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"Chronological Changes in Microanatomy of Human Foetal Liver - Current Insight"

KEYWORDS: Liver, Microanatomy, Hepatocytes, Portal triad

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ABSTRACT

Background: Liver is the largest compound gland in the body. It plays a major role in metabolism and has a number of exocrine and endocrine functions in the body. It is known that function of an organ depends on histological maturation of that organ. By studying the microscopic structure of liver at various fetal ages will help to establish the time when the liver becomes fully functional.

Aim: Study the microscopic structure of fetal liver at various gestational ages

Material and methods: In the present study 50 liver specimens were collected from formalin fixed fetuses of 10 to 40 weeks of gestation. These fetuses included spontaneous abortions, stillborn fetuses, and preterm babies and also a few autopsy specimens obtained from the various departments of our institution. Then the dissected organs were placed in containers with 10% buffered formalin solution and subjected to routine histological procedures for age related micro anatomical study.

Results: The normal sequence of micro anatomical structure of human fetal liver at different gestational ages was studied by noting the microscopic structure of liver at these stages i.e. changes in organization of hepatocytes and plates of hepatic cells, appearance of central veins and sinusoids, formation of portal triad.

Conclusion: There was local variation in the normal microscopic structure of liver observed during the present study and all findings were grossly comparable with earlier studies except few.

Our study has been planned to provide an insight into the microstructure of human fetal liver during different gestational age of fetal development and compare the findings with similar studies available in literature in an attempt to facilitate the incorporation of the new frontiers of therapy and diagnosis of diseases.

INTRODUCTION:

Liver is the largest gland in the human body [1], and it is among the few internal organs capable of natural regeneration of lost tissue as little as 25% of remaining liver tissue can regenerate [2]. It is a well-known fact that function of an organ depends on histological maturation of an organ. Liver develops as a ventral outgrowth from the gut endoderm in the region of the anterior intestinal portal during 3rd week of gestation [3, 4].

Liver is first visible during the 4th week of development as an outgrowth from endoderm of foregut, which enters the septum transversum and differentiates into masses and plates of the cells. The mesenchyme of the septum transversum forms the capsule and connective tissue elements of the liver. Within the mesenchyme of septum transversum, capillary plexuses are formed and the endodermal cells invest the branches of these plexuses. These endodermal cells are now transformed as a single layer of plates of hepatic parenchyma, while vascular channels in between will form hepatic sinusoids [5].

Liver plays an extraordinary role in metabolism and has a number of functions in the body, including decomposition of red blood cells, plasma protein synthesis, glycogen storage, detoxification and hormone production, liver consists of both exocrine and endocrine parts. The exocrine part secretes bile and endocrine part of liver secretes chemical substances like glucose from glycogen and plasma proteins. In foetal life the liver is an important site of haemopoiesis hence it is essential for all stages of life [6].

Within the adult liver, the Intra Hepatic Bile Duct (IHBD), Portal vein and Hepatic artery run together and are named them as "portal triad". The hepatocytes surround the portal triad and arranged in a single sheet of cells known as hepatic plates or cords, separated by sinusoidal spaces which are connected to a network of blood vessels/capillaries. Plasma of Blood from the portal vein enters the sinusoidal space and comes in direct contact with the hepatocyte basal surface, where metabolites and toxins get absorbed [6].

Hepatocytes are the principal cell forming majority of the (80% of the cells) mass of adult organ. The life span of the hepatocyte is 5 months on an average; they have got an ability to regenerate. Hepatocytes are organised in the form of plates/cords of cells separated by sinusoids supported by a reticulin (collagen type III) fibre network. Sinusoids show a discontinuous, fenestrated endothelial cell lining. The endothelial cells are not having basement membrane and are separated from the adjoining hepatocytes by the space of Disse, which drains lymph into the lymphatics of portal tract [7,8].

