differentiating renal corpuscles and juxtamedullary differentiated renal corpuscles. Medullary rays and collecting ducts are well formed and are dividing. Capsule was well differentiated with numerous collagen fibres running parallel to the long axis of the kidney. Beneath the capsule many developing glomeruli were seen in the nephrogenic zone which were now compactly arranged due to decrease in the interstitial tissue. Another important finding in this group was that the parenchyma of the two adjacent lobules was separated by the nephrogenic zone. This zone is similar to the nephrogenic zone seen under the capsule and extends from the subcapsular area to the pelvis of the kidney. It gives the appearance of a column or septum and is known as the primary columns Bertini. This column

marks the junction of primary lobules. Pelvis became extended and many minor calyces open into it. The epithelium lining the pelvis at this stage was multi-layered. Branching and differentiating collecting ducts and undifferentiated tubules at 16 weeks and differentiated tubules at 24 weeks could be identified. Stratified collecting ducts at 16 wks. and differentiating Henle's loops at 24 weeks were observed.

**More than 25 weeks GA group:** Nephron Induction and differentiation were observed at 28 weeks. Well differentiated Renal corpuscles, glomerulus, tubules, collecting ducts and Henle's loops could be identified at 28 wks. GA Comparison of sections of kidney at 28 and 32 weeks presented juxta-cortical ampullary arcades at 28 wks. and degenerating renal corpuscles at 32 wks. At 40 weeks there is disappearance of nephrogenic zone with continued renal vesicle differentiation and interstitial growth.

## Discussion

According to Hamilton, Mossman and Boyd textbook of embryology the renal morphogenesis starts at 5th week of gestation and extends up to last month of last trimester of pregnancy. But, there were no detailed histological depictions of microscopic observations on developing human kidneys. All interpretations were based on animal studies. As per the literature all three stages of nephrons appear during 5th week. But, in our study pro and mesonephric stages were visualized simultaneously at 5th week and meso and metanephric stages at 6th week.[8] In the literature the earliest description on nephrogenic events in human was that at 10 weeks GA.[9] In the present study the nephrogenic events in the 5th, 6th and 8th weeks of embryonic period were observed that facilitated observations on certain of the events that were reported in animal studies. The degenerating pronephros, Urogenital mesentery, pronephric tubule, pronephric tubule opening in to coelomic cavity, absence of glomerulus, presence of Renal (R), gonadal (G), and Supra renal (SR) primordia, pronephric and paramesonephric ducts, mesonephric tubules and cloaca were observed at 5 weeks GA and could be described in detail that was not reported in literature for human embryos.[9] In the present study at 6wks GA metanephric kidney, urogenital mesentery containing mesonephric kidney and gonad, degenerating mesonephric tubules, mesonephric and paramesonephric ducts, renal corpuscles, 'S' and vesicle stages of metanephrogenesis were observed. Mesenchymal epithelial transition (MET) and various stages in nephrogenesis i.e. interaction, cap, blastemal, vesicle, comma and renal corpuscle could be identified as early as 6th week. A similar description of interaction between ampulla of ureteric bud and adjacent metanephric tissue was reported at 10 weeks by Shalika sharma and Sunanda Raina [10]. Bhattam Narasinga Rao and Mantraratnam Pramila Padmini (2012)[10], reported Invagination of nephric vesicle to form S or V-shaped bodies and glomerulus at 14th week of gestation, whereas the same were observed at 6 weeks in the present study. Takano et.al., (2007)[11] observed V shaped at 13-19 wks and 'S' shaped glomeruli at 20 – 24 weeks. According to Maria et al (2002)[5] nephrogenesis in human starts at 6th week of intrauterine life and is completed by 35th week of gestation which is similar to our findings. Differentiation of nephron was not complete at 6 weeks though quoted in the literature. Cortico-medullary differentiation, primary columns of Bertini, was observed at 16 wks GA. Sabita et.al.[12] Bhattam narasinga rao reported cortico-medullary transition at 16 wks which was similar to the observation in the present study. Shalika sharma and Sunanda Raina[9] reported the same at 14 weeks. According to Hamilton, Mossman and Boyd this appears at 12 weeks GA[8] Hosapatna M et al (2015)[13] also did not observe distinct differentiation of cortex and medulla until 12th week. Anant Dinesh et.al., (2006) reported corti co-medullary demarcation at 18-25 weeks.[14] The difference in the time of appearance reported by different studies could be due to racial differences. Nephron arcades were observed at 16 weeks in the present study while the same was reported at 16-18 weeks by Shalika sharma and Sunanda raina.[9] Medullary rays could be observed at 24 weeks in the present study and were reported at 28 weeks by Shalika sharma

