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Histological and Morphometric Parameters Study of Human Neonatal Liver in Kirkuk City

Yassin Salih Abdullah[1], Dr. Elham Majeed Mahmood[2], Dr. Saad Ahmed Mohammed[3]

Abstract

Background: The liver is the largest organ in a newborn's body, and it is situated in the upper abdominal cavity, and is responsible for around (2) to (3) percent of an average person's total weight. Liver has some important roles in the body by performs several essential functions such as detoxification, protein synthesis, bile production, and nutrient storage, works synchronously with numerous other organs,, to maintenance the homeostatic mechanisms.

Aim: The current study aims studying the histological and morphometric parameters of the human neonatal livers in the Kirkuk city. Many investigators have been worked on the adult liver in human, but there are few previous studies about the neonate liver, Thus the main aim of the present study is to clarify and compare the findings about neonatal liver with that of the other researchers.

Patients and methods: An autopsy of 30 cadavers within forensic medicines unites at Kirkuk governorates had been achieved, with age ranging from 1-29 days ,50-55 cm length, and 2.7- 6 kg body weight were examined, during the period from Nov 2022 to Jun 2023. The cadavers were inspected grossly for studying the anatomical features and the tissue from each sample were fixed and examined by using leica microscope for studying the histological feature and measuring some features diameters and size.

Results: The current study showed, that the liver of the neonate is surrounded from outside by a layer of dens irregular connective tissue which represents the capsule. The microscopic examination of the capsule showed that it consists of a non-thick layer of irregularly arranged collagen fibers, the parenchyma of the liver consists of the hepatocytes, hepatocytes was arranged in palate, the classical lobule in the neonate liver was hexagonal in shape.

In conclusion, this study provides evidence of the histological feature, and other morphometric parameters of human neonatal liver. morphometric parameters of human neonatal liver by using leica microscope, applicable and reproducible estimates in calculating normal liver histological parameters. I think that my study may be a reference for similar studies to be done in the upcoming years.

Keywords: *neonates, liver, histology, classical lobule, portal triad.*

[1] Human anatomy and histology department, Tikrit university faculty of medicine, Iraq, ORCID: 0009-0000-6281-2520, yaseen07704088@gmail.com

[2] Ph.D. human anatomy and histology, Tikrit university, college of medicine human anatomy and histology department, Tikrit, Iraq, ORCID: 0000-0001-5507-8022

[3] Ph.D. human anatomy and histology, Tikrit university, college of medicine human anatomy and histology department, Tikrit, Iraq, ORCID:0000-0002-5032-9504

1- Introduction

The liver stroma is composed of connective tissue capsule (Glisson's capsule), while its parenchyma is composed of hepatocytes. When the connective tissue of liver capsule enters the parenchyma at the liver's hilum, its branches in various directions, dividing the liver parenchyma into its basic structural unit, the hepatocyte (hepatic lobule or classical liver lobule) (1)

1.1. parenchyma of the liver:

The liver parenchyma is composed of the classical lobule, the portal lobule, and the hepatic acinus. Approximately 80% of the entire hepatic volume is comprised of hepatic lobules, which are built mostly of one-cell-thick liver cell cords constructed of polyhedral hepatocytes. Both endothelial cells and Kupffer cells line the neighboring sinusoids. Between the endothelial cells and hepatocytes lies the perisinusoidal space (space of Disse). (2,3)

A-classical lobule:

The structural unit of the liver has a hexagonal structure, and at the six angles of each lobule are portal tracts including (arterioles, venules, bile ductules, and lymphatics). At the center of each lobule is the central vein (4), and the human liver includes around 1 million lobules. Plates of hepatocytes seem to radiate outward from the lobule's center region towards the central vein. . (5)

B-Portal lobule:

On the basis of biliary flow through the liver, its shape is triangular, with the central vein found at each corner of the triangle and the portal vein located at the center of the triangle. Bile produced by hepatocytes in the triangular unit is collected in the bile duct, which is located at the center of the triangle. Blood and bile migrate in opposing directions; blood goes from portal areas to the central vein through sinusoids, whereas bile produced into canaliculi is guided to portal regions by specific hepatic microvilli. . (6)

C- Hepatic acinus:

-The diamond-shaped acinus is the functional unit of the liver, with the central vein positioned at the two opposed angles of the diamond and the portal traid at the other opposite angles. The acinus is split into three zones based on the distance from the blood supply.

