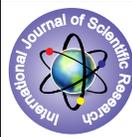


The Histogenesis of Human Liver a Perspective Study on Organization of Hepatocytes and Portal Triad



Medical Science

KEYWORDS : Histogenesis,Liver, Hepatocytes,Portal triad

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ABSTRACT

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.

INTRODUCTION:

The development of human liver is an ongoing process which begins after fertilization and continues into postnatal life. It is a well-known fact that function of an organ depends on histological maturation of an organ.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, glycogenstorage, detoxification and hormone production,liver consists of both exocrine and endocrine parts.In foetal life the liver is an important site of haemopoiesis hence it is essential for all stages of life.[1]

Within the adult liver, the Intra Hepatic Bile Duct(IHBD), portal vein and hepatic artery run together parallel and are named them as the "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. [2]

Ham and Cormack said that the liver is unique; there is no division of labour between those cells that produce the exocrine and those that elaborate the endocrine secretion. All its parenchymal cells (Hepatocytes) produce both kinds of secretions. Recent researches indicate that hepatocytes and cholangiocytes may have been derived from a common precursor or stem cell. [3]

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.[4,5]

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.[6,7]

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.[8] So the present study aims to carry out a light microscopic study in order to determine the early Histogenesis of human liver. After determining the normal histological characteristics at various stages of development, this study may provide an insight into and also help to distinguish the normal from certain pathological changes occurring in liver during prenatal period.

MATERIAL AND METHODS:

Collection of material:

In the present study about 50 fetuses were obtained from the department of OBSTETRICS & GYNAECOLOGY of our institution. 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.

Measurement of external parameters:

Crown Rump length (CRL) of these fetuses was measured using spreading Verniercalliper and an osteometric board with millimetre scale. Gestational Age of fetuseswere estimated from the chart co-relating crown-rump length and gestational age as given in text book of Embryology by Hamilton, Boyd and Mossman.

Fixation of fetuses:

The fixation of the fetuses was ensured by injecting 10% formalin locally at various sites with the help of 10 ml syringe and needle in abdominal, thoracic, cranial cavity and subcutaneously in the upper and lower limbs. After injecting formalin, fetuses were kept in 10% buffered formalin filled glass jars before dissection.

Dissection, measurement and fixation of liver:

After fixation fetuses were carefully dissected by taking bilateral subcostal (Rooftop) incision on the anterior abdominal wall and the liver was removed carefully by severing structures at porta-hepatis along with all the ligamentous attachments close to the liver. [9]

Then the organs were placed in containers with 10% buffered formalin solution for 2 to 4 days accompanied by an identifying label. The label comprised of gestational age of foetus, any clinical factors directly responsible for death and identification number.These livers were then processed for preparing paraffin sections of liver tissue. [10]

Tissue processing:

The fixed sections of liver tissue were then dehydrated in ascending grades of alcohol. They were placed in 70% alcohol for 6-8 hrs during the day, then in 90% alcohol overnight. This was followed by three changes of absolute alcohol for one hour each on the next day. Excess of alcohol was removed by keeping the tissue on blotting paper

and then placed in xylene for about 2-4 hours for clearing. Then the tissue was subjected to three changes of paraffin wax at 56 to 60°C temperature for one hour each.

Paraffin block making:

The tissue was then embedded in fresh filtered wax. 'L' shaped mould (Leuckharts Mould) was smeared with glycerin, and fresh filtered wax was poured into it so as to fill it completely. The block was then removed from the mould. The blocks were labeled appropriately with blotting paper and pencil.[11]

Section cutting of tissue block:

Finally the block was cut with the help of rotary microtome into sections the size of 5 to 7 micron in thickness in the form of ribbon. The ribbon was gently placed on the surface of water in a preheated water bath whose temperature was set at 50-60° C in order to remove folds.

Mounting of sections:

The sections were lifted from the water bath and placed on egg albumin coated slides. Slides were kept for drying on a hot plate at 45°-50°C for 2 hours or more. After this the slides were fixed with flaming over a spirit lamp.

Staining:

The sections were then stained using H-E method. Per-Iodic Acid Schiff stain was also used to observe the glycogen granules in fetal liver.(as given in Carleton's Histological Technique-1973).[10,11]

Some sections were stained with Masson's Trichrome and Reticulin stain to observe the collagen fibres in developing fetal liver. Also a few Haematoxylin&Eosin stained and special stained adult human liver sections were used to compare adult liver architecture from fetal.

Thus the following parameters were noted:

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

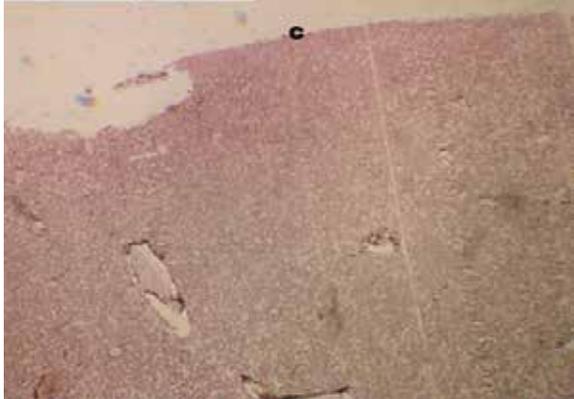
RESULTS:

Developmental process can be studied from different aspects such as increase in size, weight, microscopic structure and attainment of functions.Our study essentially comprises of studying the microscopic structure and structural maturation by appearance of central veins and sinusoids, formation of portal triad/tract.

