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Histogenesis of human fetal liver of various weeks of gestation

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Abstract
Pediatric liver transplants accounting for 10-15% of all liver transplants worldwide occur due to congenital defects. The main etiological factors behind liver transplantation are congenital liver defect. The American Liver Foundation published a 'Pediatric Liver Research Agenda which advocate that a better understanding of embryonic liver development would provide important insights into treatment and preventive strategies for pediatric liver disease. The present study aims to see the detail histogenesis and development of human liver in prenatal period.

Keywords: liver transplantation, histogenesis, perinatal period, embryonic liver development

Introduction
Liver is the largest gland in the human body [1] and it is among the few internal human organs capable of natural regeneration of lost tissue as little as 25% of remaining liver can regenerate [2]. It develops as a ventral outgrowth from the gut endoderm, in the region of the anterior intestinal portal during 3rd week of gestational age [3, 4]. Exocrine part of the liver secrets bile and endocrine part of the liver secretes chemical substances such as glucose from glycogen and most of the plasma proteins. Many functions of the body, includes glycogen storage, decomposition of the red blood cells. Plasma protein synthesis, detoxification and hematopoietic function in the foetus carried out by liver. Total hepatectomy with liver transplantation may be the most difficult operation ever derived both technically and physiologically.

Aims and Objectives
The present study aims to study histogenesis and development of human liver in prenatal period to observe microscopic structure of liver at various gestational age groups and its future implications in cadaveric liver transplantations in hepato-cellular carcinoma patients.

Materials and Methods
This was a hospital based, observational, prospective, cross sectional study conducted at Hi- Tech Medical College & Hospital, Bhubaneswar, Odisha, India by the Department of Anatomy in collaboration with Department of Obstetrics & Gynaecology from November 2011 to June 2013. The study was done on 52 normal fetuses (28 male and 24 female fetuses) without obvious congenital anomaly of gestational age between ranging from 6 weeks to 36 weeks. The fetuses were collected soon after delivery by spontaneous miscarriages & therapeutic legal abortions. Study samples were arbitrarily divided into groups of biweekly gestational age by duration of amenorrhoea from medical records & ultrasound fetometry after receipt of informed consent from mother and legal guardians. The foetal age was measured by Crown-Rump Length (CRL) in foetuses of 6-20 weeks gestational age whereas Crown-Heel Length (CHL) was more accurate in foetuses of 20-36 weeks. This was observed by the chart given in the text book of Human Embryology by Boyd, Hamilton and Mossman CRL of fetuses which varied from 80mm to 338mm. Consents were taken by principal researcher herself. All participants were clearly explained that they reserve the right to refuse to participate in the study. A copy of the consent form was given to each participant. The study was continuously being monitored by the IEC during the study period. It was registered as ECR/273/INT/OR/2013 issued under 122 DD of Drugs and Cosmetic rule 1945 GOI and approved by IEC. The rules and guidelines for disposal of human anatomical waste were strictly followed during the study. The liver were dissected under dissecting microscope within three hours of death from foetuses of each gestational week and were fixed in 10% formalin for 2-4 days. The liver tissues were cut in 5 mm thickness. A few drops of glycerol were added as formalin itself produces little shrinkage. After fixation by formalin, the tissues were transferred to 30%, 50%, 70%, 90% and Absolute alcohol each for 30 minutes. Then the tissues were put in xylol for 24 hours to clear the residual alcohol. The liver tissues were processed for paraffin sections by tissue blocking (Paraffin Embedding). 3 pots of hard paraffin were taken; paraffin was melted in the incubator at 56 degrees, as hard paraffin is ideal for materials which are to be cut in thin sections about 12 mu. The tissue was put in the first pot containing equal parts of paraffin and xylol and then changed to second and third pots containing only fresh melted paraffin at 90 minutes interval. Then the tissues were mounted in fresh melted paraffin with L-Block. The L-Block was then trimmed to a rectangular shape. Then the L-Block was fixed with the block holder (choke) and the block holder was clamped in the rotary microtome.5 mu sections were cut

