International Journal of Anatomy and Research,
Int J Anat Res 2016, Vol 4(1):1819-24. ISSN 2321-4287
DOI: http://dx.doi.org/10.16965/ijar.2015.341

Original Research Article

ESTIMATION OF GLOMERULAR SIZE BY MICROMETRY IN MIDGESTATIONAL PERIOD

Suvarna A. Gulanikar *[1], G.A. Shroff [2], A.R. kharkar [3], V.S.Mandhana [4].

*[1] Assistant Professor, Dept. Of Anatomy, MGM Medical college, Aurangabad, Maharashtra, India.

[2,3] Professor, Dept. Of Anatomy, MGM Medical college, Aurangabad, Maharashtra, India.

[4] Associate Professor, Dept. Of Anatomy, MGM Medical college, Aurangabad, Maharashtra, India.

ABSTRACT

Introduction: Changes in glomerular size play an important role in the initiation and progression of various glomerulopathies. There are few anatomical reports on the fetal glomerular size than on adult human renal glomerulus. Therefore, our study conducted, with an aim to estimate an average glomerular diameter in cortical zones of the kidney during 14th to the 28th weeks of gestation, to define periods of their most intensive growth, and to record differences of glomerular size between different cortical zones.

Materials and Methods: Thirty normal human fetuses without any congenital anomalies in midgestational period ranging from 14 to 28 weeks were selected for the study. The glomerular diameters were evaluated in hematoxylin and eosin-stained microscopic sections of the kidneys by micrometry in outer, middle, and inner zones of renal cortex. Average diameter was calculated in different groups and relation of them in all three zones of the cortex was analysed.

Results: The results showed that outer cortex glomerulus were smallest with the average diameter of 55.88 ±7.20 to 63.5±7.33 µm. At the deeper level, size of glomerulus increased. In the intermediate cortex average size of glomerulus is 69.85 ± 23.18 to 87.63 ± 9.37 µm. and the deepest juxta medullary glomerulus were largest in size with the average diameter of 94.61±14.18 to 117.47± 10.89 .

Conclusion: The present study concluded that smallest diameter of glomerulus was seen in outer cortex, while the largest one in inner cortex. In all the age groups [14-28 weeks] the glomerular diameter remained nearly same in each zone of the cortex. The Changes in glomerular size played an important role in the initiation and progression of various glomerulopathies. The importance of this study lies in determining the average glomerular diameter, which could be used as baseline values for studies of glomerular diseases. It may be possible to define the pathological changes in fetal kidney based on the present study.

KEY WORDS: Glomerulus, Cortex, Micrometry, Glomerulopathies.

Address for Correspondence: Dr. Suvarna A. Gulanikar, Assistant Professor, Dept. Of Anatomy, MGM Medical college, Aurangabad, Maharashtra, India. **E-Mail:** suvarnagulanikar@gmail.com

Access this Article online

Quick Response code	Web site: International Journal of Anatomy and Research ISSN 2321-4287 www.ijmhr.org/ijar.htm
DOI: 10.16965/ijar.2015.341	Received: 17 Dec 2015 Accepted: 06 Jan 2016 Peer Review: 17 Dec 2015 Published (O): 31 Jan 2016 Revised: None Published (P): 31 Jan 2016

INTRODUCTION

With an advent of modern technology and equipments for diagnostic and therapeutic procedures in the field of medical sciences, it becomes imperative to have sound knowledge of the basic human morphology and develop-mental anatomy. Early prenatal diagno-sis and the importance of genetic counselling are of great interest for evaluation of normal fetus anatomy.

Changes in glomerular size play an important role in the initiation and progression of various glomerulopathies. Estimation of glomerular size in the normal kidney provides baseline values for studies of glomerular diseases.

Bertram J F et al. (1995) States that the highly specialized architecture of the renal glomerulus is altered in a variety of disease states. Glomerular enlargement precedes glomerulo-sclerosis in idiopathic focal segmental glomerul-osclerosis (FSGS), diabetic nephropathy, obesity-related glomerulopathy, HIV nephropa-thy, reflux nephropathy, pre-eclampsia, sickle cell nephropathy and transplant glomerulopathy. Morphometric methods, including stereological methods, have been widely used to analyze these changes in both animal and human glomeruli [1].