-Zone 1: encircles the portal tract and is the most oxygenated owing to its proximity to blood arteries.

-Zone 2 has less oxygen than Zone 1

-Zone 3 encompasses the hepatic venule (central vein) and contains the least oxygenated blood owing to its distance from blood arteries. . (7,8)

1.2. Hepatocyte:

Hepatocytes have an average diameter of 25–40 m and are polyhedral and multidimensional. The cells are organized in one- or two-cell-thick cords with rich eosinophilic cytoplasm and a nucleus in the center. The nucleus may include glycogen and is spherical or oval in shape. (2,3)

1.3. Hepatic sinusoids

Blood passes via sinusoids from the portal vein to the central vein. They are lined by endothelial cells and Kupffer cells, which rest atop a layer of reticulin that may be readily seen using a reticulin stain. The endothelium lining is plate-like and perforated, facilitating the interchange of blood and hepatocytes through the Disse space. . (9,10,11)

1.4. Space of Disse:

The space of Disse is a thin per sinusoidal gap between endothelial cells and hepatocytes containing the milieu for blood and hepatocyte exchange. . (12,13)

1.5. Kupffer cells:

Kupffer cells are macrophages that line the sinusoid in the liver. They comprise more than 75 percent of all fixed macrophages in the body and roughly one-third of the hepatic sinusoidal cell volume. They are most prevalent in zone 1 of the sinusoidal wall, where they are attached to endothelial cells. These cells endocytose and kill microbes, eliminate endotoxins and senescent erythrocytes, and may function as antigen-presenting cells. (14)

2- Materials and Methods:

An autopsy of 30 cadavers within forensic medicines unites at Kirkuk governorates had been achieved, with age ranging from 1-29 days ,50-55 cm length, and 2.7- 6 kg body weight were examined, during the period from Nov 2022 to Jun 2023.The Medical Ethics Committee of Tikrit University College of Medicine had approved this study with the code number (IQ.TUCOM.REC.3/7/450). Ethical agreement statements were acquired from all individuals' families in this study, according to the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh. The tissue from each sample were fixed and examined by using leica microscope for studying the histological feature and measuring some features diameters and size.

2.1. Sampling and fixation

Liver specimens were taken from separately part of the liver each specimens with 1cm3 dimensions. specimens were fixed using 10% formalin solution (100 ml of 40% formaldehyde, 9 gm Sodium chloride and 90 ml of tap water), for 24hours at room temperature to fixation tissue and prevent it from autolysis.

2.2. preparation of liver tissue(samples) specimens.

The tissues were processed according to (15), as the following procedure:

1 – the tissues were removed from formalin (10 %), and washed in running tape water for 30 minutes to remove traces and fixative.

2-Dehydrated through upgrading alcohol's (70%, 80%, 90%, 95% & absolute alcohol), it took halves an hour's hour for each concentration.

3 -Cleared in xylene using two change of xylene (30 minute for each change).

4-Infiltrated in paraffin wax (B.D.H. / England, with a melting point of 55-60 C°) for 2 hours.

5-Embedded in melted paraffin wax on a steel chuck, which has already coated with glycerol and frozen on zero for 1 hour.

6-Sectiones were made of 6 μm using a microtome (Cambridge microtome) at room temperature.

7-Sections were drawen as a ribbon in a flat unwrinkled sections and placed in water bath at 45C° for 30 minutes.

8-Sections were picked up on clean slides, which were already coated slightly with egg albumin and glycrine, then mixture was filtered by using piece of gauze, finally few drops of thymol were added (act as preservative substance)

2.3. Staining methods:

1. Haematoxylin and Eosin staining: (15)

A histological representative sections from each block of the liver were taken to detect their general histological structure in a routine technique (16) Thus, 6 µm paraffin sections, were air dried on slide at room temperature and subjected to the following procedure:

1 – Deparaffinized in xylol for 2 changes, each for 5 minutes.

2 - Hydrated through graded alcohol's:

Absolute alcohol, 2 changes, each for 1 minute.

- 95% alcohol for 1 minute.

- 80% alcohol for 1 minute.

- 70% alcohol for 1 minute.

- 50% alcohol for 1 minute.

- 30% alcohol for 1 minute.

- Distilled water for 1 minute.

3 – Stained with Harris Haematoxylin using the following components: -

-Haematoxylin …… 2.5 g

-Absolute alcohol …… 25 ml.

-Aluminum potassium sulphate …… 50 g.