in rotary microtome. The microtome was revolved at 40 per min and ribbon was formed. Then the ribbon was put in tissue flotation bath. Albuminised slide was then made by putting a drop of Mayor's albumin (equal parts of glycerine and egg white) and spreading it uniformly by rubbing with finger. The piece of ribbon was then taken on the slide and dried at room temperature. The slide was then put on the slide warming table. When the paraffin melted the slide was put into xylol for 2-3 minutes because xylol removes paraffin. Then the slide with tissue was put in decreasing grades of alcohol (Absolute alcohol, 90%,70%,50% and 30%) then then was put in the prepared Harris Alum Haematoxylin (nuclear) stain for 7 minutes and lastly washed with distilled water.2-3 drops of 1% acid alcohol (1cc Hcl in 75% alcohol) was added to remove the excess stain beyond the nucleus. The slide was then put in running tap water for 30 minutes to develop haematoxylin colour (bluish).

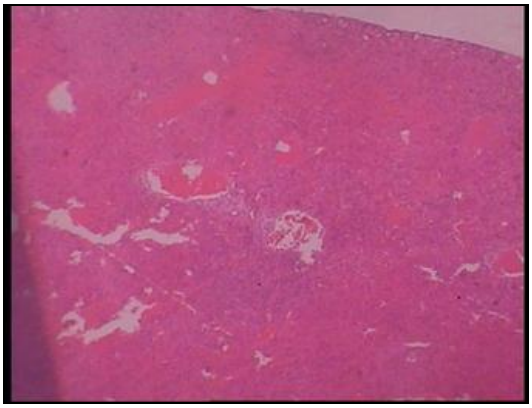
Then the slides were again dipped in ascending grades of alcohol (30%, 50%, and 70%) and then put in eosin Y (cytoplasmic) stain for 30 seconds. Then the slide was washed with absolute alcohol for a few seconds so that excess of eosin was removed and lastly the slide was placed in xylol. The slide was then taken out from xylol and then put in 1-2 drops of DPX (Adhesive agent) and a cover slip was put on it and pressed slightly so that air bubbles were removed. Sections were then seen in light microscope under low power 10X followed by high power 45X magnification. Thereafter photomicrographs were taken by camera using microscope adapter [5].

Observations

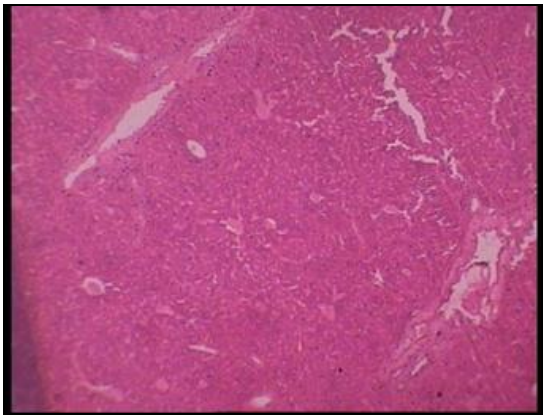
In the present study, a total of 52 aborted embryos and foetus of different gestational ages of both sexes and normal abnormal were observed. The prenatal specimens are categorized in to gestational age groups of 6 – 12 weeks, 12 – 24 weeks, 24 – 36 weeks and more than 36 weeks. One representative sample of liver tissue from each gestational age group was proceeding for histological examination.

Table 1

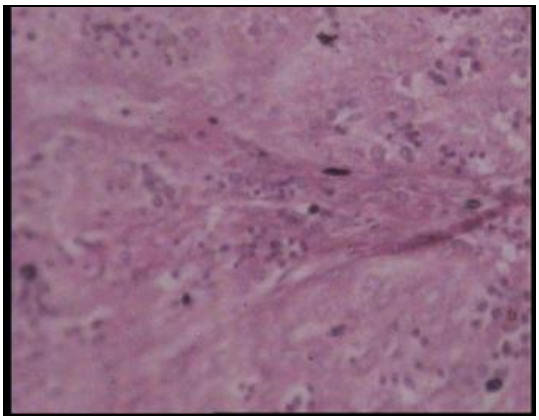
Gestational age (Weeks)	Male (28)	Female (24)	Total (52)
0 – 12	4	4	8
12- 24	8	2	10
24 – 36	9	12	21
> 36	7	6	13