There are few anatomical reports on the fetal glomerular size than on adult human renal glomerulus. The objective of the present study was to calculate an average glomerular diameter in cortical zones of the kidney during midgestational period, to define periods of their most intensive growth, and to record differences of glomerular size between different cortical zones.

Nephrogenesis during kidney development involves reciprocal molecular signalling between cells of the metanephric mesenchyme and epithelial cells at the tips of the ureteric branches [2]. The formation of nephrons lasts from week 9 to week 44 after conception, with each newly formed nephron opposed to the periphery of each renal lobe. Consequently, the earliest formed glomeruli are located centrally. Approximately 70–75% of the nephrons are found in the outer and midportions of the renal cortex [3].

Inner cortical (juxtamedullary) nephrons form first, while the most superficial outer cortical nephrons form last. In fetal kidneys, juxtamedullary glomeruli have been reported to be larger than outer cortical glomeruli [4].

Souster and Emery et al. (1980) [2] have shown that initially the glomerular size increases rapidly, while the difference between size of juxtamedullary and superficial glomeruli tapering progressively and disappearing after the age of 3 years [4].

Olivetti and co-workers et al. (1980) found a linear transcortical gradient of glomerular diameters in both young and old rats, with a mean volume of juxtamedullary renal glomeruli twice that of subcapsular glomeruli [5]. According to Arataki et al. (1926), the glomeruli of rats continue to increase in number and size until the age of 100 days [6].

Tsuda et al. (1943) described that in all cases the central glomeruli had a greater diameter than the peripheral ones, in some cases twice as great, as but never more than this [7].

Shimada K. et al. (1993) states that, The glomeruli were arranged in the uniform fashion on the medullary ray with the smallest in the most superficial cortex and the largest in the juxtamedullary zone. The superficial glomeruli remained at the surprisingly same size up to birth. Juxtamedullary glomeruli showed no significant differences before birth, either [8].

Dakovic-Bjelakovic M. et al. (2006) studied on changes of glomerular size during different gestational ages and concluded that superficial glomeruli had the smallest diameter, while juxtamedullary glomeruli were the largest. The average glomerular diameter increased during intrauterine development in all zones, most intensive in the X lunar month [37-40 weeks]. There was a significant difference of the glomeruli between different cortical zones in the young foetuses. Such significant difference receded as gestational age increased [9].

Tank K. C. et al. (2012) showed in their study that histologically the glomeruli were arranged in such a way that the smallest in the most superficial cortex and the largest in the juxtamedullary zone. The mean glomerular size in the outer, middle, and inner zone of the cortex was 43.8 µ, 51.5 µ, and 59.7µ respectively, and it remained nearly same in a particular zone of cortex at different weeks of fertilization from 12 to 36 weeks of fertilization [10].

Number of glomeruli increases gradually between 10^{th} and 18^{th} of gestation and then increases rapidly until 32 weeks then an upper limit is reached. At term nephron formation is complete, with each kidney containing about 2 millions of nephrons [11].

Once glomerular production is brought about to a halt, it never resumes subsequently. Increase in kidney size is the result of increasing length of tubules and associated increase in blood vessels and connective tissues [12].

MATERIALS AND METHODS

Thirty human fetal kidneys were studied in the present study. Thirty aborted fetuses of midgestational age without any congenital anomaly were collected from the Department of Obstetrics and Gynaecology MGM medical college, Aurangabad after taking written permission of parents. Each specimen fetus was tagged with an identification number.

The proper fixation of fetus was done with 10% formalin solution for 2 to 3 days. Macrodissection was done to extract both kidneys enblock. Only normal kidneys were included in the study. The associated fat, fascia, nerves and other unwanted tissues were removed from the dissected fetal kidneys.

