Original Article

Indian Journal of Anatomy
Volume 9 Number 4, October – December 2020
DOI: http://dx.doi.org/10.21088/ija.2320.0022.9420.2

Morpho–Metrical and Stereological Analysis of Human Fetal Kidney During Mid-Gestation Period Ranging from 12th to 35th Weeks to Study Growth Pattern of Kidney in Qualitative and Quantitative Aspects

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How to cite this article:

Kirti Sudhakarrao Solanke, Rajan Bhatnagar, Boruah Dibyajyoti, et al. Morpho–Metrical and Stereological Analysis of Human Fetal Kidney During Mid-Gestation Period Ranging from 12th to 35th Weeks to Study Growth Pattern of Kidney in Qualitative and Quantitative Aspects. Indian J Anat. 2020;9(4):243–250.

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Abstract

Morpho–metrical and stereological analysis of human fetal kidney during mid-gestation period ranging from 12th to 35th weeks of gestation to study growth pattern of kidney in qualitative and quantitative aspects.

Aims and objective: Carry out Morpho-metrical Analysis of human fetal kidney during mid-gestation period ranging from 12th to 35th week and evaluating following parameters:

a. Physical dimensions and weight of each kidney.

b. Glomerular area for each kidney at different gestational ages.

c. The numerical density of glomeruli in the cortex of each kidney at different gestational ages.

d. Volume of glomeruli in the 3 different zones of each kidney at different gestational ages.

Methods: For study purpose we divided 31 aborted fetuses into six groups according to gestational age. After measuring external parameters such as CRL of fetuses; they were injected with 10% formalin and fixed in 10% formalin for 3 days. Kidneys were procured; physical dimensions, volume and weight of kidney were measured. After tissue processing, blocks were cut; sections were mounted and H&E stained. Using software analyzer density, area, volume of glomeruli at three different zones i.e. cortex, juxtramedullary and medullary regions were calculated. Glomerular area and volume of cortical, juxtamedullary and medullary region for each kidney at different gestational ages was estimated by measuring the area and volume of 100 glomeruli per fetal kidney under 20X objective lens of image analyzer. Comparison was done for three different zones.

Keywords: Kidney; Glomeruli; Density; Steriology; Volume; Area; Cortex; Medulla.

Introduction

With prevalence of end stage kidney disease on the rise worldwide and facilities of kidney transplantation being available in a large number of centres; demand for donor kidneys are increasing at the rate unrecorded in the history. There have been some approaches to development of kidney or kidney like structures in vitro.

The size[1] of kidney is dependent on the number[2] and size of nephrons[3] and is presumably influenced both by genetic and environmental factors and so are the number of glomeruli at birth.[4]

The total filtration surface area of kidney depends on the glomerular density and the glomerular surface area.[5] Any variation in these factors alters the total filtration area which is thus a useful indicator of renal development and function. Many methods have been used to derive glomerular sizes and all of them have their own shortcomings.[6] The estimation of renal glomerular volume is a useful technique with clinical, diagnostic and prognostic relevance in several conditions including renal artery stenosis, glomerulosclerosis and glomerulomegaly.[7] Some authors have presumed that the retardation of renal development, as occurs in individuals of low birth weight, gives rise to increased postnatal risk of systemic and glomerular hypertension, as well as enhanced risk of expression of renal diseases such as aplasia, hypoplasia, cystic diseases or renal agenesis.[8] Thus, in utero detection of anomalies will prevent delay in postnatal diagnosis and enable early surgical repair of significant lesions.[9]

One in three adults worldwide is suffering from high blood pressure. Forty percent of adults worldwide aged 25 and above had raised blood pressure in 2008. Theme by WHO for 2013 on world health day is "The Global brief on hypertension: silent killer, global public health crisis".[10] One of the causes for high blood pressure is reduced nephron number during fetal life. Studies showed that retarded intrauterine growth may be associated with significant reduction in nephron number and size.[11,12] Knowledge about number, size, density, volume and distribution of kidney thus renders significant knowledge about organization of kidney.

