Histogenesis of developing human liver in Marathwada region of Maharashtra

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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 . Microscopic structure of liver at various gestational age groups. The size of hepatic lobule with the help of micrometer scale and eyepiece reticle. Total 40 human fetuses (19 male and 21 female) were procured from Obstetric/Gynaecology department of GMC Aurangabad. Consent was taken from respective parents with approval of Institutional Ethical Committee of GMC Aurangabad. The size of classical liver lobule was measured by using micrometer scale and the eyepiece reticle. After passing through different stages of staining, the slides were prepared from the liver tissue in which haemopoiesis was found abundant at early stages of gestation and decrease as the age of liver advances from 12th to 36th week of gestation. Connective tissue elements increase from 12th week onwards showing thick capsule and thickened trabeculae. Portal tracts start appearing from 18th week of gestation. Kupffer's cell appears at around 22nd week of gestation and thereafter a gradual rising pattern is found up to 34th week of gestation. Portal triad are seen distinct by 22nd week of gestation therefore the hepatic lobule size can be measurable from 22nd week onwards. In our study the size of the hepatic lobule at 22nd week of gestation is 0.715 mm. The size of hepatic lobule then increases gradually up to 36th week of gestation and is 1.244 mm.

Keywords: Histogenesis, Haemopoiesis, Hepatic lobule, Congenital defects, Liver development, Micrometer scale, Eyepiece reticle, Portal tracts, Kupffer's cell.

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Introduction

The fundamental processes involved in the development are growth, differentiation and metabolism. "Growth" is increase in the special dimensions and weight. Differentiation is an increase in complexity and organization. "Differentiation" may be manifested as an increase in morphological heterogeneity resulting in the assumption of form and pattern and in the appearance of recognizable organs or organ primordia (organogenesis). "Metabolism" includes the chemical changes in the developing organism[1]. In the present study, while studying the development of liver in antenatal period, different histological parameters of liver were considered. Of all liver transplantation worldwide, pediatric liver transplantation is 10-15%. The main etiological factors behind liver transplantation are congenital liver defect. In 2002 the American liver foundation published 'Pediatric liver research Agenda'. This agenda advocate that a better understanding of embryonic liver development would provide an important insight into the treatment and preventive strategies for pediatric liver disease. After knowing the entire normal histological characteristic at various stages of development this study can be proposed to distinguish from certain pathological changes occurring in liver during prenatal period. In intrauterine life haemopoisis is one of the most important function of liver[2]. It starts from 10th to 12th week of intrauterine life and gradually subsides during last two months of intrauterine life and only scanty foci remains at birth. The liver primordium appears in the middle of the third week as an outgrowth of the endodermal epithelium at the distal end of the foregut called as hepatic diverticulum which proliferates and forms epithelial liver cords which ultimately differentiates into hepatocytes and form the lining of the biliary ducts. Blood cells, kupffer cells and connective tissue cells are derived from mesoderm of septum transversum[3]. The classic description of liver morphology is centered on hepatic lobule, a region of liver tissue of something like pinhead size and hexagonal shape with a central vein and plates or cords of hepatocytes separated by sinusoids radiating from the vein to the periphery of the lobule[4]. About 80% of the liver volume and 60% of its cell numbers are formed by hepatocytes which are polyhedral with 5-12 sides and are from 20-30 micro meter across[5]. As far as

liver histogenesis is considered to some extent it correlates with the morphological parameters of liver development which has been studied in the past by different authors[6,7,8].