The kidneys were kept in 10% formalin for 24 hours and then processed for dehydration, clearing, embedding. Then paraffin blocks were prepared. Tissue blocks were, serially sectioned in a transverse plane parallel to the long axis of the kidney. 7 µm thick sections were taken using a rotary microtome. After affixing sections on glass slides, tissue was stained with Haematoxylin- Eosin (H&E) and Masson's trichrome stain.

All specimens, divided into 8 groups according to gestational age of the fetus for the purpose of histomorphometrical study of glomerulus. Group 1 - 14 weeks, Group 2 - 16 weeks, Group 3 - 18 weeks, Group 4 - 20 weeks, Group 5 - 22 weeks, Group 6 - 24 weeks, Group 7 - 26 weeks and Group 8 - 28 weeks.

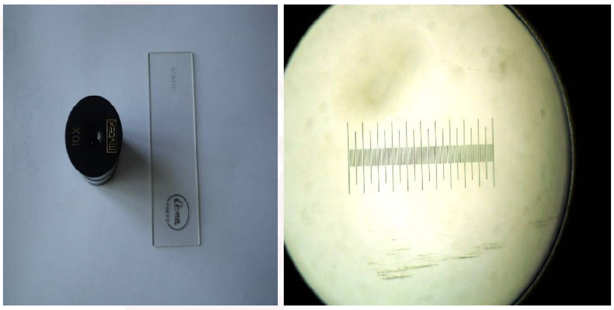
Method used for Micrometry [11]: Micrometry is a two-dimensional quantitative method that uses a gauge with a calibrated micrometer for the measurement of objects under microscopic observation. The Micrometer has two parts, a stage micrometer and an eyepiece reticle. The use of a stage micrometer to check the divisions and measurements on the eyepiece reticle is the best way to achieve this calibration.

Stage Micrometer (fig. 1): A Stage Micrometer is a microscope slide with a finely divided scale marked on the surface. The scale is of a known true length and used for calibration of optical systems with eyepiece reticle patterns.

Eyepiece Reticle (fig. 1): A linear measurement scale fitted into one eyepiece of the light microscope. The eyepiece scale is called as the reticle. This eyepiece reticle is interchangeable. The reticle is used to measure any plantar dimension in a microscope field, since the ocular can be rotated in any direction and the object of interest can be repositioned with the stage manipulators.

The present study used the micrometer for micrometry. The divisions and measurements on the eyepiece reticle are used to achieve the calibration.

Fig. 1: Stage Micrometer and Eyepiece Reticle.



Conversion Factor: To get the conversion factor, we calibrated the microscope and its associated reticle with a stage micrometer.

The following was the procedure: 1. Select normal or lowest magnification. 2. Ensure the eyepiece reticle is in sharp focus, 2. Place the stage micrometer on microscope stage. 3. Adjust position and focus so the stage scale is clearly visible, 4. Rotate the eyepiece reticle and position the stage in the field of view so that the two scales appear parallel, one positioned above the other 5. Adjust the alignment of the scales so that the zero values correspond.

With the zero values aligned, the stage micrometer scale will either appear longer than the eyepiece scale or alternatively it may appear the same length or shorter than the eyepiece scale. In the present study the conversion factor calculated as given below.

The stage scale appears shorter than the eyepiece scale: Note how many eyepiece divisions match the full length of the stage micrometer scale. Using a Stage Micrometer with 100 divisions of 0.1mm, which is the total length of the scale equal to 1000 µm. So, 1 division is equal to 10 µm.

Selected objective magnifications 4X: The full length of the stage micrometer scale covered 29 divisions of the reticle. So the first 29 divisions of the reticle scale are 0.1mm long which is equal to 100 divisions the full length of the reticle scale equals 1000 µm. So each reticle division is equivalent to (1000/29) = 34.5µm. For an eyepiece reticle with 100 divisions, each division will measure 34.5 µm at the stage for this magnification.

Selected objective magnification 10 X: The stage scale now covers 79 divisions of the reticle scale. For an eyepiece reticle with 100 divisions, each division will measure [1000/79] = 12.7ìm at the stage for this magnification.

Therefore, we can apply these conversion factors to state what each division of the eyepiece reticle is measuring for a selected magnification.