6 to 8 Weeks



10 Weeks



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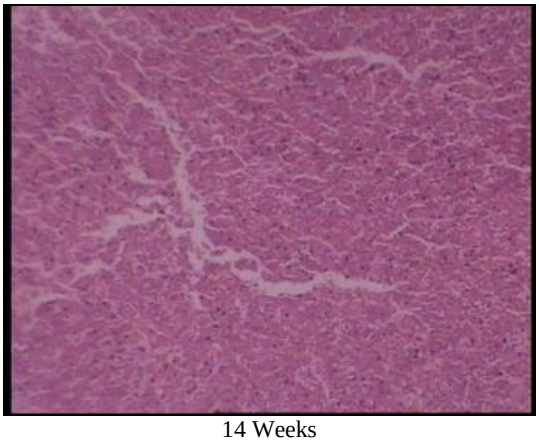
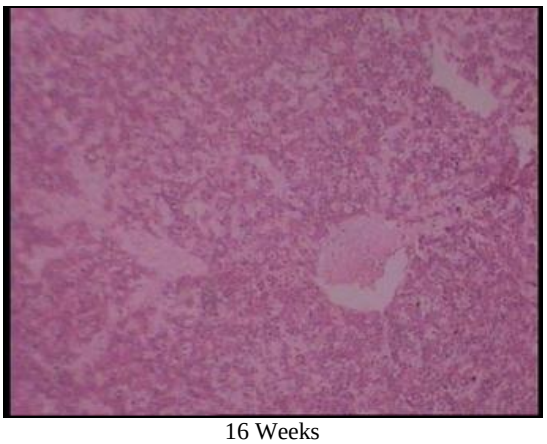
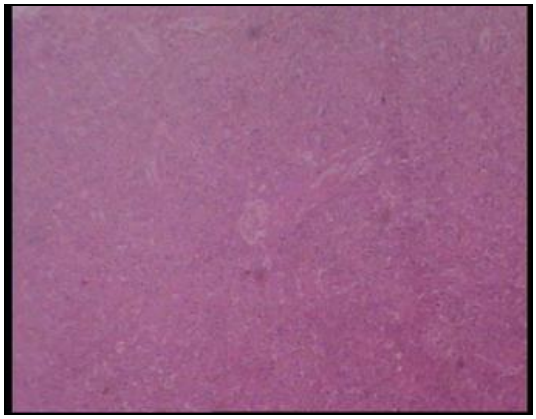
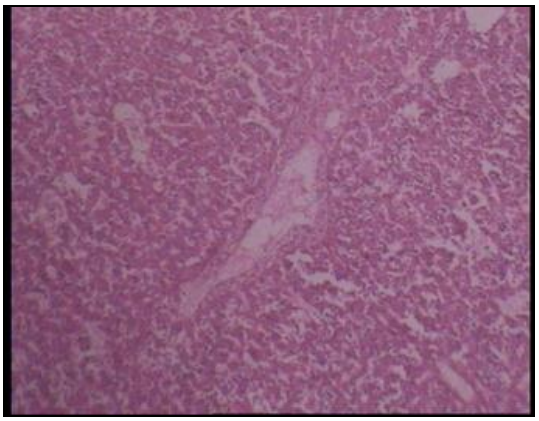