-Distilled water…… 500 ml.

-Mercuric oxide …… 1.25 g.

-Glacial acetic acid …… 20 ml.

4 – Washed in running tap water for 5 minutes until sections become blue.

5 – Differentiated in 1% acid alcohol (1% HCL in 70% alcohol).

6 – Counter – stained in 1% eosin for 1minute.

7 – Washed in tap water for 5 minutes.

8 – Dehydrated in ascending alcohol's (30%,50%,70%, 80%, 90%, & 95%) for 1 minute each; then in absolute alcohol for 2 changes, two minutes for each.

9 – Cleared in a –xylene: alcohol for 30 seconds.

b –then xylene only for 30 seconds.

10- slides were covered with a mixture of glycerine and thymol granules to prevent fungal growth.

11 – Finally sections were air dried and mounted in Canda balsam.

2.4. Microscopical inspections and Descriptive histology:

Morphometric and descriptive histology are both used in the microscopic analysis of liver tissue sections. Microscopical examinations in this work were conducted using a Leica light microscope. Histological characteristics of newborn livers were analyzed by microscopic inspection of tissue sections (obtained from liver tissues used in the current research).

2.4.1. Measuring Technique:

Measurement Livers capsule thickness, classical lobule size and portal traid size, was made per the section using 50X objective lens for these measurement. The thickness of liver capsule was measured by using an ocular and a stage micrometer. Six different

locations were used to measure each species of liver capsule thickness from the outer to inner part. The mean and standard deviation (±SD) value were then computed.

Each line on the calibrated ocular lens is equivalent to 2.4 m, and at 40X magnification, the calibrated stage was placed in a position of supremacy between the ocular lens and the objective lens. The classical lobule & portal traid diameter measured by measuring perpendicular dimensions, the following formula was used to determine the diameter.

Diameter of classical lobule &portal traid = (Maximum transverse diameter + Maximum perpendicular diameter) ÷ 2

3- Results

3.1. Descriptive histology;

A-Liver capsule (Glisson capsule);

The current study showed, that the liver of the neonate is surrounded from outside by a layer of dens irregular connective tissue which represents the capsule. The microscopic examination of the capsule showed that it consists of a non-thick layer of irregularly arranged collagen fibers as it appears in the (figure 1).

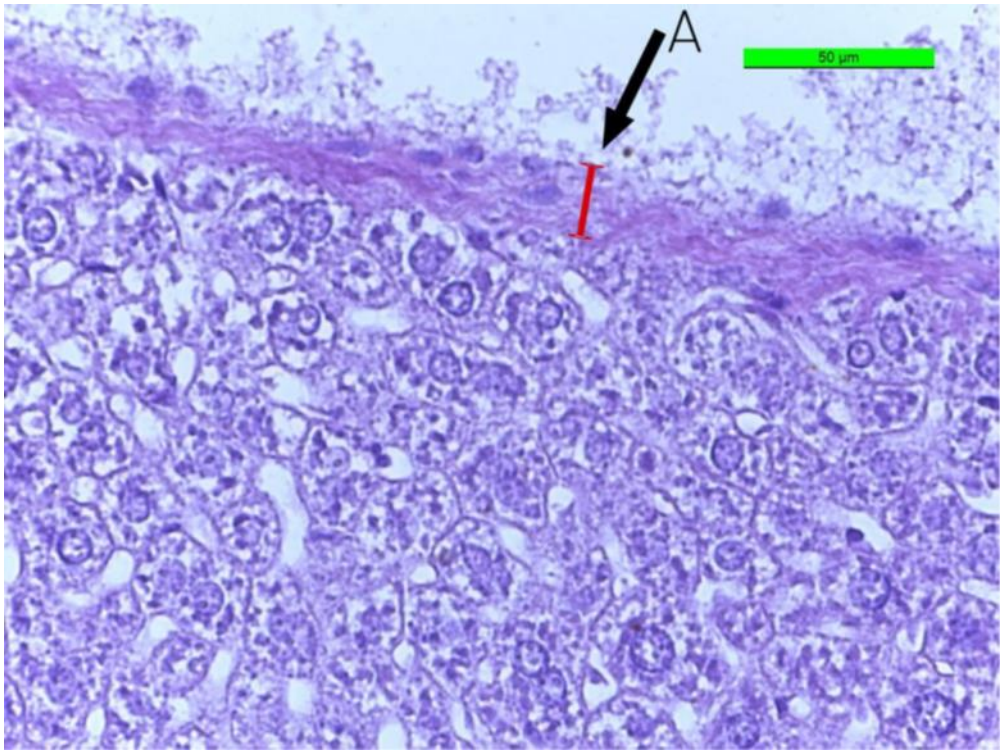

Figure 1: shows liver capsule (“Glisson capsule”) (H&E stain, 50X)