4X - 1 division = 34. 5 µm
10X - 1 division = 12.7 µm
If the stage scale appears longer than the eyepiece scale: Count the number of eyepiece reticle divisions that cover the stage micrometer scale.

The Conversion Factor: A Stage Micrometer with 100 divisions in 0.1mm having a total length of 0.1m.m.

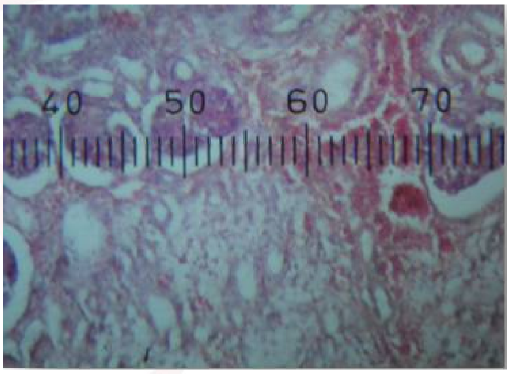
Selected objective magnification 40 X: The full length of the reticle scale only spans the first 30.5 divisions of the stage scale. We know that 100 divisions of stage scale are equal 0.1 mm = 1000 µm. And 1 division =10 µm. So 33.5 divisions of stage scale equals to 305 µm, which is equivalent to 100 divisions of eyepiece reticle. So 1 division of the reticle scale will measure [305/100] =3. 05 µm.

The conversion factor is: **40X - 1 division = 3. 05 µm**

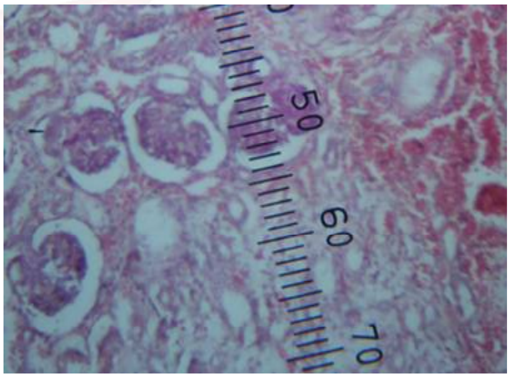
Histomorphometry of glomerulus: In each histological section the renal cortex was divided into three evenly spaced zones, Outer zone (superficial glomeruli), middle zone, and inner zone (juxtamedullary glomeruli). Outer cortical glomeruli were within 3-4 glomerular diameters of the capsule, while juxtamedullary glomeruli were within 3-4 glomerular diameters of the corticomedullary junction. The diameters of individual glomeruli from each zones of the cortex from each group were measured by using ocular micrometer [8]. [fig 2]

For measurement of glomerular diameter five random approximately circular glomeruli from each zone of the cortex were analysed. Two fetal kidney samples were studied in each group of gestational week. Total ten glomerular diameters were measured in each cortical zone in different groups. Average diameter of the three cortical zones was analysed and compared with the other studies. The data obtained showed specific rate of growth of fetal kidney in different gestational ages.

Fig. 2 : Measuring diameter of Glomeruli- Diameter of glomerulus= 7× 12.7 = 88.9 µm.



10 X- [14 weeks , middle cortex]



10 X- [14 weeks , middle cortex]

RESULTS

After staining with H and E and Masson's trichrome stains the following things were observed.

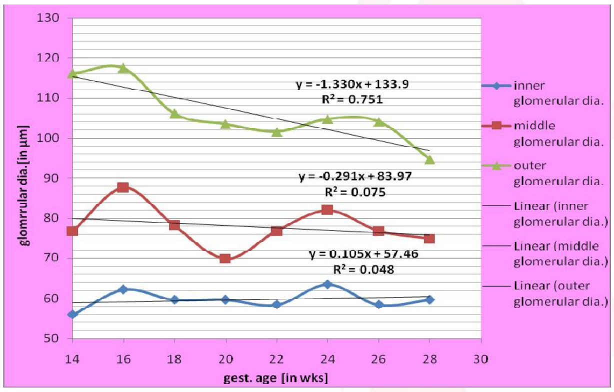
Glomerular Size: With the help of micrometry we calculated the diameters of individual glomeruli from outer, middle and inner zones of the cortex.