In the era of modern technology and equipments developed for diagnostic and therapeutic procedures in the field of medical sciences and improved survival of premature neonates in recent years, it is worthwhile to investigate the effects of premature delivery on the kidney, in which nephrogenesis is still ongoing during the third trimester.[13] In fetuses with no abnormality; nephrogenesis is completed between 32 weeks to 36 weeks, nephrons are not formed after birth.[3] It was found that nephron number in stillborn infants with intra uterine growth retardation (IUGR) was significantly reduced compared to infants that were appropriately grown-for gestational age.[14] Intra uterine growth retardation affects nephrogenesis. In case of preterm infant, nephrogenesis is seen even after birth and is evidenced by active nephrogenic zone which is observed along with increased number of immature glomeruli.[15,16] Thus, it becomes important to have sound knowledge of the basic human morphology and developmental anatomy.

Though reports pertaining to histogenesis of human foetal kidney do exist; not much work has been carried out covering both morphological and stereological aspects of development.[17] The present study aims to study the histogenesis as well as carry out morpho-metrical analysis of density, area and volume of glomeruli of human foetal kidney during intrauterine period ranging from 12th to 35th week.

Aim and Objectives

Carry out Morpho-metrical Analysis of human foetal kidney during mid-gestation period ranging from 12th to 35th week and evaluate following parameters:

a. Physical dimensions and weight of each kidney.
b. Glomerular area for each kidney at different gestational ages.
c. The numerical density of glomeruli in the cortex of each kidney at different gestational ages.
d. Volume of glomeruli in the cortex of each kidney at different gestational ages.
e. The total volume of each kidney to be estimated using Archimedes' Principle.

Materials and Methods

Collection of Material: Thirty one aborted human foetuses were collected from labour room of Obstetrics and Gynaecology department of a tertiary care hospital in, Wanvorie, Pune, after taking informed consent of parents and following all ethical norms and clearances. Foetuses with different gestational age ranging from 12 weeks to 35 weeks which showed no abnormalities on macroscopic examination were taken.

These foetuses included spontaneous abortions and stillborn foetuses. Twins and foetuses with gross anomalies of urogenital system were omitted from our study. 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.

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.

The physical dimensions and weight of each kidney were measured using a linear scale, vernier callipers, divider, thread and a balance. Volume of each kidney was ascertained by Archimedes's principle. Kidneys were preserved in 10% formal saline.

Estimation of total renal volume: Total volume of each kidney was measured by Archimedes principle. Whole kidney was immersed in measuring flask and displacement of water was seen and measured.

After measuring volume, fixed enblock kidneys were processed to prepare paraffin blocks. Sections were cut with rotary microtome 5 to 7 micron thick then mounted and stained.

Morphological Analysis: The histological sections of kidneys belonging to foetuses of different age of gestation were studied using a Olympus binocular light microscope under 4X, 10X, 40X objective lenses with the aid of an interactive image analyzer and observations were noted down.

Stereological Procedures

Estimation of numerical density of glomeruli in cortex, juxtamedullary and medullary regions: The numerical density of glomeruli in cortex, juxtamedullary area and medulla of each kidney at different gestational ages was estimated by observing 10 microscopic fields per foetus under 20X objective of image analyzer with aid of specific software package Dewinters Biowizard 4.2.

Microscopic field area was kept constant and the counting was done for all fields using grids to reduce counting errors. The average particle count was estimated and numerical density was calculated with the aid of software Dewinters Biowizard 4.2.

Estimation of glomerular area and volume: Glomerular area and volume of cortical, juxtamedullary and medullary region for each kidney at different gestational ages was estimated by measuring the area and volume of 100 glomeruli per fetal kidney under 20X objective lens of image analyzer with the aid of software package Dewinters Biowizard 4.2.

Steps using Dewinters Biowizard 4.2 software: Steps of estimating density, area and volume of glomeruli using software package Dewinters Biowizard 4.2 are given below:

1. Photograph of H and E stained slide was taken:

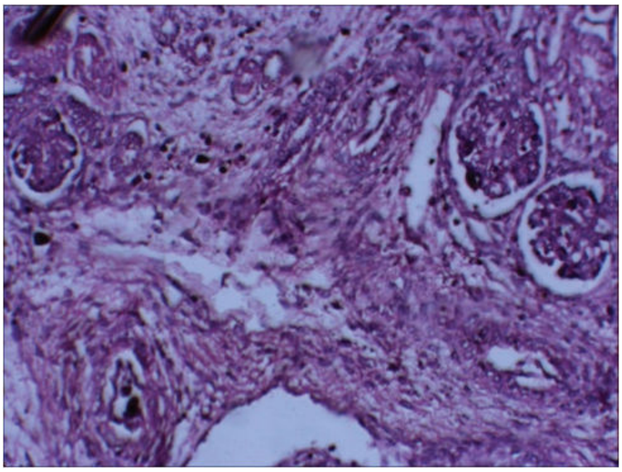


Fig. 1: Photograph of H and E Stained Slide.