B-Parenchyma of the liver and portal triad:

The parenchyma of the liver consists of the hepatocytes, hepatocytes was arranged in palate that were one or two cells thick and was separated by sinusoids. The current study showed that the neonatal liver parenchyma consists mainly of polygonal cell with an acidic cytoplasm, and most of them bi-nucleated as in (figure:2).

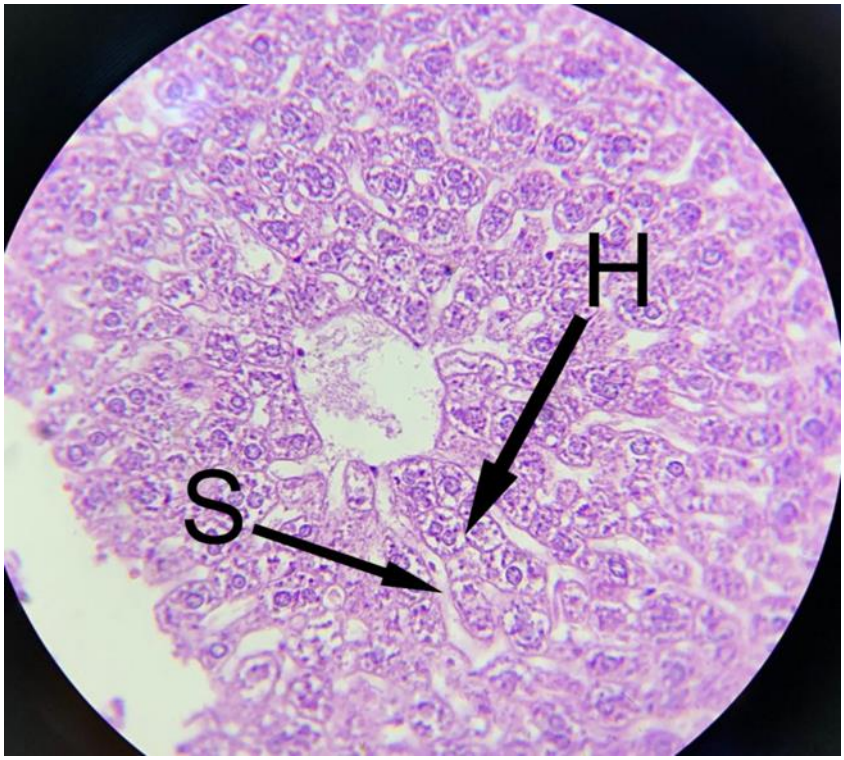

Figure 2: Hepatocytes (H) are distributed in plates separated by the sinusoids (S)

(H&E stain, 40X)

In the portal area (portal vein, hepatic artery, bile duct) the three structures were clearly visible. Portal area shows abundant amount of connective tissue with extension towards the hepatic lobule although these extensions are very minimal and little weak these areas also show presence of high amount of fibroblasts with round to spherical nuclei as in (figure 3).