These observations suggest that Glomeruli in the superficial cortical zone had the lowest

Table 1: Relation of glomerular size in outer middle and inner cortex with gestational age of fetus.

Gestational age [In weeks]	Outer cortex		Middle cortex		Inner cortex	
	[In µm]	±SD	[In µm]	±SD	[In µm]	±SD
14	55.88	±7.20	76.83	±6.31	116.2	±11.61
16	62.23	±8.36	87.63	±9.37	117.47	±10.89
18	59.69	±7.09	78.1	±5.49	106.04	±10.42
20	59.69	±7.45	69.85	±23.18	103.5	±11.22
22	58.42	±8.36	76.83	±8.17	101.6	±10.36
24	63.5	±7.33	81.91	±8.70	104.77	±10.47
26	58.42	±6.55	76.83	±6.31	104.14	±10.017
28	59.69	±7.45	74.93	±8.36	94.61	±14.18

Graph 1: Relation of glomerular size in outer, middle and inner cortex with gestational age of fetus.



average diameter that is 55.88±7.20 to 63.5±7.33 µm. In the intermediate cortex average size of glomerulus is 69.85 ± 23.18 to 87.63 ± 9.37 µm. and the deepest juxta medullary glomerulus were largest in size that is 94.61±14.18 to 117.47± 10.89.

In present study, the period of intensive growth is between 14-16 weeks of gestation, while the size of the glomeruli in a particular zone does not significantly alter with different gestational age groups.

DISCUSSION

Dakovic-Bjelakovic M et al. (2006) [9] states that the average glomerular diameter in the outer zone continually increased from IV lunar month [13-16 weeks] (0.057 +/- 0.004 mm) to X lunar month [37-40 weeks] (0.082 +/- 0.004 mm), with highly significant correlation with gestational age (r=0. 755; p<0.01) . In the intermediate zone diameter increased from 0.081 +/- 0.004 mm (IV lunar month) to 0.096 +/- 0.004 mm (X lunar month) with low linear correlation with gestational age (r=0. 161). Juxtamedullary glomeruli were the biggest ones with their average diameter, during the IV LM ranged from 0.093 +/- 0.006 mm to 0.101 +/- 0.004 mm. In the IV and V lunar months [13 – 20 weeks] of gestation, there was a significant difference (p<0.01; p<0.05) between the average glomerular diameter in the different zones of the kidney cortex.

Tsuda et al. (1943) [7] stated that in all cases the central glomeruli have a greater diameter

Table 2: Comparison of average Glomerular diameter at outer middle and inner cortex with the study of Tank K.C et al. [10].

Gestational Age [In weeks]	Average outer glomrular Diameter [In µm]		Average middle glomerular Diameter [In µm]		Average Inner Glomerular Diameter [in µm]	
	Tank K.C et al. [10]	Present study	Tank K.C. et al. [10]	Present study	Tank K.C. et al. [10]	Present study
14	46.76	55.88	51.99	76.83	59.78	116.2
16	44.32	62.23	52.4	87.63	63.55	117.47
18	44.95	59.69	52.09	78.1	62.22	106.04
20	- - -	59.69	- - -	69.85	- - -	103.5
22	44.82	58.42	53.45	76.83	62.56	101.6
24	41.94	63.5	44.68	81.91	51.17	104.77
26	- - -	58.42	- - -	76.83	- - -	104.14
28	42.53	59.69	52.75	74.93	64.49	94.61

than the peripheral ones as high as twice. Robert L. Vernier et al. (1962) [12] observed that glomeruli of all stages of maturity from the most primitive to the mature, adult type of glomerulus were founded in the renal cortex of fetuses of all ages. Glomerular maturation was noted to proceed progressively from about 1 1/2 months (origin of metanephros) to about 35 weeks of gestation.