Microscopic structure of liver at different stages of gestation:

Histological observations made are as follows:

Fig 1: (30 X) Microphotograph of 10th week liver. H & E Stain showing thin capsule(C)



10th and 13th week stage of liver:(Fig 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 number of haemopoietic cells are seen scattered around. Thin vascular ill-formed spaces are present. Central veins and portal triad were not observed.

14th and 15th week stage of liver:

Histological features are similar to that of the 13th week stage. However additionally few small blood vessels surrounded by connective tissue are also seen.

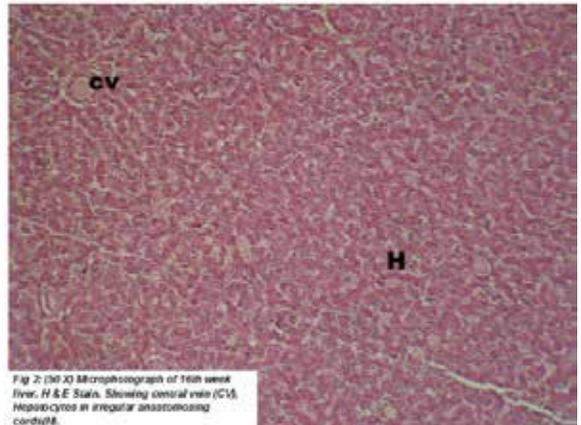


Fig 2: (30 X) Microphotograph of 16th week liver. H & E Stain. Showing central vein (CV). Hepatocytes in irregular anastomosing cords(H).

16th week stage of liver: (Fig 2)

Capsule is somewhat thickened by deposition of collagen fibres. 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 clearcut lobular architecture is not evident. Portal triad was not seen.

17th week stage of liver:

Histological structures are more or less similar to that of the 16th week stage liver except central veins are more numerous.

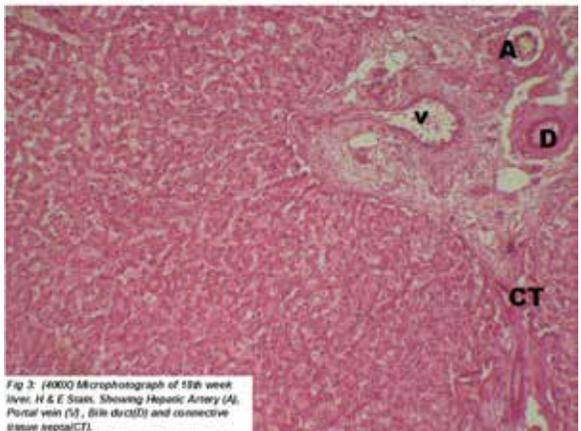


Fig 3: (400X) Microphotograph of 18th week liver. H & E Stain. Showing Hepatic Artery (A), Portal vein (V), Bile duct(D) and connective tissue septae(CT).

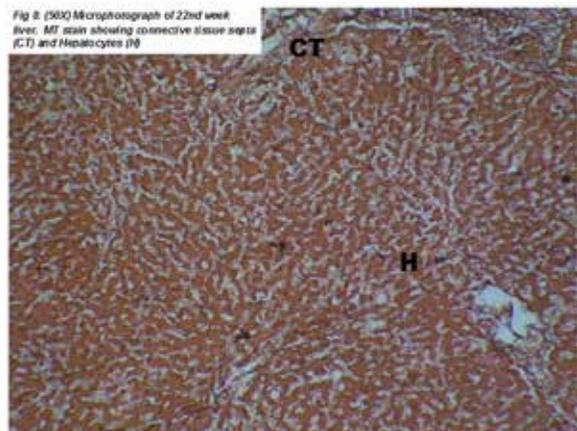
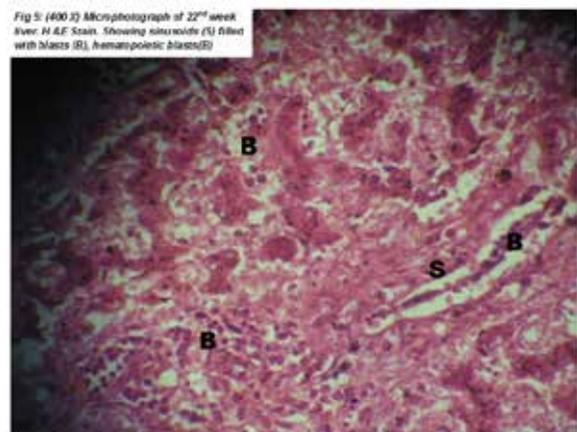
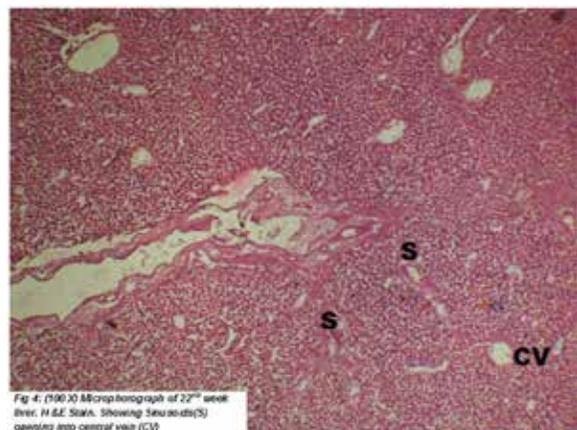
18th week stage of liver: (Fig 3)