Aims and Objectives

The present study aims to study histogenesis and development of human liver in prenatal period

a. To observe microscopic structure of liver at various gestational age groups.
b. To measure the size of hepatic lobule with the help of micrometer scale and eyepiece reticle

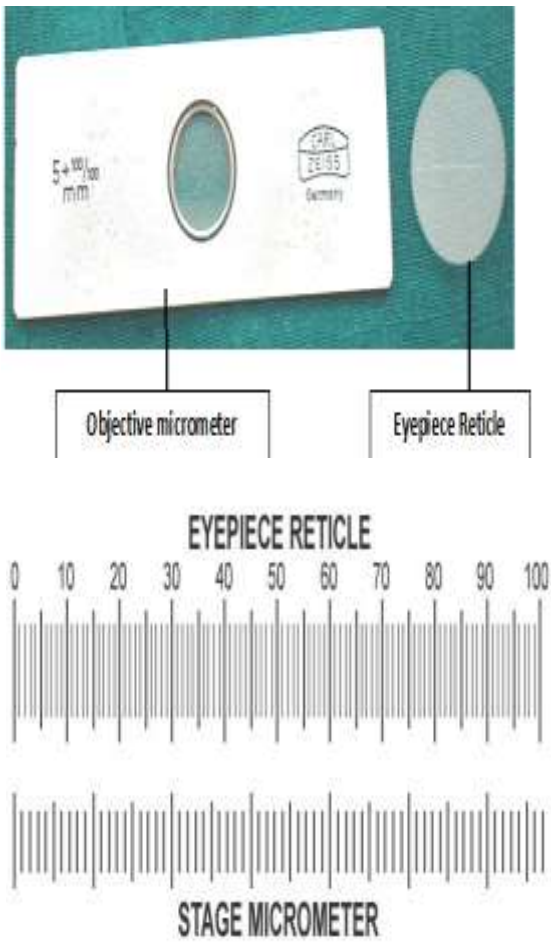
Materials and Method

Forty human fetuses (19 Male & 21Female) of different gestational age group ranging from 12th to 36th weeks of gestation were used in the present study. These fetuses were procured from department of Obstetrics & Gynecology, Government Medical College and hospital Aurangabad. Spontaneous abortions and stillborn fetuses were included in the study. Twins and fetuses with gross anomalies were discarded from the study. Consent was taken from respective parent with approval of the Ethical Committee of GMC Aurangabad. Gestational age and Body Weight (BW) of the fetuses were measured. CRL was measured by thread and osteometric board with millimeter scale. Weight of the fetuses was measured in gram on double pan balance. Fixation of the liver tissue was done by injecting 10% formaline locally on various sites with the help of 10 ml syringe and 20 G needles. Fetuses were dissected by taking simple midline and oblique incisions on anterior abdominal wall & right hypochondric region respectively. Small pieces of liver tissue were cut and then passed through the different stages of haematoxylin and eosin staining and finally slides were prepared and observed under 10X, 40X and 100X.

Procedure for the measurement of diameters of hepatic lobules.

Calibration of objective

Accurate measurement of microscopic objects requires the use of an eyepiece reticle (a.k.a. eyepiece micrometer) and a stage micrometer. The eyepiece reticle is a round glass disk on which divisions are itched. The eyepiece reticle is inserted into the eyepiece and held in place in the correct focal plane of the eyepiece. The eyepiece and eyepiece reticle can be rotated throughout all 360 degrees in the eye tube, so that measuring scale can be aligned with or superimposed over the image of the specimen. An eyepiece reticle has a linear scale featuring 100 divisions. Before using the eyepiece reticle for accurate measurements it is necessary to calibrate the eyepiece reticle using a stage micrometer. A stage micrometer is simply a microscope slide with known dimensions itched upon its surface, having calibration of 100 lines in a 1 mm, equivalent of 0.01mm i.e. 10 µm. The stage micrometer is placed directly on the stage of the microscope and focused. By rotating the eyepiece both scales can be positioned parallel to each other aligned at zero.



Diagrammatic representation of calculation of eye piece and stage micrometer

To calculate the value of one division of the eyepiece reticle 1000µ was divided by 70 and the result was 14.3 µ per division, i.e. the reticle value, in this case 14.3µ, would apply only to the objective (10x) for which the calibration was done. In 40X objective and 10X eyepiece, 14 divisions of the eyepiece reticle corresponded with 5 divisions of the stage micrometer. Each division of the stage micrometer equals 10µ, so 5 divisions of the stage micrometer are equivalent to 50µ. To calculate the value of one division of the eyepiece reticle 50µ was divided by 14 and the result was 3.6 µ per division. The reticle value, in this case is 3.6µ.

