Original Article

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Histogenesis of Liver in Human Fetuses

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Abstract

Introduction: The present study attempted to find out the histological changes of liver during its development in human fetuses. *Methods:* Liver from 10th–40th GW fetuses were studied after staining with Hematoxylin and Eosin, and Masson's Trichrome stains. *Results:* The liver from 10th week onwards to term consists of two different cellular elements, larger cells with pale staining centrally located nuclei, hepatocytes and dark staining smaller cells with dark rounded nuclei, the haemopoietic cells. The haemopoietic cells occupy larger area from 10th week onwards till 22nd week when both the elements are equal in quantity, after that hepatocytes quantity increases gradually and the haemopoietic cells decreases in amount and at term only little haemopoietic cells remains. Sinusoids are irregular and having wide lumen from 10th to 18th week, after that the lumen become narrow and regular. Within the sinusoids lies the haemopoietic cells and all the sinusoids are lined by fenestrated endothelium. Hepatic lobules become apparent after 22nd week and kupffer cells appear after 25th week. Bile duct, hepatic artery and portal vein all identified from 14th week onwards till term. *Discussion:* During early development, liver was composed of collagen fibers with fibroblast cells, hepatocytes and bigger haemopoietic cells. Haemopoietic cells could be detected till 36th GW fetuses. Even though immature RBCs and haemopoietic stem cells were detected, hemopoiesis in the liver along with it hepatocytes could be ascertained in the present study.

Keywords: Haemopoietic Cells; Hepatocytes; Kupffer Cell; Stem Cell; Haemopoiesis.

Introduction

The earliest development of the liver in human is indicated by the appearance of the hepatic diverticulum, first recognizable as an anlage lying ventral to the endoderm of the foregut in the anterior intestinal portal in 5 somite embryo (2mm). This diverticulum is an endodermal thickening known as hepatic endoderm, originating from the primitive streak and is believed to be induced by mesoderm in the cardiac area [1]. The combined anlage of the bile duct and liver is known as hepatic diverticulum. The hepatic duct and glandular tissue develops from the cranial portion of the hepatic diverticulum [2]. The hepatic diverticulum consists of rapidly proliferating endodermal cells lining the primitive foregut and gives rise to cellular branches which invade the mesenchyme of septum transversum. These cellular branches are termed hepatic cords and they intermingle with the umbilical and vitelline veins forming the hepatic sinusoids. Liver cords differentiate into the parenchyma and forms the lining of the biliary ducts. Haemopoietic cells, kupffer cells and connective tissue cells are derived from the mesoderm of the septum transversum [3]. The mesenchyme of the septum transversum forms the endothelial cells and the connective tissue frameworks of the liver and the endoderm differentiates into hepatocytes. The proliferation and bulging of the hepatic diverticulum stimulates the production of blood islands in the investing mesenchyme [1]. The liver remains proportionately large during its development and constitutes a sizeable organ dorsal to the heart at stage 14 then more caudally placed by stage 164 [5]. By this stage hepatic ducts can be seen separating the hepatic epithelium from the extra hepatic biliary system [6]. At 3 months' gestation, the liver almost fills the abdominal cavity and its left lobe is nearly as large as

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its right and it constitute 10% of the whole body weight [7,8]. Although its haemopoietic functions cease before birth its enzymatic and synthetic functions are not completely mature at birth [4].

Materials and Methods

The material studied consisted of seventy two (72) normal fresh human fetuses, of different gestational ages ranging from 10 weeks (CRL-6cm) to 40 weeks (CRL-39.5cm), collected from the Department of Obstetrics and Gynecology, RIMS, Imphal, which were the products of terminated pregnancy under the Medical Termination of Pregnancy act of India, 1971 and stillbirths. The specimens were utilized for the present study after seeking permission from the institutional ethical committee. The age of the fetuses were calculated from the obstetrical history, gross features and crown-rump (C.R.) lengths. Thereafter they were fixed in neutral buffered formalin for two to seven weeks and then the dissection was carried out.