Kupffer Cells or Browicz-Kupffer cells or stellate macrophages, are specialized macrophages situated in the liver lining the sinusoidal walls, that form part of the reticulo endothelial system (RES) (aka: mononuclear phagocyte system).Their role is endocytic against blood-borne materials entering the liver. These cells were first identified by Karl Wilhelm von Kupffer in 1876.He named them as "sternzellen" (star cells or stellate cells).In 1898, after many years of research, TadeuszBrowicz, a Polish scientist identified and named them correctly as macrophages [9,10].

Though it is known that liver is relatively large in size in prenatal period, not many details are known about the microscopic structure of liver at different stages of development in the prenatal period [11]. So the present study aims to carry out a light microscopic study of fetal liver of different gestational age in order to determine the normal histological characteristics at various stages of development. This study may provide an insight into light microscopic study in order to determine the sequence of histological differentiation of exocrine and endocrine components of liver and also help to distinguish the normal from certain pathological changes occurring in liver during prenatal period.

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MATERIAL AND METHODS:

In the present study 50 human fetuses were obtained from the department of obstetrics & gynecology of our institution.This work conforms to the values laid down in the Declaration of Helsinki (1964). The protocol of this study has been approved by the institutional ethical committee in which it was performed. Informed written consent was obtained from the parents of fetuses. These fetuses included spontaneous abortions, stillborn fetuses, preterm babies and also a few autopsy specimens. Twins and fetuses with gross anomalies were omitted from our study.

Fetuses were grouped under five groups based on their gestational age (10-20, 20-35, 25-30, 30-35 and 35 weeks above till 40). Gestational age of foetus was obtained from the case sheet of the hospital and further confirmed by measurements of crown rump length (CRL) of these fetuses using spreading Vernier caliper and an Osteometric board with millimeter scale. Gestational Age of fetuses was estimated from the chart co-relating crown-rump length and gestational age using reference from Hamilton and Boyd's text book of embryology [3].

The fixation of the fetuses was done by injecting 10% formalin locally at various sites with the help of 10 ml syringe. After injecting formalin, fetuses were kept in 10% buffered formalin filled glass jars [12]. Then dissected by bilateral subcostal (Rooftop) incision and the liver were removed carefully by severing structures at porta-hepatis along with all the ligamentous attachments close to the liver [13].

Then the organs were placed in containers with 10% buffered formalin solution for 2 to 4 days accompanied by an identifying label. These livers were then processed for preparing paraffin sections. One representative sample of liver tissue from each gestational age group was processed for microscopic examination. The sections were then stained using Haematoxylin–Eiosin(H&E) method [14]. Per-Iodic Acid Schiff stain was also used to observe the glycogen granules in fetal liver. Some sections were stained with Masson's Trichrome(MT) and Reticulin stain to observe the collagen fibers in developing fetal liver [15]. Also a few Haematoxilin&Eosin stained and special stained adult human liver sections were used to compare adult liver architecture from fetal [16] and the following parameters were noted:Observations were noted under a light microscope.

- Organization of hepatocytes and plates of cells
- Appearance of central veins and sinusoids
- Formation of portal triad / tract

RESULTS:

Developmental process was studied from different aspects such as increase in size, weight, microscopic structure and attainment of functions. The present study essentially concentrating on studying the microscopic structure,structural maturation by appearance of central veins and sinusoids, formation of portal triad/ tract. One representative sample of liver tissue from each gestational age group was processed for micro anatomical examination. The results were as follows

1) Histological observations of liver at different stages of gestation are as follows:

10th to 13th week stage of liver: (Fig 1) and (Fig 1.1)

A thin capsule is seen. The parenchymal cells of the liver are oval in shape. Cells show faint pink cytoplasm and large rounded faintly blue stained nuclei, with prominent nucleoli. These cells are arranged in irregular clumps and cords. Large numbers of haemopoietic cells are seen scattered around. Thin vascular iII-formed spaces are present. Central veins and portal triad were not observed.