2. Photograph was edited in paint. We marked thin lines with white colour around each glomerulus in each photograph as depicted in photograph below. After this photographs were opened with the help of software package Dewinters Biowizard 4.2.

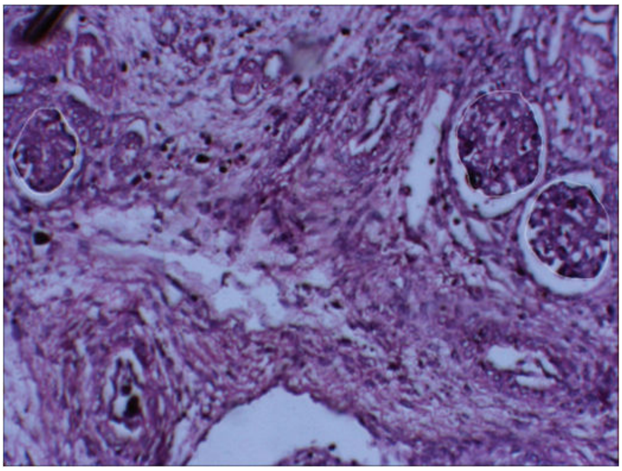


Fig. 2: Glomeruli Marked with White Color.

3. Software read glomeruli and gave all calculations about it as shown below:

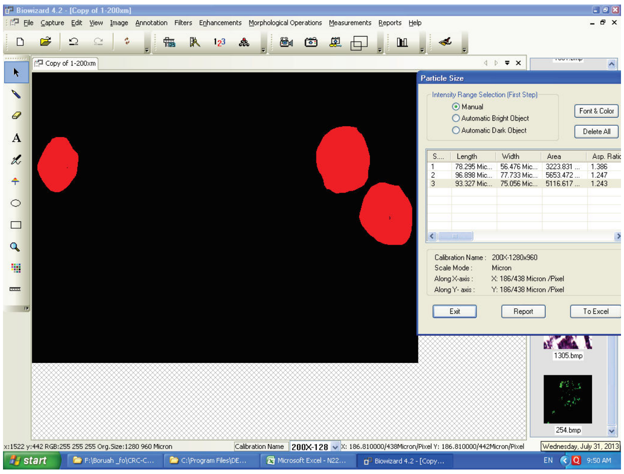


Fig. 3: Image on Software with Particulers.

Observations and Results

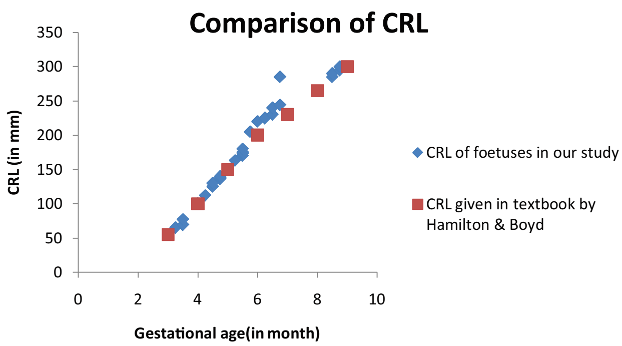
Physical Dimensions: The CRL was correlated with the gestational age of the foetuses and is shown in Table 1.

Table 1: The CRL was correlated with the gestational age of the foetuses.

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 2: 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: Crown-rump length (CRL).

Crown-rump length (CRL) of foetuses in our study was correlated with CRL given in text book. Foetuses which were small for age were removed from the study (Graph 1).

We measured weight with digital weighing machine and volume by Archimedes principle. All data was tabulated in annexure-1. Width, thickness were measured at upper pole, hilum and lower pole and height was measured using vernier calliper and collected data was depicted in Table 3.

Steriological Observations

We divided our study samples in six groups for summarising the findings:

Table 3: Morphological Observations.