Only those fetuses which were free from any gross anatomical abnormality were selected for the present study. The specimens were categorized into different age groups for easier study and observation as there will be similar features and finding in the adjacent age groups. After proper fixation, the tissue were trimmed and cut and prepared for histological studies. The slides were stained with routine haematoxylin and eosin staining. Van Giesons staining were also done to differentiate the collagen fibres and reticulin fibres. Slides were then examined for general morphology and cellular details under low power (10X), high power (40X) and oil immersion (100X).

Results

10 to 16 weeks

In the panoramic view, hepatic parenchyma is recognized with both cellular and fibrous component, where plates of hepatic cells are arranged in linear and branching pattern and anastomosing between each other (Fig. 1a). There is homogenous parenchyma with early sign of lobulation indicated by tiny central vein in the centre (Fig. 3a), inside the lobule hepatocytes are in different stages of differentiation showing labyrinthine network as well as cord like pattern interspersed with haemopoietic cells within the space, the future sinusoids (Fig.1b). In between the cords there are spaces where other different types of cell are present (Fig. 2a). In the plates of cell there is presence of epitheloid glandular cell with early myocytes, also some mesenchyme to fibroblast cells. The hepatic lobule is yet to appear clearly and the kupffer cells are not yet developed. There are no interlobular septa in between the immature lobule. The capsule of the liver is poorly defined and it is made up of single layer of cell with flattened nuclei and there are thin layer of connective tissue demonstrated by Van Gieson's stain(Fig. 4a).

17 to 20 weeks

Hepatic parenchyma is recognized with a number of incomplete hepatic lobule, the shape of which ranges from circular to polygonal with ill defined

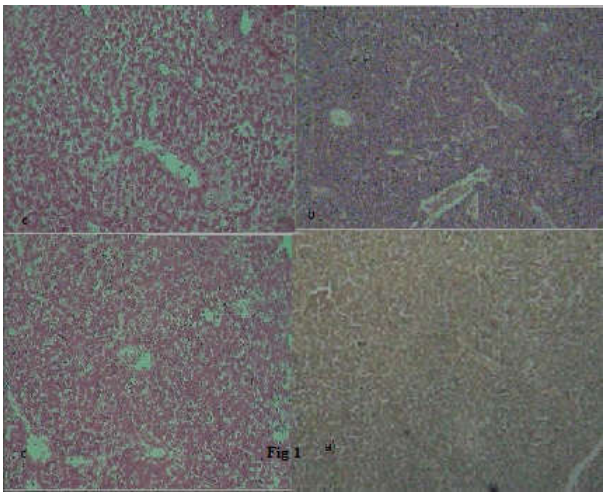

Fig. 1a: Panoramic view showing initial lobulation at 10th week
b. Showing the appearances of proper central vein and hepatic triad area at 14th week
c. Showing the sinusoidal appearance and network of hepatic cords at 22wk
d. Increase in size of the individual lobule at term liver

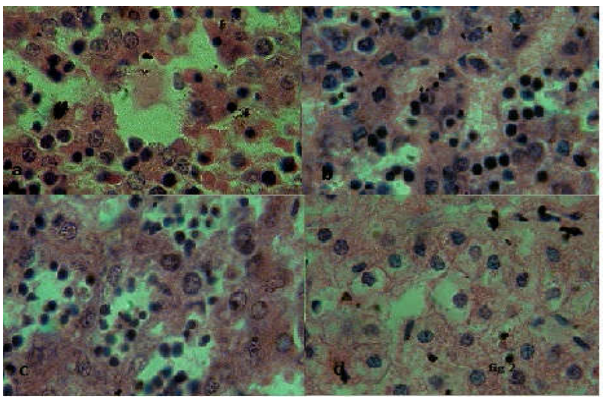

Fig. 2a: Showing irregular hepatic cords with 2-3 cell thick at 14th week
b. sinusoids are filled with haemopoietic cell, darker than hepatic cells at 20th week
c. anastomotic network of sinsuoids and hepatic plates at 30th week
d. well defined hepatocytes and hepatic plates are at 40th week

portal triad in the corner. Each lobule has rows of hepatocytes arranged radially from the central vein and separated from each other by empty spaces, future sinusoids (Fig. 2b). The hepatic columns are more in number and there is increase number of hepatocytes around the central vein. Haemopoietic cells are dominant in the parenchyma. There are empty spaces between the hepatic plates, these spaces are future sinusoids(Fig. 2b). In these sinusoidal spaces, there are abundant darkly nucleated smaller group of cell, haemopoietic in origin. The sinusoidal spaces are very prominent near the central vein but it is less obvious peripherally. The capsule is well defined and consists of connective tissue fiber and single layer of flattened cells as identified by Van Gieson's and Massons Trichrome stain (Fig. 4b).