16th week stage of liver: (Fig 2)

Capsule is somewhat thickened by deposition of collagen fibers. A few blood vessels are seen. At few places central veins are seen. Cells of liver parenchyma are present in the form of irregular anastomosing cords. Initiation of formation of bile duct is seen at some places. Sinusoids start appearing in between these cords and are lined by endothelial cells which can be identified. A clear-cut lobular architecture is not evident.

18th week stage of liver: (Fig 3)

Portal tracts can be identified surrounded by connective tissue. At few places bile ductules can be identified in portal tracts. Hepatocytes are still arranged in irregular clumps and cords and lobular architecture was not evident. Central veins are more numerous

19th and 20th week stages of liver:

More number of central veins is seen. More number of portal tracts could be identified. Portal tracts can be identified.

21st and 22nd week stages of liver: (Fig 4)

Hepatocytes are regularly arranged in cords around the central veins; sinusoids can be clearly identified and are seen to be communicating with central vein. Sinusoids are filled with haemopoietic cells. Central veins and portal tracts can be clearly identified. Portal tracts show all the three elements. Hepatic lobules are clearly demarcated.

28th to 40 week stage of liver: (Fig 5) and (Fig 5.1, Fig 5.2)

All the features are well defined. Haemopoiesis reduces after 34th week of gestation. Sinusoidal walls are lined by endothelial cells. Plates of hepatocytes are seen radiating from central vein. Hepatic cells show vacuolated cytoplasm.Haemopoiesis is seen in all stages of developing liver from 12th to 30th week and no obvious change in volume of haemopoietic tissue could be detected in any stages described above.

MT stain and Reticulin stain (Fig 6 and 7)

Summary:

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

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

IMAGES:

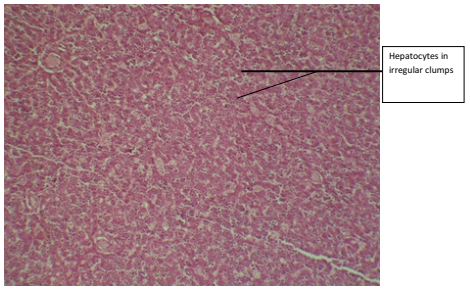

Fig 1: (100 X) Microphotograph of 12th week liver. H & E Stain.

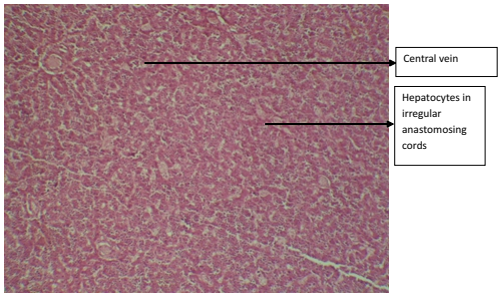

Fig 2: (50 X) Microphotograph of 16th week liver. H & E Stain.

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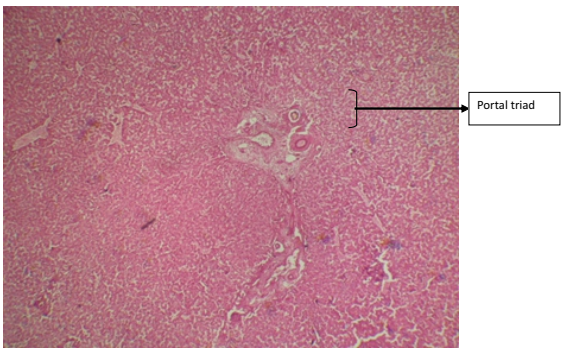

Fig 3: (100 X) Microphotograph of 18th week liver. H & E Stain.