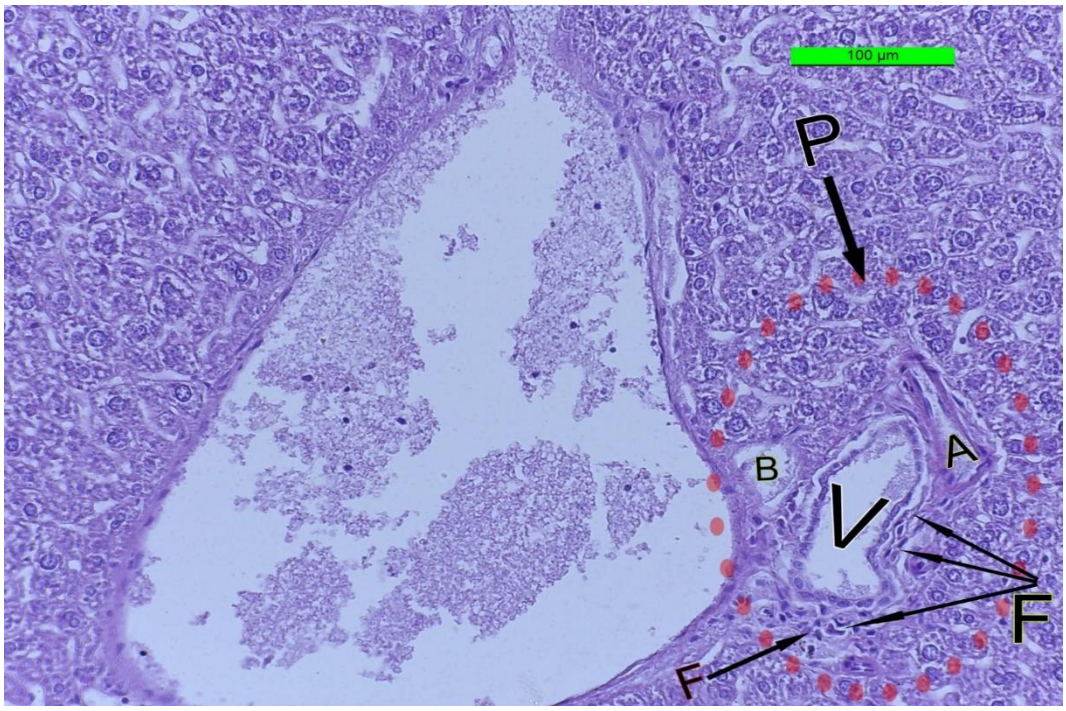

Figure 3: Neonatal portal region “portal triad” and their structure (H&E stain, 20X)

Portal vein(V), Bile duct(B), Hepatic artery(A), fibroblast (F), portal area (P)

C-Hepatic Sinusoids:

The histological findings of the current study revealed that the hepatic sinusoid were present in between the hepatic plates when the blood passes through the sinusoids the hepatocytes exchange metabolites from the blood, the sinusoids were found to be dilated along with the central vein and portal radical. This sinusoid was lined by endothelial cells and kupffer cells. (Figure 4).