21-24 weeks

The lobulation is better identifiable than the previous age group fetuses. Hepatic plates are 2-3 cells thick and shows irregular branching pattern and in between the plates there are sinusoids, filled with abundant dark staining cells, which are developing blood cells in various stages of maturation (Fig. 1c). The parenchymal tissue is more than previous age group but the sinusoidal spaces are less abundance. Portal area is recognized with a number of structures within connective tissue fiber which are both mature and immature. Some of the hepatic plates as well as sinusoids are continuous with surrounding lobule indicating immaturity of the lobule. The hepatocytes are round to ovoid in shape with spherical and vesicular nuclei (Fig. 1c).

The cell boundary is better defined in this age group than the previous weeks. The nucleus is lightly stained and occasionally presents bi-nucleated appearance but number of bi-nucleated hepatocytes reduced than the earlier weeks. Kupffer cells are appears for the first time at 22nd week in the sinusoidal wall (Fig. 3b). Kupffer cells are identified as larger cells with irregular and elongated nuclei which stained darker than the endothelial cells.

The hepatocytes and haemopoietic cells are almost equal in number, but the hepatic plate's areas are more than the sinusoidal areas (Fig. 5a). Elastic lamina is seen around the central vein and surrounding the hepatic artery in a small amount by Voeroff's stain. The capsule is well developed with thick connective tissue fiber.

25 to 32 weeks

Hepatic lobule is well organized and hepatocytes are seen to arrange in columns, which form

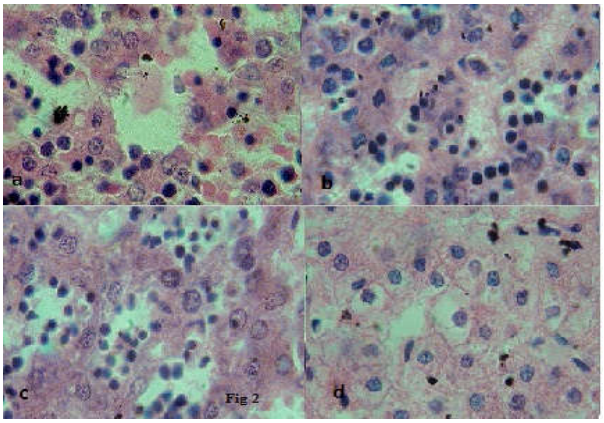

Fig. 3a: Irregular sinusoids and ill defined central vein at 14th week
b. Trabeculated appearance of hepatic cords with abundant haemopoietic cells and kupffer cell at 22nd week
c. Reduced haemopoietic cells at 30th wk
d. Abundant hepatocytes and kupffer cells with less blood cells at term liver

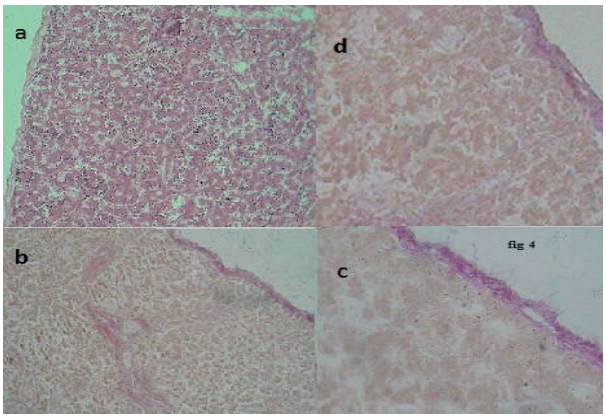

Fig. 4a: at 14th week ill defined thin hepatic capsule
b: Increase thickness of capsule with connective tissue element at 18th week
c & d: Well defined capsule at 30th and term liver