Observation and Results

Haematoxylin-eosin slides were prepared from fetal liver specimen and observed under low and high power of light microscope. Following were the observation of various stages.

Liver at 12th and 13th week of gestation: A thin capsule is seen. Large numbers of haemopoietic cells of different types are seen scattered around. The parenchymal cells of the liver are arranged in irregular clumps and cords (Fig. 1A). These cells are oval in shape with faint pink cytoplasm and large rounded nuclei, with prominent nucleolus. (Fig. 1B) Few connective tissue cells and associated fibers are scattered diffusely amongst these. Central veins, Kupffer cells and portal triad were not observed.

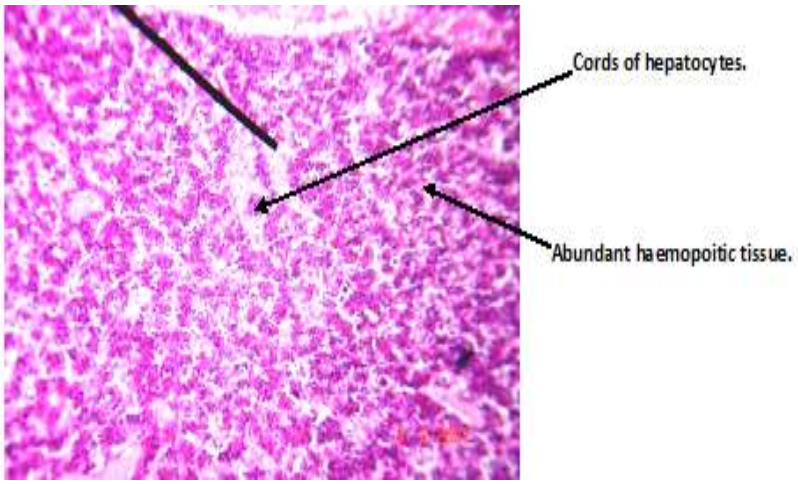


Fig. 1A: At 10x; 12 weeks

Slide of Liver of 12th week's gestation; stained with Hematoxyline and Eosin