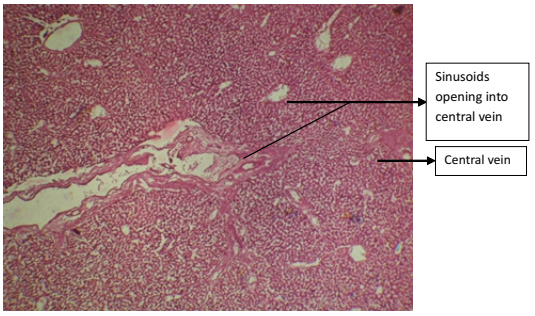

Fig 4: (100 X) Microphotograph of 22nd week liver. H & E Stain.

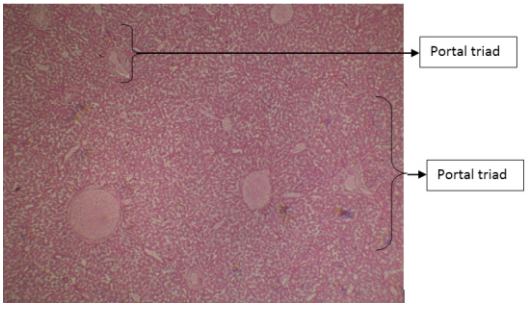

Fig 5: (100 X) Microphotograph of 40th week liver. H & E Stain.

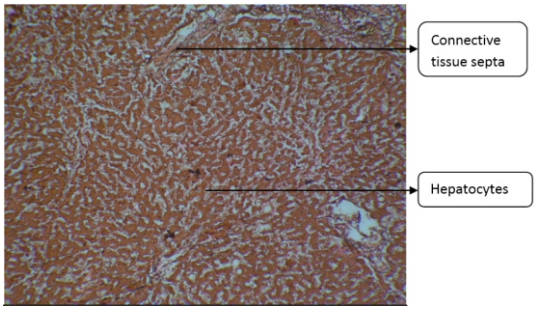

Fig 6: (50X) Microphotograph of 22nd week liver. MT stain

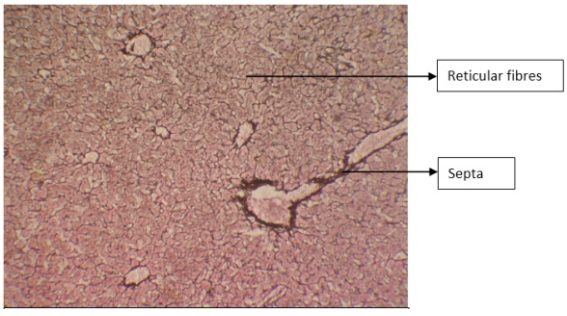

Fig 7: (100 X) Microphotograph of 18th week liver. Reticulin Stain (counter stain Eosin). Showing connective tissue elements

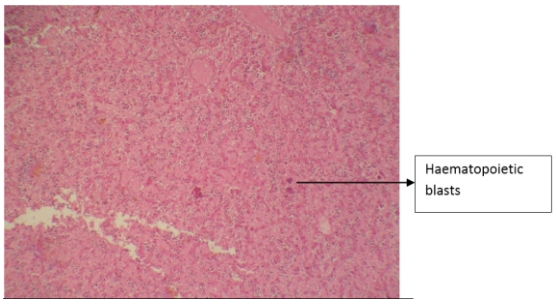

Fig 1.1: (100 X) Microphotograph of 12th week liver. H & E Stain. Showing hematopoietic activity.

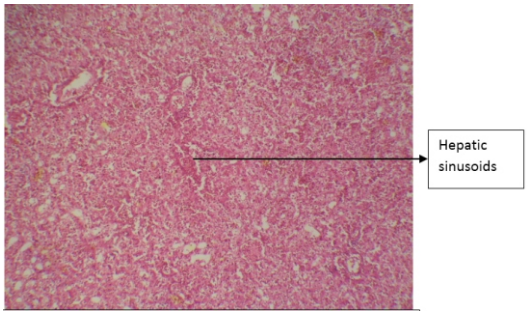

Fig 5.1: (100 X) Microphotograph of 36th week liver. H & E Stain. Showing hematopoietic activity.