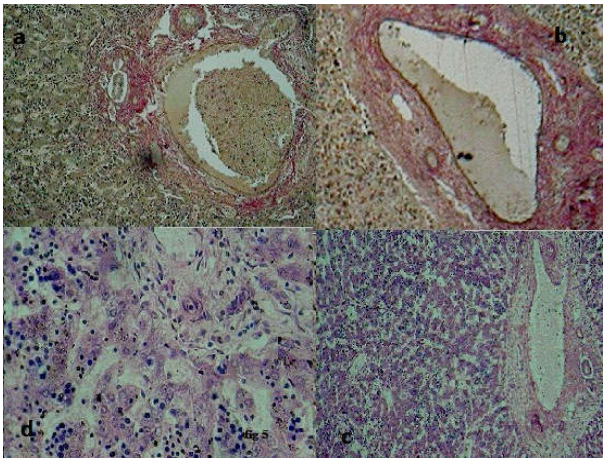

Fig. 5a: Developing portal triad area at 22nd week, showing irregular and immature connective tissue cell
b: at 26th week definite pattern has been formed
c: Well defined portal triad area at 34th week
d: Term liver showing endothelial cells, kupffer cells and hepatocytes near the portal area

multiple branching pattern. Bile ductules are seen in the portal areas with spherical nucleus and cuboidal epithelium (Fig. 2c). Individual hepatocytes and cell boundary are better defined in this age group. Near the central vein, the hepatic plates are arranged as cords but towards the periphery they are arranged as branching pattern. Haemopoietic cells presents near the central vein are very less and are smaller than the hepatocytes having dark staining round nuclei (Fig. 3c). Portal area shows connective tissue elements, mostly mesenchymal cells with spherical nucleus and some fibroblast cells (Fig. 5b).

33 to 38 wks

The size of the lobule as well as the central vein is larger than the previous age group and the sinusoids are better defined. Surrounding the portal triad, hepatic plates are solid with no or little intervening sinusoids. Hepatic cords are 1-3 cell thick and they shows branching pattern. Individual hepatocytes are round to polygonal in shape with vesicular nucleus which is round and eosinophilic in character (Fig. 5c). The hepatocytes are irregularly arranged and lined by flattened cell with flattened nuclei, these cells are endothelial cells. Between the lining endothelium and hepatocytes a small space is present, known as perisinusoidal space. Inside the lumen of the portal vein, there are both nucleated and non-nucleated erythrocytes (Fig. 4d).

Term Liver

The liver parenchyma is almost like adult liver architecture but the hepatic lobules are smaller in size. Boundaries between the adjacent lobules are more prominent than the previous age group fetuses (Fig. 1d). The glandular elements increased much more than the sinusoidal area and the hemopoietic tissue still present although much lesser than the previous age group fetuses (Fig. 3d). The hepatic cords are one to two cells thick. In the portal area all the three structures are clearly visible, and the size of all the structures are almost adult shaped but smaller in diameter than the adult liver (Fig. 2d). Portal area shows abundant amount of connective tissue with extension towards the hepatic lobule although these extensions are very minimal in comparison to the adult liver (Fig. 5d). These areas also show presence of fibroblasts with round to spherical nuclei. These areas also show abundant amount and presence of muscular fiber as depicted by special staining method of massons Trichrome. The capsule is very well developed as identified by its thickness and increase in its layers of connective tissue (Fig. 4d).

Discussion

The report and data on the development of the liver in human fetus by various author revealed that hepatic primordium was derived from proliferation of cell from the blind end of a Y shaped diverticulum, which grows from the foregut into the septum transversum [9,10]. From the cranial part of that diverticulum the liver develops and the caudal part developed into gall bladder and bile ducts [6]. Liver primordium was first identified in a 5-somite embryo as a flat plate of endodermal cell lying ventral to the endoderm of the foregut at the anterior intestinal portal. The same result also obtained by other at 18cm CVR length fetuses [11,12]. In the present study, earliest fetus of our series the liver was easily identified and it was found occupying the abdominal cavity extending from the diaphragm above to the pelvic cavity below, similar findings was reported by Enas Abdul et al. [13]. During the early stage, at 10th week both the right and left lobes are identified and both are of same size. The liver of camel is covered by the thin Glisson's capsule, which first appeared in fetuses of 75mm CVR length [13]. The glisson's capsule was formed of only one layer of flattened cell with oval nuclei, the same result also observed by Moustafa et al in the dog [14]. In our present study, glisson's capsule was observed 14th week onwards till term. At 14th week it was very thin but gradually increased its thickness and term capsule is very prominent and thick.