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 is not evident.

19th and 20th week stages of liver:

More number of central veins are seen. More number of portal tracts could

be identified. Portal tracts can be identified.

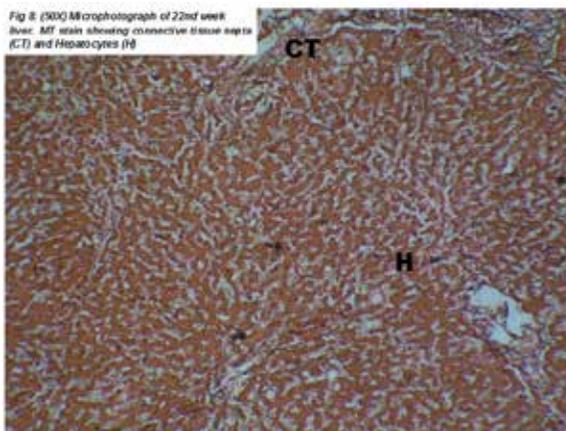
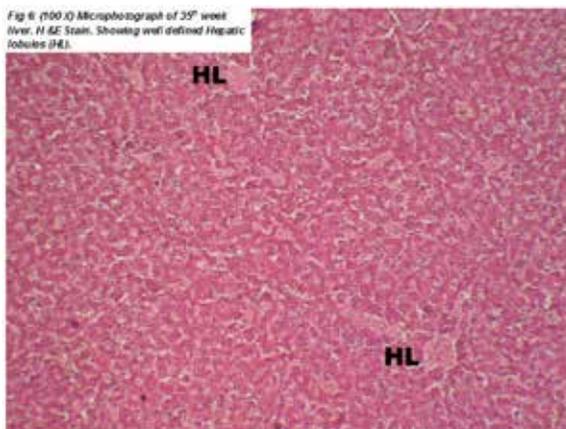


21st and 22nd week stages of liver: (Fig 4, 5, 8)

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.

23rd week to 27th week stage of liver:

The microscopic structure of liver is similar to that of 22nd week.



28th to 40 week stage of liver: (Fig 6)(Fig 7)

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.

Summary of Development from 12th to 36th week Stage of Liver:

Central Vein: Central vein appears at around 16th to 17th week 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.

DISCUSSION:

50 human fetuses of different age groups were studied. Structure of foetal liver was observed under light microscope by differential staining procedures in fetuses of 10 to 40 weeks of gestation. The following were observed.

The microscopic structure of liver at different stages in prenatal period i.e. Changes in organization of hepatocytes and plates of hepatic cells

At 12 to 15 weeks a thin fibrous capsule and irregular clumps and cords of hepatic parenchymal cells were present.

Appearance of central veins and sinusoids, & Formation of portal triads.

Central veins appeared at 16th week. Sinusoidal wall lined by endothelial cells were also identified first at 16th week of gestation.

Portal tract comprising of the branches of portal vein, hepatic artery and bile ductules could be identified first at 18 weeks but all the structures of classical liver can be clearly identified first at 22nd week stage, the size of lobule only increases thereafter.

CONCLUSION

The normal sequence of histogenesis of human 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. These findings were in concurrence with studies done on the subject by earlier workers.

In addition to Haematoxylin&Eosin(H&E) stain, Periodic Acid Schiff(PAS) stain was used to demonstrate glycogen granules within the developing liver cells and at different gestational ages, findings were in agreement with what has been noted by workers on the subject earlier.

There was local variation in the normal histogenesis observed during the present study and all findings were grossly comparable with earlier studies except one 32 week foetus which showed a variation in deposition of glycogen granules. This was correlated clinically and biochemically as a case of deficiency of phosphorylase kinase enzyme (Glycogen Storage Disease).

Of late liver biopsy performed during neonatal period is been used to presumably diagnose certain congenital abnormalities like intra-hepatic bile duct obstruction and Glycogen storage

diseases. Early diagnosis of these conditions can help prevent their progression into chronic stage.

An understanding of normal histogenesis 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 electron microscopy with special staining techniques will add to this understanding and aid in the early definitive diagnosis of certain liver diseases.

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