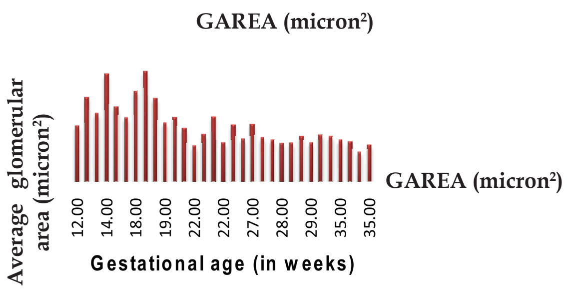
Age in weeks	Kidney width(mm)			Kidney thickness(mm)			Height (mm)
	At upper pole	At hilum	At lower pole	At upper pole	At hilum	At lower pole	
Group1(12-14 weeks)	3.75-6.04	3.38-5.69	3.49-5	3.64-3.86	3.06-3.86	2.73-4.65	6.93-9.3
Group 2(16-18 weeks)	5.56-6.15	4.9-5.9	5.6-7.04	4.41-6.43	4.51-7.83	3.83-7.29	12.82-12.87
Group 3(19-21 weeks)	8.83-11.37	8.12-10.12	9-9.55	8.55-9.18	7.45-8.82	7.03-8.62	18.86-20.52
Group 4(22-27 weeks)	11.37-15.7	10.1213.45	9.55-14.48	8.55-10.71	7.45-13.19	7.03-12.7	20.52-27.22
Group 5(28-29 weeks)	11.91-17.97	14.07-18	13.51-17.7	13.09-14.78	14.13-17.13	14.5-14.51	28.15-31.9
Group 6(34-35 weeks)	18.24-18.29	12.88-18.28	15.15-17.87	11.34-13.12	9.97-17	10.09-13.88	33.66-34

Table 4: Steriological Observations.

Age in weeks	Average glomerular area (micron²)	Estimated average glomerular volume (micron³)	Average glomerular density(GD)(per mm²)
Group1(12–14 weeks)	3814.52	198.990 x 10^3	34.93
Group 2(16–18 weeks)	4094.36	205.955 x10^3	30.26
Group 3(19–21 weeks)	2485.60	150.366 x10^3	35.43
Group 4(22–27 weeks)	2298.20	101.003 x 10^3	35.99
Group 5(28–29 weeks)	1945.15	73.397 x 10^3	44.66
Group 6(34–35 weeks)	1929.69	73.797 X 10^3	38.13

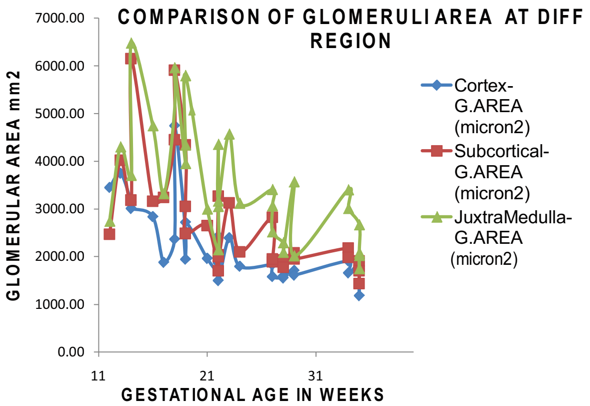
Table 4, annexure 3, 4 and 5 show glomerular area varied from 2000–4000 micron², estimated glomerular volume varied from 75.000 X 10^3 to 200.000 X 10^3 micron³ and glomerular density ranged between 30–45 per mm². In group 2 i.e. from 16–18 weeks, minimum estimated glomerular volume was 61.632 X 10^3 micron 3 at 17 weeks and maximum 361.267 X 10^3 micron 3 at 18 weeks and average density 30.26 per mm². In group 3 i.e. from 19–21 weeks minimum glomerular area was 1940.94 micron 2 and maximum was 5796.60 micron 2 with average of 2485.60 micron 2 that of group 4 (22–27 weeks) minimum glomerular area was 1499.94 micron² and maximum was 4570.53 micron². In group 5 (28–29 weeks) minimum density was 22.62 per mm² and maximum was 72.40 per mm².

Glomerular Area



Graph 2: Showing changes in average glomerular area with gestational age.