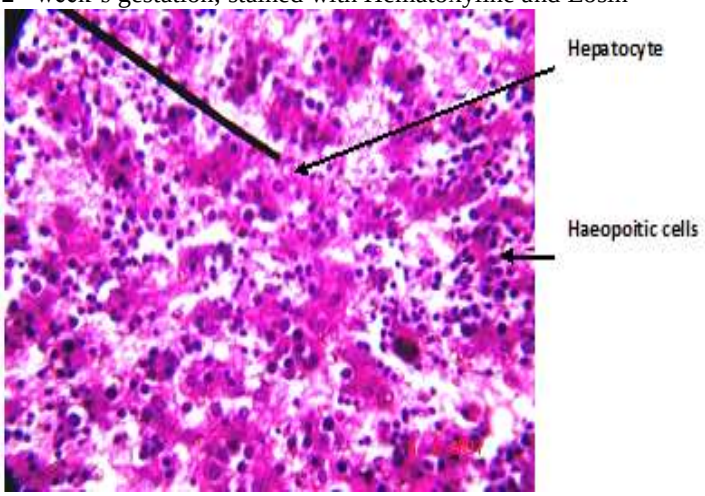


Fig. 1B: At 40X; 12 weeks

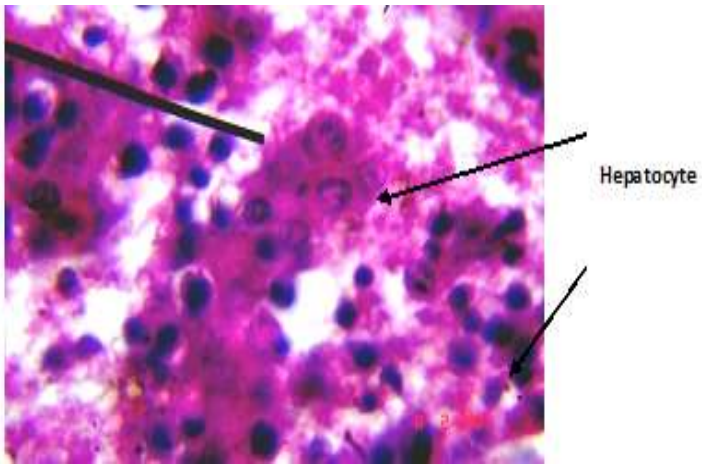


Fig. 1C: At 100X; 12 weeks

Liver at 17th and 18th week of gestation: Histological structure is more or less similar to that of the previous stage except that central veins are more numerous and Portal tracts could be identified where the branches of portal vein, hepatic artery are surrounded by connective tissue. At few places a bile ductule could be identified in portal tracts. Hepatocytes are still arranged in irregular clumps and cords. Sinusoids are well defined, filled with haemopoitic cells, lobular architecture is not evident (Fig. 2B).

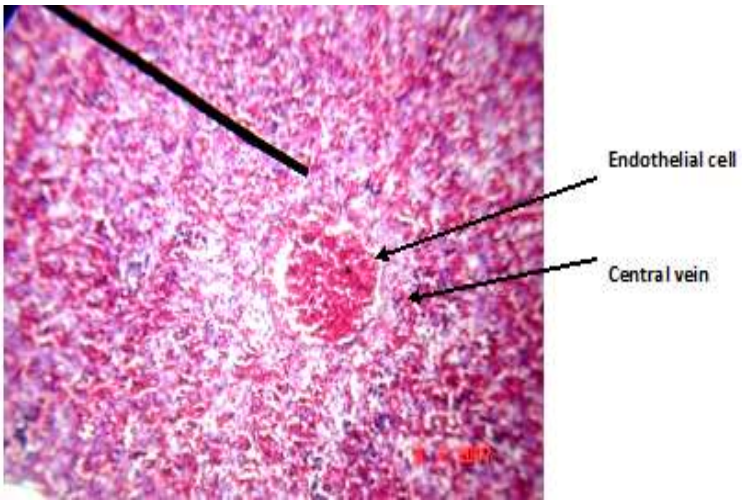


Fig. 2A: 10X; 18 weeks

Slide of Liver of 18th week's gestation; stained with Hematoxyline and Eosin.