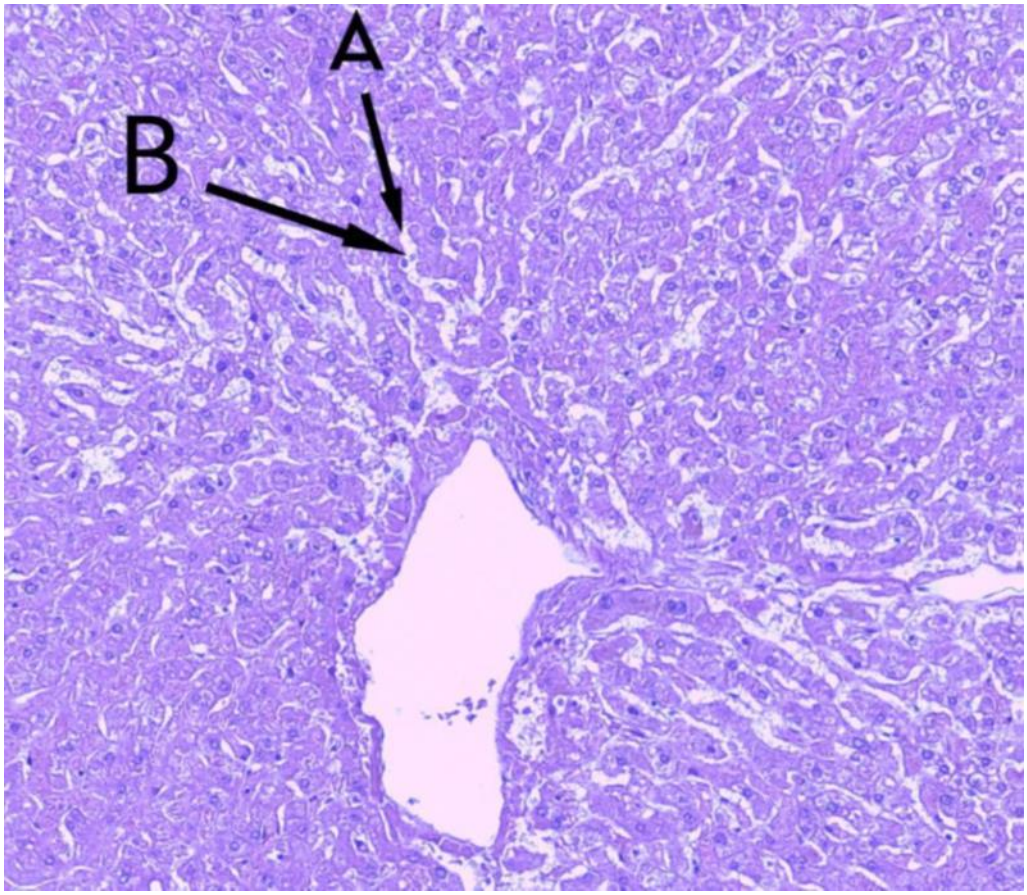

Figure 4: shows the liver sinusoid in between the hepatic palate (H&E, 20X)

A-hepatic sinusoid B – kupffer cells

D- classical liver lobules or hepatic lobules:

The present study shows the classical lobule in the neonate liver are hexagonal in shape. And in the three to five corners of its corners are found portal triads, which contain a branch of portal vein, hepatic artery, a bile duct and occasionally a lymphatic vessel. And in the center of each lobule there were the central vein. As in (Figure 5).

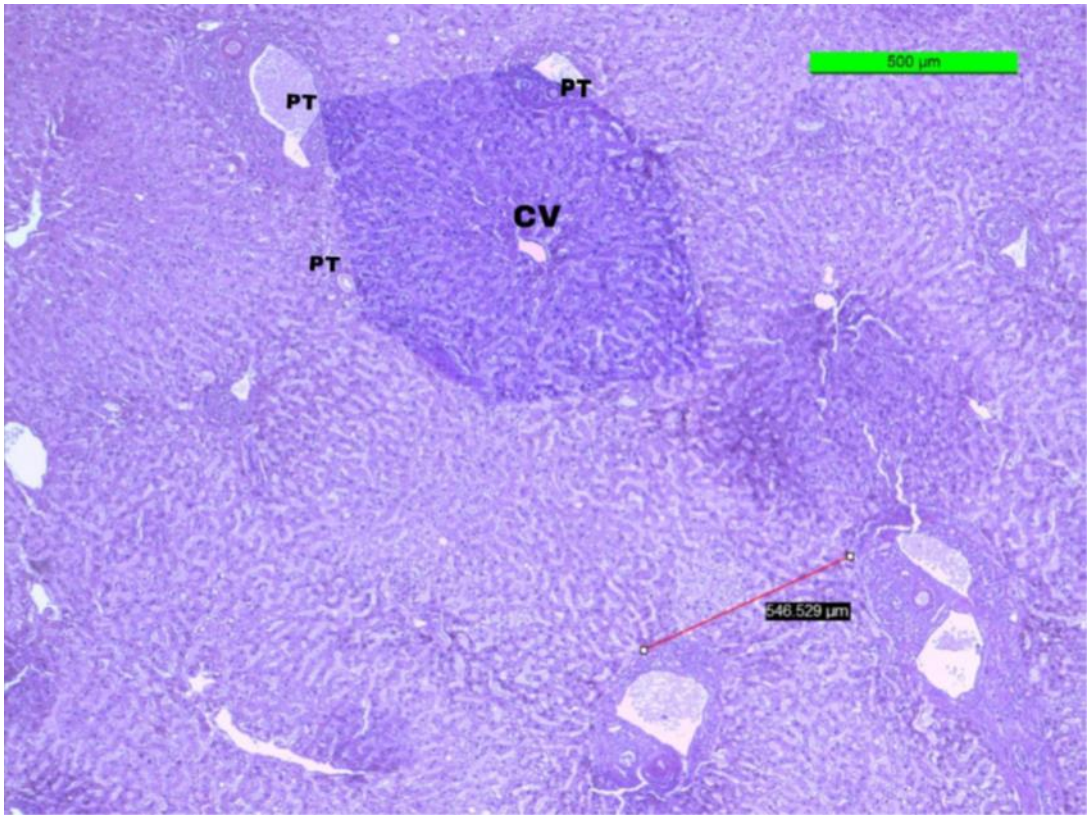

Figure 5: Section of the liver with classical lobule has been marked in

(H&E stain, 5X), portal triad(PT), central vein(CV)

3.2. Morphometric histology:

A- Measurement of capsule thickness:

The histological findings of the current study of 30 samples of neonatal liver revealed that, the capsule consists of connective tissue layer that covering the liver. The means ±SD of neonate liver capsule thickness were 20.95±2.85 µm. as in (figure 6, and table 1)