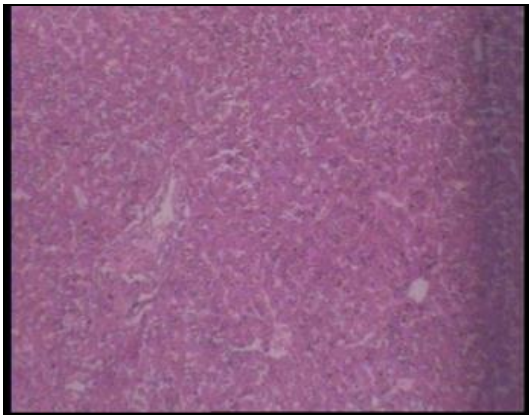
14 Weeks

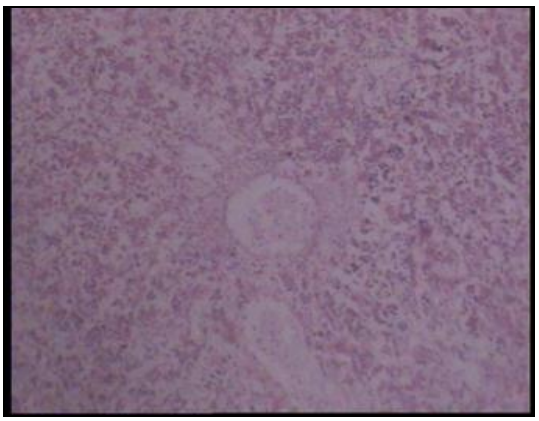


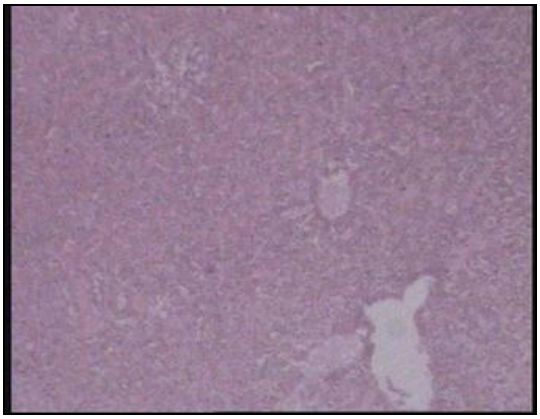
16 Weeks


20 Weeks


24 Weeks


28 Weeks


32 Weeks


36 Weeks

In foetuses of 6 to 8 weeks sinusoids and aggregation of hepatocytes central vein was seen. In 10th week of gestation Central vein was well formed with radiating cords of cells towards the periphery was seen, portal triad is visible. In 12th week Hepatic lobule formation, scattered Kupffer cells on the margin of sinusoids and formations stage of radiating cords of hepatocyte was seen. In 14th week well marked central vein with definite cords of hepatocytes connective tissue replaced well marked, portal triad was seen. In 16th week well marked central vein with radiating cords of cells with portal triad was seen. In 20 weeks, Reticular fibres with Kupffer cell was seen. In 24 weeks Portal triad and central vein with sinusoid surrounded by periportal connective tissue was observed. In 28 weeks Increased Reticular fibre with Kupffer cells, Central vein, radiating cords of hepatocytes, portal traid, Glisson's capsule or hepato-biliary capsule covering portal triad. In 36 weeks Portal triad, binucleated hepatocytes, Kupffer cells, portal canal, central vein and sinusoids were observed. In foetuses of >36 weeks Radiating cords of hepatocytes, Kupffer cells along with central vein were observed.

Discussion

The structural units of liver, hepatic lobule was first reported by Wepfer (1664), later by Malphigi (1666). Kierman (1833) defined the hepatic lobule as hexagonal structure that has central vein in its centre. He also that the boundaries are clearly defined by connective tissue only in few species like pig and it is sparse in humans except in the portal traids. Elias (1949) described the cord like arrangement of hepatocytes that branch and anastomose enclosing sinusoids between them. He Suyan (1983) described that the lobular formation in the liver starts between 9-12 weeks of gestation. Anne *et al.* (1996) described different stages of liver development. By the time bone marrow haemopoietic function starts liver haemopoeisis regresses by 28-32 weeks of gestation (Linda *et al.* 2011). The intrahepatic arterial radicles and branches of portal vein appear within the liver parenchyma at 10 weeks of gestation (Sophie *et al.* 2008). Initially they are located in the centre of fetal liver, later they reach the periphery of liver at 15 weeks (Gouysee *et al.* 2002). The intrahepatic capillaries which are developed from embryonic vessels are lined by continuous endothelium at 8 weeks of gestation, later they are differentiated into sinusoids with fenestrated endothelium at 17 weeks (Marchiarelli *et al.* 1988). The hepatocytes of foetal