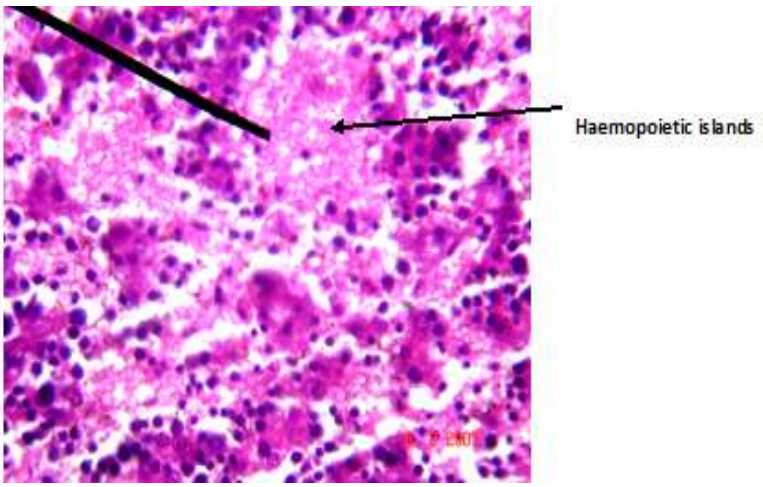


Fig. 2B: 40X; 18 weeks

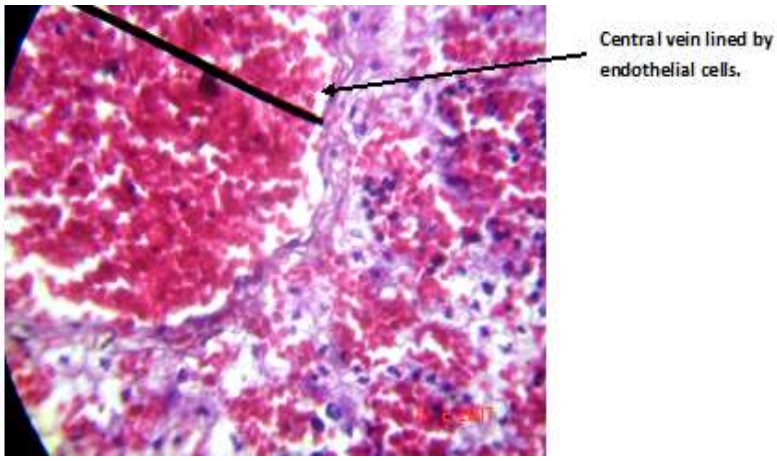


Fig. 2C: 100X; 18 weeks

Liver at 23rd week to 27th week of gestation: The microscopic structure of liver is similar to that of 22nd week. Only size of the classical liver lobule is seen to be increasing (Fig. 4A). This increase in size of hepatic lobule is studied separately. Details about size of hepatic lobule are given in the Table 1 and Graph 1.

Liver at 28th week to 36th week of gestation: All the features are well defined. Haemopoiesis reduces after 34th week of gestation (Fig. 5B). Sinusoidal walls are lined by endothelial cells. Stellate shaped Kupffer cells are seen attached to the sinusoidal walls and also found in lumen of sinusoids. Plates of hepatocytes are seen radiating from central vein (Fig. 5C). Hepatic cells show vacuolated cytoplasm, an indicator of glycogen presence.

Slide of Liver of 28th weeks gestation; stained with Hematoxyline and Eosin.