From the 6th week onwards, the liver primordium was composed of two different cellular elements: the hepatocytes and the hemopoietic cells, in between there are irregular blood spaces. The haemopoietic cells appeared dispersed between the liver parenchyma [3,13]. From the 2nd to the 7th month, blood cells are actively differentiating between hepatic cords and the sinusoidal lining [1,15,16,17]. Hematopoesis begins during the sixth week [18]. Early in the development liver is the primary site of haemopoiesis; in the 7th week, hematopoietic cells outnumber the functioning hepatocytes in the parenchyma [19]. Our present study also supported the above statement as we observe in our series that, 10th week onwards liver parenchyma consists of 2 different cellular elements: hepatocytes and haemopoietic cells. Up to 16th week of gestation haemopoietic cells are more than the hepatocytes and after that hepatocytes element increase and at 22nd week both the elements are equal in the parenchyma.

At first the hepatic cells are small and irregular in shape and form an irregular network of cords separated by islands of hemopoietic cells, but there is little organization into plates and sinusoids that are clearly defined [20]. The hepatocytes were in the form of coalesced liver cords in 12th week onwards [21]. At 12th week there is proliferation of endodermal cells in the form of solid hepatic cords and at 14th week clusters of small mesenchymal cells arranged in the form of vesicles have been seen but endothelial lining was not visible. Hepatic cords were solid at first then acquired lumen [15]. Our present study also support the above statement as we found that at 14th week there is coalesced liver cord and the cords are irregular in shape extending from the central vein and shows anastomotic pattern and the cords are separated by irregular blood spaces. This finding support the above mentioned statement that, hepatic cords were solid at first and acquired lumen and this process start near the central vein towards the periphery.

According to Bates et al., the hepatic lobules are identifiable in the 6th gestational week [19]. Ansari et al stated that, hepatic lobule was measureable from 22nd week onwards in human fetuses [22], but Krause and Cuttis mentioned that the lobulation seen in the adult is not present in the developing embryos [20]. In our present study immature liver lobule was identified from 18th weeks onwards but there was no definitive intralobular boundary and sinusoids were continuous between adjacent lobule and definitive adult architecture was not achieved at term liver also. In fetal life, the hepatocytes plates are two cells thick, a normal finding up to age of 7 yrs [5,17,23]. In our present study we observe that the liver cords were 2-3 cells thick in the earlier period but gradually becomes 1-2 cells thick at term. Kupffer cells are derived from the mesoderm of the septum transversum [10] and kupffer cells appeared at 22nd week of gestation and their number increased till 34th week of gestation [13]. In our present study we observe kupffer cells at first after 25th week of gestation only.

Bile canalicular structure appear very early in the gestation at 6-7th week [19]. Intrahepatic bile duct formation is complete by 3rd month. Bile secretion is noted at 3rd month3 more precisely at the 12th gestational week [24,25]. But some author believes that major bile ducts at the porta hepatis are fully formed by the 16th week of gestation [17,24]. In our present study we found, bile duct in the portal area appeared in 14th week onwards but fully formed at 16th week similar to Vijayan et al. [25,26]. A given volume of liver is comprised of 50-70% of hepatocytes at midterm and 90-95% at term. The remaining cellular elements of those livers are hemopoietic cells which are arranged in clusters containing a variety of developmental stages [27,28,29]. With the increase in gestational age, there is diminution of relative liver weight and this diminution is due to a real diminution in the proportion of true hepatic tissue present in the fetus [30,31,32]. In our present study also we observe that in the first half of pregnancy haemopoietic cells are abundant and reduced in amount during near term.

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