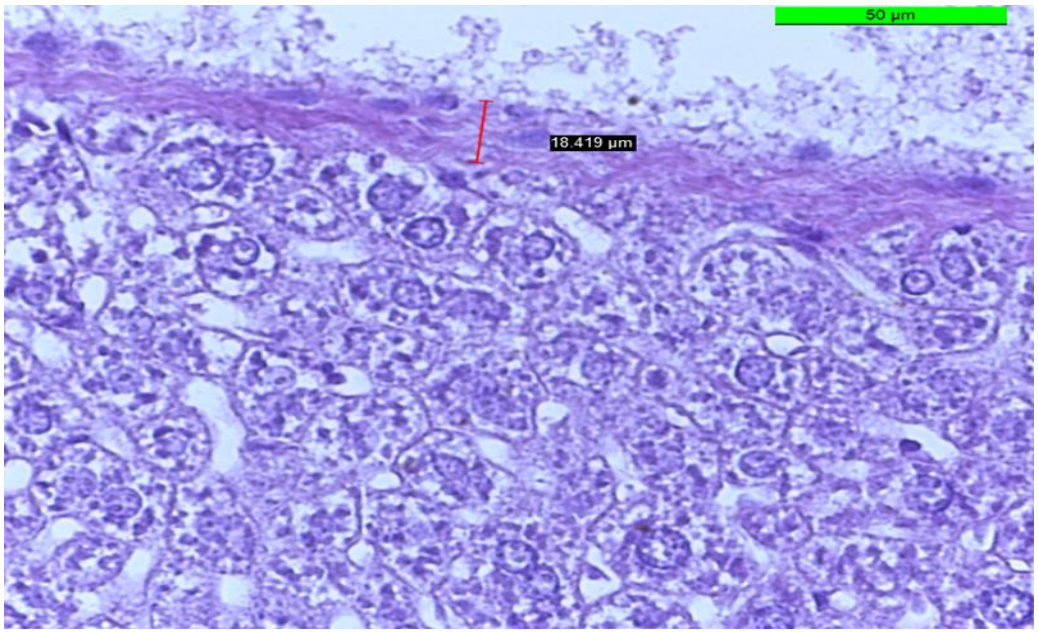

Figure 6: Liver section by Leica microscope under high-power objective 50X (H&E stain) show capsule thickness measuring.

B-Diameter of the classical lobule:

In this part of study, the diameter of the classical lobule was measured by measuring the perpendicular dimensions of the lobule. by Leica microscope measurement tool, the diameter was 0.75 – 1mm with means ±SD were 0.75 ±0.84 as in (figure 7, and table 1).

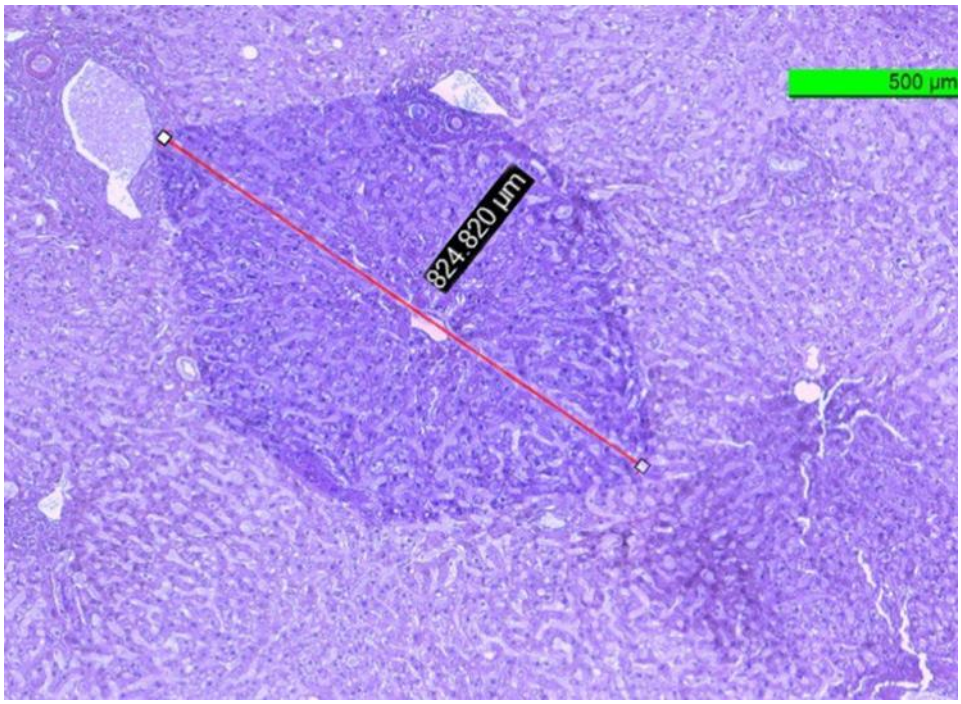

Figure 7: Diameter measuring of classical lobule by Leica microscope

(H&E stain 5X)