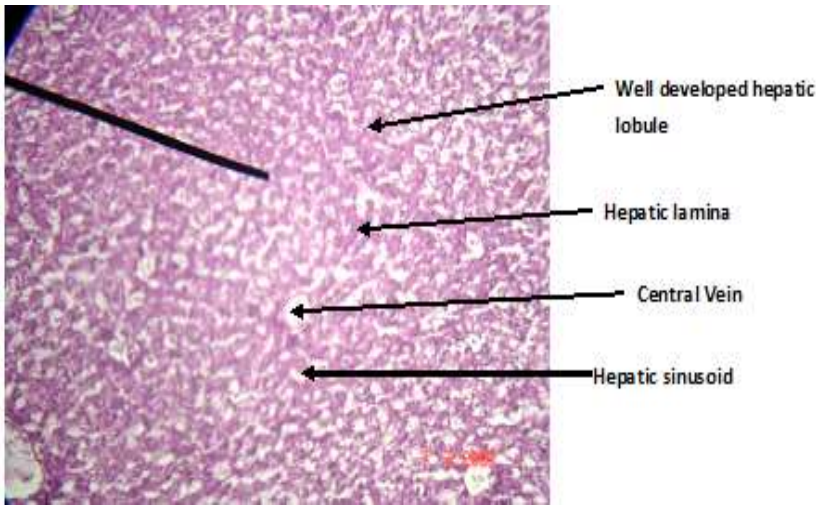


Fig. 4A:10X; 28 weeks

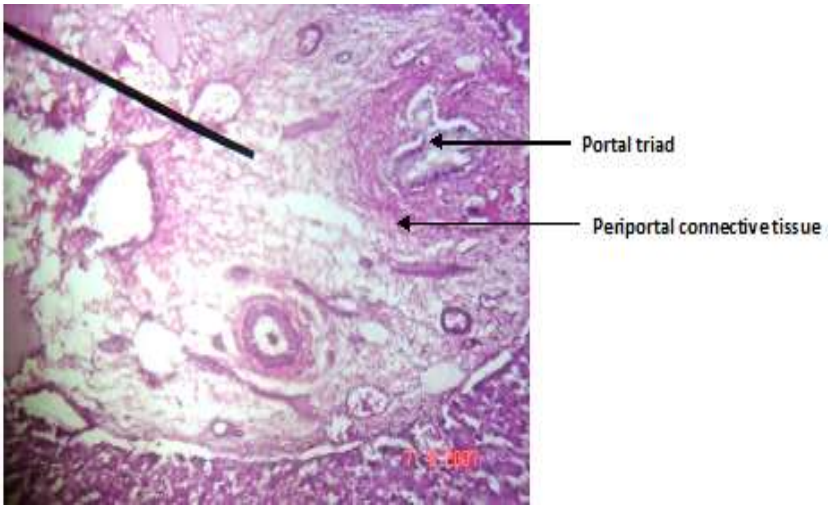


Fig. 4B: 40X; 28 weeks

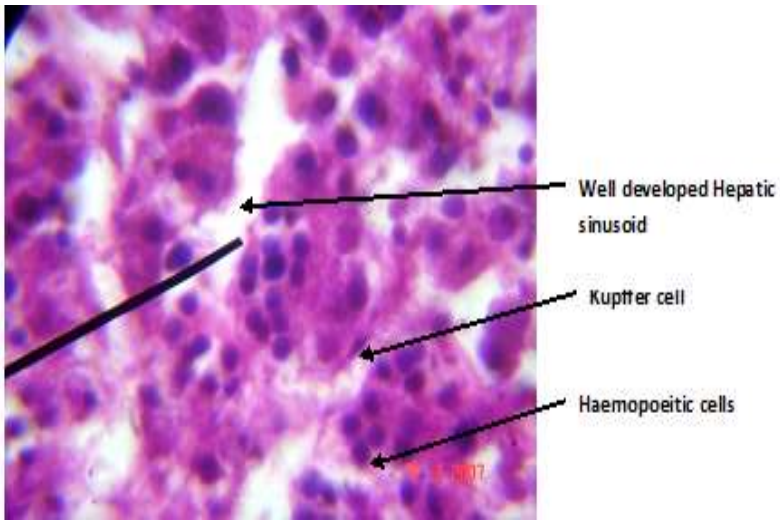


Fig. 4C:100X; 28 weeks

Slide of Liver of 36th week's gestation; stained with Hematoxyline and Eosin.

