ORIGINAL ARTICLE

Anthropometric and Histologic Study of Liver and Kidney of Foetuses and Their Correlation With Gestational Age

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ABSTRACT

Introduction: It is very important to accurately estimate the age of foetus for various medicolegal cases. This study is an attempt to establish a relationship between anthropometric measurements and histologic analysis of liver and kidney to identify gestational age of the foetus. **Methods:** The study was carried on 30 fetuses. Their anthropometric measurements were done using Vernier calipers. The data was statistically analyzed by computation to find out its normative value. Histologic analysis was done by preparing hematoxylin and eosin stained slides and looking under light microscope. The relationship between gestational age and data thus obtained was determined. **Results:** Size of liver and kidneys increased with every trimester. The kidney showed immature duct system and clustered glomeruli with lack of differentiation into cortex and medulla in first trimester. Tubular differentiation started in second trimester which finished in third trimester with formation of juxtaglomerular apparatus. Size of glomerulus was, however, maximum during second trimester, followed by first and third trimester. In liver, haemopoeisis was observed in first trimester which decreased with subsequent trimesters. Lobular differentiation increased with each trimester. However, full term liver did not have the classic lobular pattern. Size of sinusoids decreased with every trimester. Abundant fibrous tissue was observed around portal triad. **Conclusion:** There is a relationship between the gestational age and anthropometric measurements and histologic features of liver and kidney of the foetus. This will help in identifying foetal age as well as any congenital kidney and liver diseases.

Keywords: Liver, Kidney, Anthropometry, Glomeruli, Medicolegal

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INTRODUCTION

Estimation of gestational age of fetus is medico legally very important in cases of illegal abortion, suspected infanticide, and in cases of medical termination of pregnancy (1). The parameters which are generally used for estimating the gestational age are fetal weight, crown rump length, crown heel length, foot or hand length etc (2). But this estimation cannot always be accurate and it requires validation by additional figures. The histology of the embryological development of various organs and their anthropometric measurements can be used in identification of the gestational age.

Sometime a problem can arise during mass calamities, explosions, and assault cases where the dead body is disfigured to hide the identity of the victim and you may find only the organs of the victims and the fetuses in those cases the anthropometric measurements of these organs liver and kidney will help us in identifying the approximate age of the fetuses as compared to crown heel length.

Now a days premature babies can survive successfully. Because of that it is very important that doctors should have proper information in relation to gross and histological development of kidney and its physiologic functional activity at any point of gestational age (GA). Permanent kidneys appear from metanephros in the fifth week. Peripheral tubule formation takes place till fifth month. Continuous lengthening of tubules leads to development of proximal convoluted tubule (PCT), loop of Henle and distal convoluted tubule (DCT). Kidneys appear lobulated throughout intrauterine period as well as birth. These lobules get dissolved during infancy due to development of already existing nephrons. Number of nephrons, however, do not increase after birth (3).

Liver is positioned underneath the right dome of the

diaphragm and it is situated in the right hypochondriac and epigastric quadrants of the abdominal cavity. It has two anatomical lobes i.e right and left lobes which are separated by the line which pass through the attachment of various ligaments on liver like falciform, ligamentum venosum and ligamentum teres.1 Liver is proportionately huge during its embryological development as compared to other organs of the abdominal cavity. At around 12 weeks of gestation, it nearly fills the abdominal cavity. The left lobe during that time is same in size as the right because of the hematopoietic function of the liver. Later as the spleen and bone marrow take over, the left lobe regresses in size and degenerates (4).

Studies have been done to get insight into the normal histology of the foetal liver at different phases of embryonic development to understand the morphology of foetal liver and different functions it carries out in foetal life. Hepatic diverticulum arise in the third week, as an extension of endodermal epithelium, consisting of rapidly proliferating cells which enter septum transversum. Epithelial liver cords differentiate into hepatocytes and lining of bile ducts. Hepatic sinusoids are formed by epithelial liver cords along with vitelline and umbilical veins (4).