Shimada K et al. (1993) [8] reported that the glomeruli were arranged in the uniform fashion on the medullary ray with the smallest in the most superficial cortex and the largest in the Juxtamedullary zone

According to Tank K. C. et al. (2012) [10] the glomeruli are smallest in the most superficial cortex and the largest in the juxtamedullary zone. The mean glomerular size remained nearly same in a particular zone of cortex at different weeks of fertilization from 12 to 36 weeks of fertilization.

The present results correlate all the above workers study. It observes smallest diameter glomerulus in outer cortex, while the largest one in inner cortex. In all the groups [14-28 weeks] of the present study, all stages of maturation of glomerulus are seen. The glomerular diameter remains nearly same in different zones of the cortex, which supports the observation of Tank K. C et al. [10]. However, not follow the results of Dakovic-Bjelakovic M et al. [9].

Average glomerular diameters in outer, middle and inner zones of the cortex are comparable with the study of Tank K.C et al. [10]. It is possible to analyse functional maturation of fetal kidney with the help of glomerular diameter in different zones of the cortex.

CONCLUSION

The present study showed the significant difference of the size of glomeruli between different cortical zones with the presence of the smallest diameter glomerulus in outer cortex, and the largest in the inner juxtaglomerular cortex. The period of intensive growth is between 14-16 weeks of gestation, while the size of the glomeruli in a particular zone does not significantly alter with different gestational age groups.

The present study indicate that the micrometry of fetal glomerulus constitute an important tool which could extract as much information as possible from tissue specimens and set objective criteria for the definition of glomerular disease. It can complement molecular data with information on quantity that are amenable to statistical evaluation.

It may be possible to analyse functional maturity of fetal kidney with the help of change in glomerular diameter, which could be used as baseline values for studies of glomerular diseases.

Conflicts of Interests: None

REFERENCES

[1]. Bertram J F. Analyzing renal glomeruli with the new stereology. Int Rev Cytol. 1995;161:111-72.

[2]. Clark AT, Bertram JF. Molecular regulation of nephron endowment. Am J Physiol. 1999;276:F485-F497.[PubMed]

[3]. Moore L, Williams R, Staples A. Glomerular dimensions in children under 16 years of age. J. Pathol. 1993;171:145-150.

[4]. Souster LP, Emery JL. The sizes of renal glomeruli in fetuses and infants. J. Anat. 1980;130:595-602.

[5]. Olivetti G, Anversa P, Melissari M, LoodAV. Morphometry of the renal corpuscle during postnatal growth and compensatory hypertrophy. Kidney Int. 1980;17:438-454.

[6]. Arataki M. On the postnatal growth of the kidney, with special reference to the number and size of the glomeruli (albino rat). Am. J. Anat. 1926;36:399-436.

[7]. Tsuda. Histologic investigation of fetal kidney, Jap J.Obst.Gynec.1943;17:227-341.

[8]. Shimda K. , Hosokawa S., Tohda A. Histological study of fetal kidneys. Development of nephrons according to gestational weeks. Nippon Hinyokika Gakkai Zasshi. 1993;84(12):2091-2096.

[9]. Dakovic-Bjelakovic M. et al. Changes of glomerular size during fetal kidney development.S RP. Arh. Celok. Lek. 2006;134(1-2):33-39.

[10]. Tank K.C.,Saiyad S. S. ,Pandya A. M., Akbari U. J. Dangar K. P. A study of histogenesis of human fetal kidney, Int. J. of Biol. Med Res. 2012;3(1):1315-1321.

[11]. Todd J.C. Clinical Diagnosis by Laboratory Methods, Philadelphia, PA, W.B. Saunders Company, 1979

[12]. Robert L. Vernier. Studies of human fetal kidney :1 Development of glomerulus. Jour. Of Peadiatrics. 1962;60(5);754-768.

How to cite this article: Suvarna A. Gulanikar, G.A. Shroff, A.R. kharkar, V.S.Mandhana. ESTIMATION OF GLOMERULAR SIZE BY MICROMETRY IN MIDGESTATIONAL PERIOD. Int J Anat Res 2016;4(1):1819-1824. **DOI:** 10.16965/ijar.2015.341