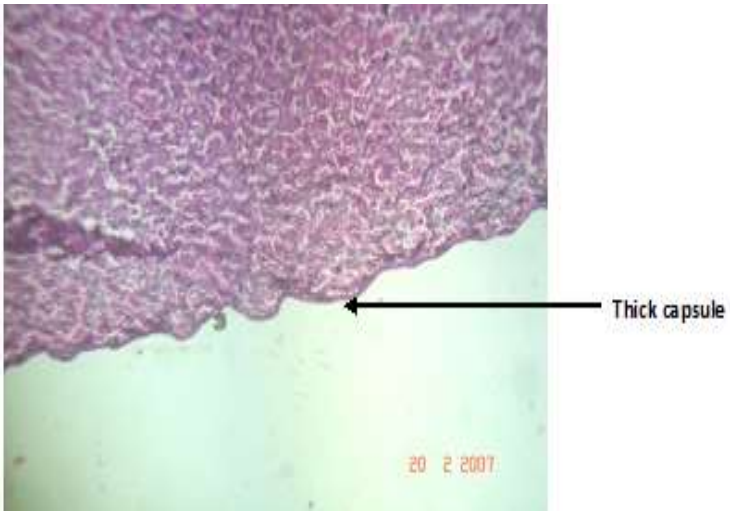


Fig. 5A: 10X; 36 weeks

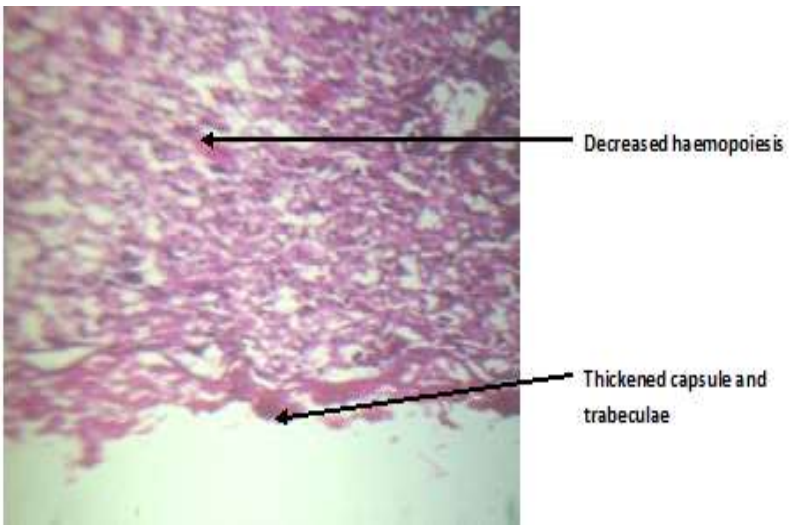


Fig. 5B: 40X; 36 weeks

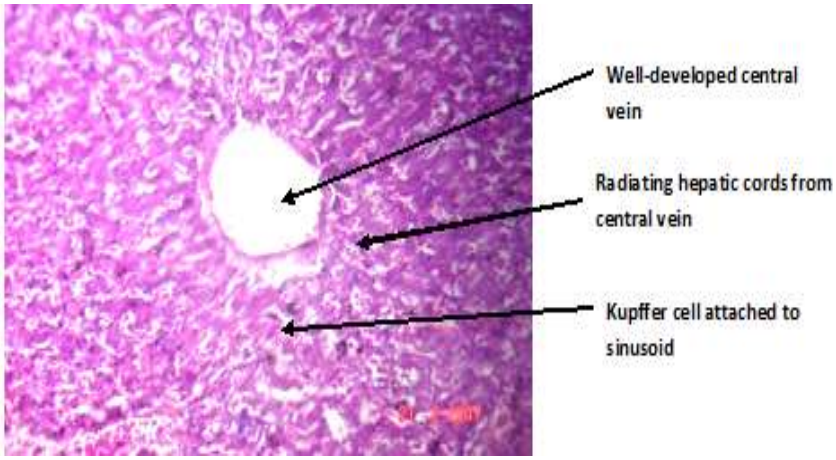


Fig. 5C: 100X; 36 weeks

Summary of Development of Different Structures from 12th to 36th week of gestation of Liver: Haemopoietic tissue: Haemopoiesis was abundant at early stages of gestation. It shows decrease as the age of liver advances from 12th to 36th week of gestation.

Connective tissue elements: It consists of thin capsule and diffuse connective tissue supporting the cords of hepatocytes at earlier stages. Connective tissue is also associated with blood vessels except central veins. Connective tissue elements increase from 12th week onwards showing thick capsule and thickened trabeculae.

Central Vein: Central vein appears at around 16th to 17th weeks of gestation. Thereafter it shows increase in size.

Portal tracts: These consist of the branches of portal vein, hepatic artery and bile ductule. They appear later during development at about 18th week of gestation.

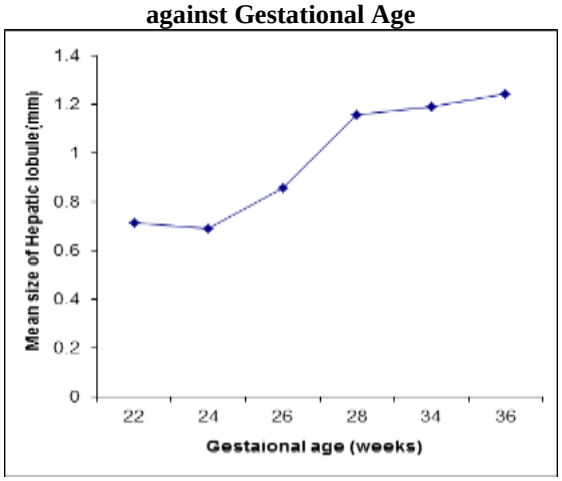
Kupffer cells: At around 22nd week of gestation Kupffer cells appear in fetal liver and are seen to increase up to 34th week of gestation.