Embryonic development is a very critical era for human development. It is significant to understand the usual developmental anatomy and histogenesis of kidney and liver to have a well understanding of different congenital anomalies in both organs (5). Currently, there is no research regarding the anthropometric measurements of liver and kidney in each trimester. By knowing the anthropometric changes and histologic pattern forensic experts can get a rough estimate of the age of a dead found foetus and various congenital anomalies of liver and kidney. This study is an attempt to establish a method which can complement the inference made by crown-heel length measurement. By knowing the anthropometric variation, age of foetus in-utero can be determined any deviation from the generalized pattern can be suspected for abnormality.

MATERIALS AND METHODS

The present study was carried out in the Department of Anatomy, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, with due permission from Ethical Committee (Registration No. ECR/146/Inst/KA/2013). A total of 30 formalin fixed foetal cadaveric livers and kidneys (n=30), irrespective of the sex, were taken for this study. 10 foetal cadavers were taken from each trimester. (1st trimester- 1-12 weeks, 2nd trimester- 13-24 weeks, 3rd trimester- 25-40 weeks).

Livers and kidneys were taken out and their length (cm), breadth (cm) and thickness (cm) were recorded using Vernier calipers. Weight (g) was recorded using a weighing scale and volume (mL) was checked in a measuring cylinder using water displacement method. The liver and kidney specimens were subsequently processed for histologic study. They were passed through alcohol solutions of variable concentrations and xylol solution before fixing in paraffin. Thin sections were made using micro-dissector which were put on slides; stained with hematoxylin and eosin; and mounted. These slides were viewed under inverted microscope and Image pro premium 9.1 software were used to record the observations and measuring area (um2) and diameter (um) of kidney glomeruli. The measurements were tabulated and their mean and p value (in relation to each other) were calculated using SPSS.

RESULTS

Anthropometry of liver, right and left kidneys show that length, breadth, thickness, weight and volume are maximum for the third trimester, followed by second and first trimester respectively (Table I, II and III).

Table I: Showing Anthropometric data of liver

Trimester	Length (Mean± SD) (Cm)	Breadth (Mean± SD) (Cm)	Thickness (Mean± SD) (Cm)	Weight (Mean± SD) (Gm)	Volume (Mean± SD) (Ml)
First	2.62±0.53	3.70±0.84	1.94±0.35	101.80±51.7	10.5±4.9
Second	2.95±0.75	4.35±0.53	2.26±1.05	163.10±98.9	16.11±10.24
Third	3.88±1.44	6.31±1.25	3.11±1.09	457.75±31.2	44.45±28.2

Table II: Showing Anthropometric data of right kidney

Trimester	Length (Mean± SD) (Cm)	Breadth (Mean± SD) (Cm)	Thickness (Mean± SD) (Cm)	Weight (Mean± SD) (Gm)	Volume (Mean± SD) (Ml)
First	1.61±0.38	0.98±0.23	0.8±0.18	94.03±54.2	1.3±0.48
Second	2.15±0.54	1.23±0.33	0.92±0.28	177.41±0.13	2.22±1.39
Third	3.23±1.01	2±0.4	1.28±0.18	580.56±0.3	5.89±2.84

Table III: Showing Anthropometric data of left kidney

Trimester	Length (Mean± SD) (Cm)	Breadth (Mean± SD) (Cm)	Thickness (Mean± SD) (Cm)	Weight (Mean± SD) (Gm)	Volume (Mean± SD) (Ml)
First	1.67±0.4	0.93±0.24	0.84±0.18	89.55±48.3	1.3±0.48
Second	2.08±0.59	1.22±0.41	0.90±0.26	181.48±0.13	2.33±1.65
Third	3.08±0.89	1.9±0.51	1.49±0.27	522.07±0.27	5.56±2.9

The anthropometric measurements of one kidney are not correlated to the measurements of the other kidney of the same foetus in any trimester (p value was more than 0.05).