Glomerular area showed initial increase up to 16-18 weeks and there after gradual reduction. The average was around 2000–4000 micron² (Graph 2).

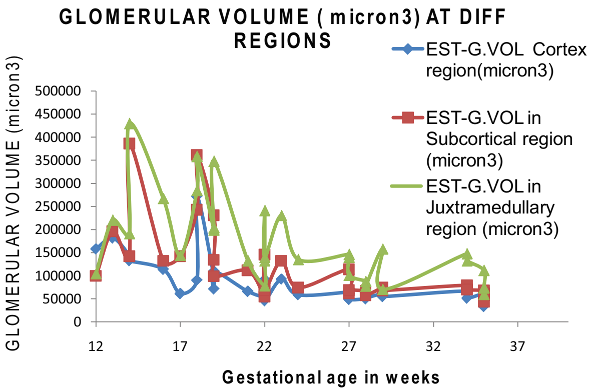


Graph 3: Showing comparisons of glomerular area at three different regions with gestational age.

The above graph 3 depicts comparison of glomerular areas at 3 different regions. It was found that glomerular area was largest in juxtamedullary region with maximum area being 6477.77 micron² and least at cortical region as 1181.14 micron². Glomerular area of subcortical region was in between. Glomeruli develop in cortical area and are then pushed towards medulla, thus area at juxtamedullary region was maximum. With increasing age, glomerular area decreases with exception at 14–18 weeks of age.

Estimated Volume of Glomeruli

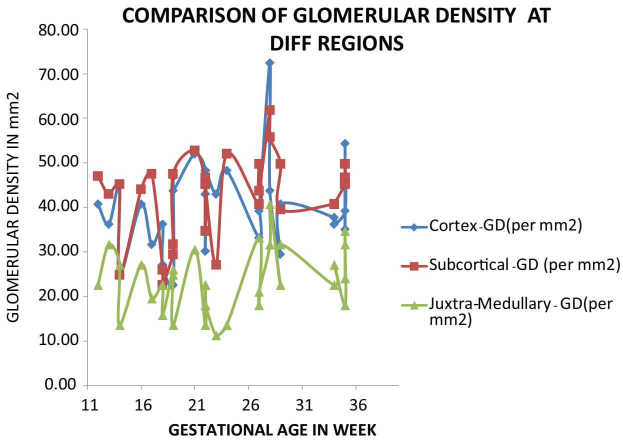
The volume of glomeruli showed gradual decrease from 12 weeks to 35 week with spurt at 14, 18, 19,24,28,34 weeks at more or less in all regions but most prominently in juxtamedullary region. Volume of glomeruli was largest with maximum of 429.991 X 10^3 micron^3 at juxtamedullary glomeruli and lest at cortical glomeruli as 33.715 X 10^{-3} micron.[3] Subcortical estimated glomeruli volume was in between (Graph 4).



Graph 4: Showing comparison of glomerular volume at three different regions with gestational age.

Glomerular Density

Graph 5 shows glomerular density constantly increases from 12 to 35 weeks, with exceptions at 14, 18 and 23 weeks and sudden spurts at 28 and 35 weeks of gestation. At 14, 18 and 24 weeks estimated volume of glomeruli increases suddenly hence decrease in glomerular density. Glomerular density was maximum at cortical and subcortical regions with maximum being 72.40 per mm^2 and was lowest being at juxtamedullary region as 11.31 per mm^2.



Graph 5: Showing comparison of glomerular density at three different regions with gestational age.

When we compared estimated average glomerular volume with that of average glomerular density, it was found that whenever estimated average glomerular volume increased, the average density of glomeruli decreased. Overall glomerular density exhibited a fluctuating trend with abrupt fall at 14, 18 and 22 weeks of gestation. Accordingly the these estimated glomerular volume showed abrupt spurt at similar weeks of gestation (Graph 6).

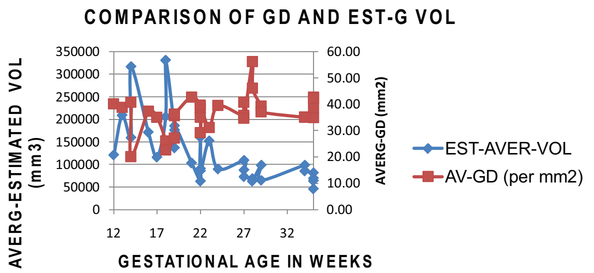


Table 5: Comparison of Glomerular Area Obtained in Present Study with Others.