Size of classical liver lobule: The size of classical liver lobule was measured by using micrometer scale and the eyepiece reticle. To determine the size of the hepatic lobule, the distance between central vein and portal tract was measured. Above distances of different slides of the same week of gestation were observed and measured. Finally means of all slides of same week of gestation were noted. The findings are given in the Table 3 and Graph 5. Portal triad are seen distinct by 22nd week of gestation therefore the hepatic lobule size is also measurable from 22nd week onwards. From the table-3 and graph-5 it is seen that, the size of the hepatic lobule at 22nd week of gestation is 0.715 mm. The size of hepatic lobule then increases gradually up to 36th week of gestation and is 1.244 mm.

Table 1: Showing mean size of hepatic lobule in different weeks of gestation

Gestational age (Weeks)	Size of hepatic lobule (mm)
12	-
14	-
16	-
18	-
20	-
22	0.715
24	0.691
26	0.858
28	1.158
34	1.190
36	1.244

Graph 1: Showing mean size of Hepatic Lobule against Gestational Age



Discussion

The microscopic structure of liver at different stages in prenatal period were observed, points are as under:

a. Capsular development.
b. Presence of haemopoisis.
c. Changes in organization of hepatocytes and plates of hepatic cells.
d. Appearance of central veins and sinusoids.
e. Formation of portal triad.
f. Size of classical liver lobule.
g. Appearance of Kupffer cells

1. **Comparison of Haemopoiesis and histological structure of liver with the studies of different workers:** According to Zamboni et al[9] haemopoiesis in liver becomes fully established around 3rd month of intrauterine life. Potter and Craig[10] have observed haemopoietic activity in liver throughout all the fetal ages. Hamilton and Mossman[11] 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. Present study is in accord with all the previous workers stated above. Haemopoiesis was seen prominently in all the stages studied but gradual decrease in haemopoiesis was found from 12th to 36th weeks of gestation, and after 34 weeks, scanty foci of haemopoietic tissues were seen.
2. **Changes in organization of hepatocytes and plates of hepatic cells were also studied and compared:** Balis JU et al[12] suggests that the liver plates are formed before the development of sinusoids. Potter and Craig reported that the liver differentiates into masses and plates of cells at 4th week. The mesenchyme of septum transversum forms capsule and connective tissue elements. Endodermal cells transform into hepatic parenchyma, while vascular channels form the hepatic sinusoids. Around the hepatic cells, bile canaliculi form bile ductules. A bile ductule along with a hepatic artery and portal vein form portal tract. In the present study, it is seen that the connective tissue elements consists of a thin capsule and reticular fibers supporting hepatic cords, but later on it is associated only with blood vessels except central vein. Connective tissue element increase from 12th weeks onwards showing thickened capsule and more thickened trabeculae.
3. **Appearance of central vein and portal tracts were also observed and compared:** Desmet VJ[13] in his study has observed that central vein starts appearing at 16th to 17th week of gestation.Sinusoidal walls lined by endothelial cells are also identified first at this stage. Portal tract can be identified first at 18th week liver, but the clear-cut architectural pattern becomes evident only at 20th to 21st week of gestation. In the present study all the structures of classical liver can be identified clearly at 22nd week. The size of lobule increases thereafter.
4. **Kupffer's cells:** According to Balis et. al Kupffer's cells are absent in early stages of gestation. As per the observation by R. N. M. Macsween[14] it has been stated that the sinusoidal endothelial cells i.e. Kupffer's cells and hepatic stellate cells appeared at 10th to 12th week of intrauterine life. In the present study it has been found that sinusoidal endothelial lining appeared at 16th to 17th week and kupffer's cells were seen at around 22nd week of gestation.

Conclusion

From the above study it can be concluded that the haemopoietic tissue was found abundant in early stage but it regressed to cease at late stages of gestation. Central vein and portal tracts appeared at around 15th to 18th weeks of gestation. Kupffer's cells appeared at 22nd week of gestation and then their number increased till 34th week of gestation. The size of hepatic lobule was measurable from 22nd-23rd week of gestation and increased in size with advancing gestation.

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