However, in liver, a correlation was found between every measurement with every other measurement (p value was less than 0.05), except between

- First trimester: Breadth and Thickness (p value 0.161)
- Second trimester: Length and Breadth (p value 0.11); Length and Thickness (p value 0.17).
- Third trimester: Length and Thickness (p value 0.31); Breadth and Thickness (p value 0.08).

Histology of first trimester kidney (under 10X) shows

glomeruli more clustered. Tubules are still developing. They lack differentiation. Second trimester kidney shows bigger glomeruli. Tubules differentiate into PCT and DCT. Medulla is still developing. In third trimester glomeruli take same size as that of a new borne child. Tubules appear developed (Fig. 1).

It is seen that the area and diameter of glomeruli increases from first to second trimester (maximum) and drastically decreases in third trimester (Table IV).

Histology of first trimester liver (under 4X) shows active haemopoeisis taking place. Lobules are not developed. There is no area of demarcation of each lobule. Abundant fibrous tissue is seen around the ill formed portal tracts. Hepatocytes are arranged as cords around the central vein and sinusoids are seen. Second trimester liver showed decreased haemopoeisis. Large sinusoids are seen. Hepatocytes are arranged as cords around the central vein. Lobular pattern is still not well defined. Third trimester liver has minimal active haemopoeisis. Vessels appear dilated. Classic lobular pattern is not evident. Hepatocytes are arranged as cords around the central vein and sinusoids are seen (Fig. 2).

Table IV. Showing mean of size of glomerulus measurements in histology of kidney

Trimester	Mean Area (Cm^2)	Mean Diameter (Cm)
First	7023.12	109.58
Second	7363.60	117.16
Third	5973.10	102.20

DISCUSSION

The American Liver Foundation issued a 'Pediatric Liver Research Agenda which promote that a good knowledge of embryonic liver development would deliver significant understandings into the management and preventive approaches for pediatric liver ailments (6).

Anthropometric measurements of both liver and kidneys length, breadth, thickness, weight and volume of fetuses increases as the gestational age of fetus increases. These findings will help us in estimating the age of fetus because as we know the mean measurements in each trimester we can identify the approximate age of the fetus. These anthropometric measurements has not been done till now by any of the authors.

We have also looked the histological development of both liver and kidney. We saw various changes in each trimester which we can utilize to find the age of the fetus and if there is any variation from these changes we can even identify any congenital anomalies in the fetuses.

In liver in 1st trimester we saw hematopoiesis which

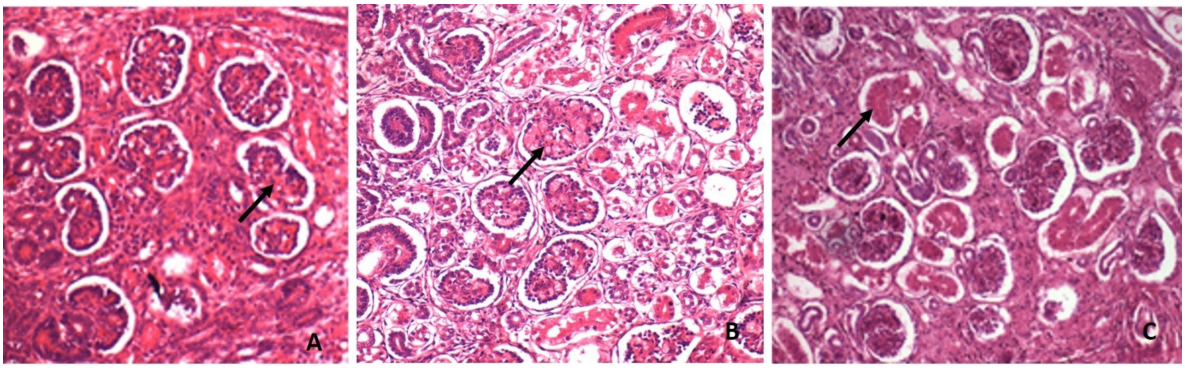

Figure 1: Showing histological sections of Kidney at 10X. A. First trimester, B. Second trimester and C. Third trimester. Arrow pointed at the glomerulus.