liver at the time of birth show glycogen vacuolization of the epithelial cells, which is the characteristic feature of foetal liver in the last weeks of gestation (Marie *et al.* 1957). The early stage of haemopoiesis along with sinusoids and aggregation of hepatocytes was observed in a specimen of 5 to 6 weeks of gestational age. According to Bhorgese [13] the development of haemopoiesis begins at about 6th week of gestational age. The findings in the present study in agreement with literature. Specimen with 20 weeks of gestational age shows early stage of reticular fibres along with Kupffer cells was observed. Bradley & Neil [14] stated that development of Kupffer cells and connective tissue cells begin at about 3rd month of gestational age, we observed that there was delay in the appearance of Kupffer cells. Gestational age of 24 weeks specimen shows portal triad with central vein and sinusoids surrounded by periportal connective tissue were observed. Blouin & Suyan [15] stated that periportal connective tissue surrounding the bile duct system observed during 8-12weeks of gestational age. There was delay in the formation of bile duct system. At 28 weeks of gestational age specimen shows increased reticular fibres with Kupffer cells was observed. According to Zhang Wenxue [16] hemopoietic cells were present from 15 - 3 5 weeks of gestational age. Portal triad, binucleated hepatocytes, Kupffer cells, portal canal, central vein and sinusoids were observed at 36 weeks of gestational age. The haematopoietic function decreased abruptly in 35-week-old fetus. Radiating cords of hepatocytes, Kupffer cells along with central vein were observed in a specimen at >36 weeks of gestational age. Balis JU *et al.* suggests that the liver plates are formed before the development of sinusoids. Potter and Craig [17] reported that the liver differentiates into masses and plates of cells at 4th week. Desmet VJ [18] in his study has observed that central vein starts appearing at 16th to 17th week of gestation. According to Zamboni *et al.* haemopoiesis in liver becomes fully established around 3rd month of intrauterine life. Potter and Craig [11] have observed haemopoietic activity in liver throughout all the fetal ages. Hamilton and Mossman states that, haemopoiesis begins very early in developing liver and reaches its peak at 6th to 7th month of fetal life and then regresses up to full term.

Notenboom [6] who also found that lobular pattern is not well defined with cells arranged in anastmosing cell pattern with single cell thick cord-like pattern. They stated that fetal period is crucial for normal liver development with differentiation in to adult phenotype from embryonic foregut being a multistep process. Payushina [7] found in their study that fibroblast and myofibroblast were present around portal triad and confirmed immuno histochemically. Early work on histology of fetal liver was done by Lipp [8] who described the embryology of the liver and stated that hepatic parenchymal plates develop from the cell cord organized along their nutrient vessel. The authors also noticed the cells of liver parenchymal cord and were uniform throughout the liver and also demonstrated anastomosing cord like pattern at places as described by Severn [9]. Villeneuve *et al.* [10]. described that fetal hepatocytes were arranged in anastomosing sheets separated by capillaries. Several workers also described the hemopoietic function of liver [9, 13]. Emura *et al.* [11]. noticed the different erythrocytes series in hemopoietic system during early stage of hepatic hemopoiesis. Presence of Kupffer cells has also been described by Naito *et al.* [12]. who found that macrophage develops in yolk sac and migrates to fetus liver.

Conclusion

The findings in the present study stating that there was a delay in the formation of sinusoids and kupffer cells along with the biliary duct systems. We observed the aggregation of hepatocytes and early stage of haemopoiesis at 5-6 weeks of gestational age which is in agreement with literature. Delay in the histogenesis and development of the liver cells and bile duct system leads to histopathological and developmental abnormalities gives knowledge to the clinicians during clinical procedures.

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Conflicts of Interests- None

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