and Sunanda raina. According to Sadiqali, et al. (2012).[15] and Hosapatna M, et al. (2015).[13] at 14 weeks of gestation the section of kidney showed a nephrogenic zone containing undifferentiated mesenchymal cells just underneath the capsule and their thickness decreased at 32 weeks. In the present study the nephrogenic zone was observed at 16 weeks and was absent at 40 weeks. In 8 wks embryo differentiating Renal corpuscle (RC), Proximal convoluted tubule (PCT), Distal convoluted tubule (DCT), Collecting Duct (CD) could be identified in the present study. At 12 week there is central to peripheral progression in metanephric kidney development and there is no cortico-medullary differentiation of functional components. According to Tank, et al. (2012).[6] at 12 weeks the kidney was covered by a thin capsule with an undifferentiated corticomedullary junction. Shalika Sharma, Sunanda Raina (2014)[9] reported very small and undifferentiated renal pelvis at 10 weeks and fully differentiated at 18 weeks. They reported Zone of transition between cortex and medulla starting at 14 weeks, presence of lobulation as early as 10 weeks and lobular fusion at 15 weeks of gestation. In our study these were observed at 16 weeks GA. According to Maria et al (2002)[5] Mishra S, et al. (2006)[16], Sadiqali, et al. (2012)[15] and Hosapatna M, et al. (2015)[13] corticomedullary differentiation occurs between 20- 24 weeks. According to Maria H et al (2002) the corticomedullary differentiation completes between 25-30 weeks.[5] Similar findings were observed in the present study. By 40 wks GA disappearance of nephrogenic zone and continuation of vesicle differentiation was observed which was similar to that reported by Sadiqali, et al. (2012)[15] and Hosapatna M, et al. (2015).[13] According to DakoviæBjelakoviæ, et al. (2005)[17] in the superficial part of the cortex, nephrogenic zone was very large at lower weeks of fertilization but as weeks of fertilization increased size of nephrogenic zone decreased and it was absent at 36 weeks of fertilization. Shimada K et al (1993).[18] Observed nephrogenic zone in all kidneys before 34 weeks of gestation, and disappeared in all kidneys after 37 weeks. Potter L (1965).[19] Tank K. C. (2012)[6], Morag S (1959)[20] in their study observed the disappearence of nephrogenic zone by 36th week where as In the present study the it was observed till 32 weeks.

## Conclusion

We concluded that the two weeks delay in the appearance of pro and mesonephric components that were observed at 5th week instead of 3rd and 4th weeks. Meso and metanephros appeared during the 6th week instead of 4th and 5th weeks. Differentiation of other components has not completed by 6th week. There is delay in further differentiation of metanephric components. Cortico-medullary differentiation (16 wks. Vs 12 wks.), demarcation of renal pelvis (16 wks. Vs 10-12 weeks), Pelvi-calyceal system (16 weeks Vs 12-14 weeks), morphologically recognizable nephrons (16 weeks Vs 12-14 weeks) and stage of complete differentiation of metanephric components (24-32 weeks). Major part of development was observed between 16-28 weeks instead of 16-24weeks as reported in literature. Ampulla division continued beyond 24 weeks, increased number of mature nephrons is seen between 24-28 weeks instead of 16-20 wks. Nephron arcades were observed between 24-28 weeks instead of 14- 22 weeks when compared with literature, delay of 2 to 6 weeks in the chronology of appearance/disappearance/differentiation/maturation of various morphological components in the ontogenesis of metanephric kidney while recapitulating its ancestral history (phylogeny) were observed in the present study.

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