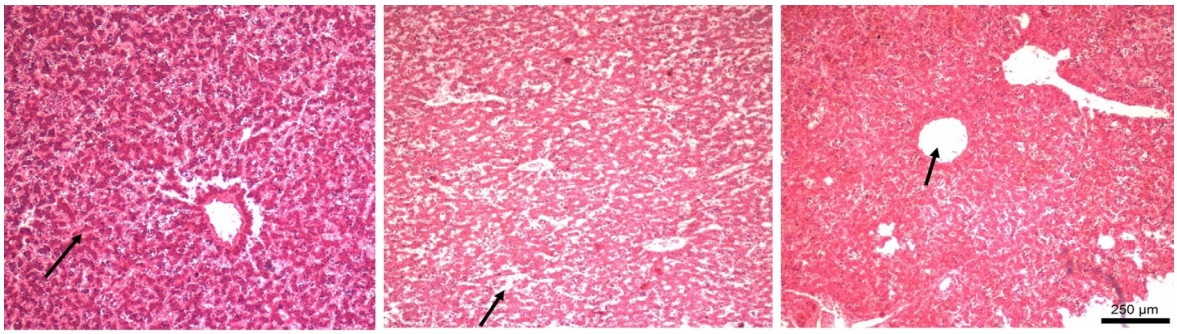

Figure 2: Showing histological sections of Liver at 4X. A. First trimester, arrow showing haemopoeisis B. Second trimester, arrow pointed at sinusoids and C. Third trimester. Arrow pointed at the Central vein.

decreases in 2nd trimester and still reduced in 3rd trimester. Hematopoiesis was seen in liver in 1st trimester as in fetus we know initial developing sites for blood cells was liver later this function will be taken by bone marrow as the fetus develops and therefore hematopoiesis decreases in 2nd and 3rd trimesters. Hepatocytes are arranged as cords around central vein and sinusoids are seen in 1st trimester itself.

In kidney glomeruli are clustered in first trimester kidney. Tubules were not differentiated. Second trimester tubules got differentiate into PCT and DCT. Medulla is still developing. In third trimester glomeruli take same size as that of a new borne child. Tubules appear developed. We also found that the area and diameter of glomeruli increases from first to second trimester (maximum) and then drastically decreases in third trimester. These all features will help in identifying the age of fetus.

Histology of liver

1st trimester

Sahoo S et al found at 6 to 8 weeks sinusoids and aggregation of hepatocytes, central vein was seen. In 10th week central vein was well formed with radiating cords of cells towards the periphery was seen, portal triad is visible. These results were similar to our results. (6) Jaiswal et al found that portal triad, hepatocyte arrangement around central vein, sinusoids all were started visible in 1st trimester which was similar to our findings (7).

2nd trimester

Sahoo S et al found in 12th week Hepatic lobule were formed. In 24 weeks central vein with sinusoid surrounded by periportal connective tissue was observed. These results were similar to our results (6).

3rd trimester

Sahoo S et al found in 28 weeks Glisson's capsule or hepato-biliary capsule covering portal triad was seen. In 36 weeks Portal triad, binucleated hepatocytes, central vein and sinusoids were observed. These results were similar to our results. (6) Jaiswal et al studied found that lobular pattern of liver was not defined till the third trimester which was similar to our study (7).

Datta AK suggested a developmental reason for the lesser size of the left lobe of liver. He mentioned that the hematopoietic function of the liver reduces significantly during the last two months of pregnancy. That's why there is reduction in the size of liver and mostly it involves the left lobe. Similar findings were observed in the presenting study (8).

Nayak, B.S observed a liver which had left lobe which was enormously elongated and another liver which was flat similar to a pancake during their study. Variation in liver size was noticed during the present study as well. Certain livers were occupying most of the abdomen even during second and third trimester and certain livers were flat irrespective of the trimester they belonged to (9).