Gestational Age (Weeks)	12Weeks			22 Weeks			28 Weeks			34 Weeks		
Authors	Souster et al	Sabita Mishra	Present study	Souster et al	Sabita Mishra	Present study	Souster et al	Sabita Mishra	Present study	Souster et al	Sabita Mishra	Present study
Cortical area (micron^2)	4891.1		3450.33	4086		2429.67	4492.7		1590.18	4383.3		1909.64
SD	1565		1408.19	276.7		1085.03	856.4		529.68	497		840.24
Subcortical area (micron^2)	7031		2464.82	4528	15313.71	3263.10	4578.8	6280.76	1773.21	4889.7		2175.48
SD	1525.9		1024.18	676.7		1320.18	745.7		781.19	764		1060.77
Juxtamedullary area (micron^2)	9736.5		2744.76	6961		4357.23	7537		2096.37	6951		3401.44
SD	1549.3		1916.71	214.4		1430.94	1059		754.83	1176		1822.48

Graph 6: Showing comparison of glomerular density (GD), estimated glomerular volume with gestational age in weeks.

Discussion

Stereological

Sabita Mishra et al. in 2006 stated volume of kidney showed linear growth from 14th week to 28th week with growth spurts during 22nd and 28th week. The present study delineated similar findings but a growth spurt was not appreciated. This might be because of difference in methods used for measuring volume of kidney. Same author also stated that glomerular density exhibited a fluctuating trend with abrupt fall during 16th, 22nd and 28th week. Present study showed similar fluctuating trend with abrupt fall during 14th, 18th, 23rd week and an abrupt rise during 28th and 35th week. This variation could be because of the number of foetuses studied. Sabita Mishra et al measured glomerular area with average being around 6000–7000 sq. microns. Glomerular area showed initial increase and then gradual fall. In the present study average glomerular area was around 2000–4000 sq. microns and showed a similar trend as that noted by Sabita Mishra et al. 18.

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. With increasing gestational age kidney weight and volume were seen increasing gradually and glomerular area and glomerular volume were seen decreasing with some exceptions. Glomerular density showed a fluctuating trend.

Clinical correlation

The Present work is an attempt to study and analyse the growth pattern of kidney in human foetuses in qualitative and quantitative aspects which may be helpful in defining foetal kidney diseases such as agenesis, hypertension, hypoplasia, renal artery stenosis.[3]

Comparison of Glomerular Area Obtained in Present Study with Others

Volume measured by Archimedes' principle was seen constantly increasing with gestational age; these findings are in accordance with the study by Sabita Mishra et al.[18]

In the present study, kidney weight and kidney volume were seen increasing steadily but the same could not be seen with respect to glomerular volume and glomerular area. Nyengaard JR et al stated, that number of glomeruli and size of glomeruli showed significant negative correlation to age and a significant positive to kidney weight [19]. Reinaldo Manalich et al. 2000 measured the volume of glomeruli at 36 to 38 weeks as 158.8+/–49.89 μ^3 X 10^{-3} and stated that there was an inverse correlation between the number of glomeruli and volume of glomeruli and weight at birth and glomerular volume 11. Negative correlation between weight and glomerular volume was appreciated in our study as well.

Souster L P and Emery J L described that juxtamedullary areas were larger than cortical and subcortical region. Juxtamedullary and subcortical glomeruli showed initial decrease from 12 to 20 weeks but superficial glomeruli remained of same size from 12 to 40 weeks of gestation. In the present study juxtamedullary glomerular area was found to be larger.[6]

In the present study volume and weight of kidney increased with gestational age whereas glomerular area and volume were seen decreasing constantly with some exceptions at 14 and 18 weeks. Glomerular density showed a fluctuating trend in all three different regions i.e. cortical, subcortical and juxtamedullary. Area and volume of juxtamedullary glomeruli was largest while that of cortical regionthe smallest. Density was highest at cortical region.

Limitations: Foetuses included in current study were of gestational age between 12 weeks to 35 weeks. Foetuses of earlier weeks of gestation (first trimester) were not included because of scarcity of such aborted foetuses.

Conflicts of interest: The authors declare no conflicts of interest.

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