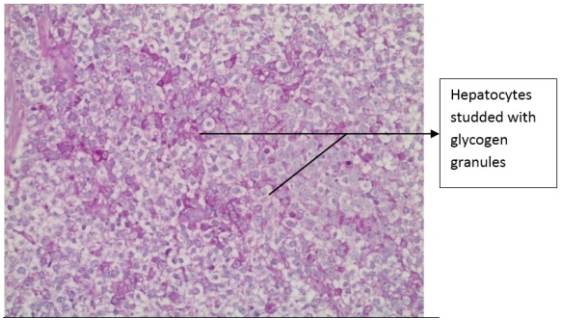

Fig 5.2: (400 X) Microphotograph of 40th week liver. PAS Stain. Showing highly concentrated hepatic glycogen granules.

Discussion:

Potter and Craig reported that the liver differentiates into masses and plates of cells at 4th week. Mesenchyme of septum transversum forms capsule and connective tissue elements. Endodermal cells transform into hepatic parenchyma while vascular channels form the hepatic sinusoids. Bile canaliculi form bile ductules around hepatic cells. Bile ductules along with hepatic artery and portal vein form portal tract [17].

According to bhorgese the developmental signs of haemopoiesis begin at about 6 weeks of gestational age. As we didn't get a specimen lesser than 10 weeks of gestational age,this finding was not noted down [18].

Bradley & Neil stated that the development of Kupffer cells and connective tissue cells begin at about 3rd month of gestational age. We observed Kupffer cells at 20th week and connective tissue cells at 16th week of gestation [19, 20].

Balis et al suggest that the liver plates are formed before the development of sinusoids [21].

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Blouin and Suyan stated that peri-portal connective tissue was observed during 8-12 weeks of gestational age.in our study it was seen at 16th week stage of development [22].

In the present study, it is seen that the connective tissue elements consist of thin capsule and reticular fibres supporting hepatic cords, but later on reticular fibres are associated only with blood vessels excluding central vein.

From 13th to 15th week the thin capsule and irregular clumps and cords of hepatic parenchymal cells are present.

According to Zhang Wenxuehaematopietic cells were seen from 15-35 weeks of gestational age [23]. Findings of the present study are in accordance with all the previous workers have stated above. Haemopoiesis is present prominently in all the stages studied. But more characteristically observed in the liver from 13th to 36th week of gestation Central veins were visualized for the first time at 16th week of gestation. Sinusoidal wall lined by endothelial cells were also identified at this stage for the first time.

This observation of appearance of central veins and sinusoids is not mentioned in any of the literatures i have referred.

Portal tract could be identified earliest at 18th week of gestation, but the clear cut architectural pattern was not evident at this stage of development. All the structures of classical liver could be identified clearly at 22nd week. The size of hepatic lobule only increased thereafter.

Conclusion:

The normal sequence of microanatomy of human fetal liver at different gestational ages was studied by noting the following findings in its order of appearance i.e. changes in organization of hepatocytes and plates of hepatic cells, appearance of central veins and sinusoids, formation of portal triad, and presence of haemopoietic tissue.

These findings were by and large comparable with earlier studies and are in concurrence with studies done on the subject by earlier workers with slight changes in the order of appearance of structures.Of late liver biopsy performed during neonatal period been used to presumably diagnose certain congenital abnormalities like intra-hepatic bile duct obstruction and Glycogen storage diseases. Early diagnosis of these conditions can helpto prevent their progression into chronic stage.

An understanding of normal sequence of micro anatomical changes of liver along with some common variations possible is a stepping stone towards this correlation. However a study group involving a larger sample size and the use of more detailed biochemical and ultra-structural analysis using electron microscopy with special staining techniques will add more to this understanding and aid in the early definitive diagnosis of certain liver diseases.

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