Histology of kidney

Loss of glomeruli eventually leads to renal ailment, it is probable that people who are born with large numbers of nephron will be comparatively safe from renal ailment in future life, while people who are born with low numbers of nephron they are likely to be more susceptible. Therefore, to have long-term good renal health it is vital to have large number of nephrons at birth. So, to develop plans for doing this, it is vital to first understand the normal kidney development (10).

1st trimester

Tank KC et al found that there were more number of immature developing glomeruli in the 1st trimester. In our study we found that during 1st trimester glomeruli were more clustered (5). Sharma S and Raina S found that in a two and a half month old fetus, the kidney is in an undifferentiated form. These findings were similar to our study we also found that in 1st trimester kidney was not fully differentiated into cortex and medulla (13).

2nd trimester

Ryan D et al found that there was a strong positive association between gestational age and kidney weight from 20 weeks of gestation up to full term in our study also the weight increases with the gestational age. They also found that in female foetuses there was a positive association between renal corpuscle cross-sectional area and gestational age but in male foetuses renal corpuscle size remained comparatively same over the gestational period. In our study we found that glomerulus size increases from 1st to 2nd trimester but in 3rd trimester it decreased (10).

Hosapatna M et al observed that by 16 weeks well differentiated PCT (with distinct brush border) and DCT were formed. Immature duct system was noticed in the medulla. Similar features were observed in the present study also (11). Tank KC et al found that during 2nd trimester they found that PCT and DCT were identifiable which was similar to our study (5).

Mishra S et al found that volume of kidney showed a linear growth from 14th week to 28th week of gestation. The glomerular area showed an initial increase up to 16-18 weeks and then a gradual fall at 28th week. (14) These all findings are similar to our study we also got increase in volume of kidney s the age advances and glomerular area initially it increases in 2nd trimester but in 3rd trimester the area decreases.

3rd trimester

Hosapatna M et al observed that by 28th week the sections of DCT were seen next to to the renal corpuscles signifying the emerging juxtaglomerular apparatus.

Similar features were observed in the present study also (11). Ram KS et al found that PCT and DCT were more noticeable after 18th week of gestation. In our study also we noticed that tubules differentiate into PCT and DCT during the 2nd trimester (12).

Tank KC et al found that during 3rd trimester they found that the medulla has well differentiated collecting tubules and thick and thin segment of loop of henle in our study also we found that tubules were well developed in 3rd trimester. They also found that size of glomerulus increases with advancing gestational age but in our study we found that glomerulus size increases from 1st to 2nd trimester but in 3rd trimester it decreased (5).

Sharma S and Raina S found that cortex and medulla can only be differentiated at 14 weeks of gestation. At 14 weeks of gestation enormous number of glomeruli, proximal convoluted tubules and distal convoluted tubules can be seen in cortex and it is well differentiated. In medulla portions of collecting tubules and the loop of henle can be seen at the end of 14 weeks of gestation (13). These findings were similar to our study we also found that PCT and DCT develops in 2nd trimester.

The results of our study will help in determining the age of the foetus in-utero and any deviation from the generalized pattern which was seen in our study will help in identifying any abnormality and congenital anomalies. It can be used by forensic experts to estimate the age of dead found foetus.

CONCLUSION

In our study we found that all anthropometric measurements of foetal kidney and liver increased with gestational age. Glomerular size was increased in 2nd trimester but decreased in 3rd trimester. In liver in 1st trimester we saw hematopoiesis which decreases in 2nd trimester and still reduced in 3rd trimester. Hepatocytes are arranged as cords around central vein and sinusoids are seen in 1st trimester itself. These anthropometric changes and histologic pattern in each trimester, will help in determining the age of the foetus in-utero and any deviation from the generalized pattern can be suspected for abnormality and congenital anomalies. It can be used by forensic experts to estimate the age of dead found foetus.

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