C- Measurement the diameter of the portal triad:

In this investigation, 3 slides from each liver sample were selected, and a power goal of 10,20X was used to measure the diameter of the portal area by measuring the perpendicular dimensions of the portal triad and calculating their diameter that were <100 µm, and the means ±SD were 2.4±0.4 mm (table 1).

D- Counting the Numbers of portal triads in each classical lobule:

In this part of the study, the number of portal triad in each classical lobule were counted by selected 4 slide from each sample of the liver and after the histological slides examination by the light microscope (Leica), The histological findings of the current study revealed that there are from 3-5 portal triads in each classical lobule. As in (figure 8, and table 1).

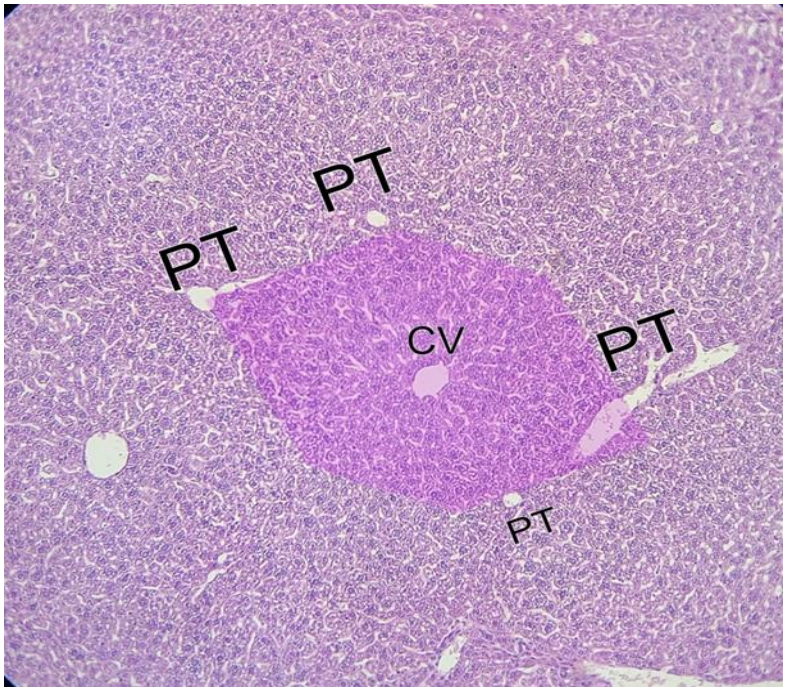

Figure 8: Histological liver section show the hepatic lobule (hexagonal shape)

Portal triad(PT), Central vein(CV) (H&E stain, 10X).

4- Discussion

An examination of histological sections of the liver of a human newborn showed that the liver is surrounded by a layer of dense, irregular connective tissue that represents, the capsule, and that the capsule consists of a thin layer of collagenous fiber. This agree with Guido M et al (17), as well as Islam MJIJoA (18). The present findings about the liver parenchyma and hepatocyte of neonatal liver coincided with Jaiswal A et al (19), who said that the parenchyma of the liver is composed mostly of hepatocyte that are distributed in a cord-like manner and are one to two cells thick. The fetal hepatocytes were big, oval to polygonal, with extensive granular eosinophilic cytoplasm; the nuclei were large, round to oval, and this agree with Islam MJIJoA. (18).

The findings of the current research concurred with those of Islam MJIJoA (18), who said that the three structures in the portal region were clearly apparent and resembled adult shapes but were smaller in diameter.

In the current investigation, hepatic sinusoids of neonatal liver were seen between the hepatic plates; the sinusoids were also shown to be dilated, along with the central vein and portal triad. In accordance with Bhadoria P et al (20)., the sinusoid was bordered by endothelial cells and kupffer cells.

It was revealed that the traditional lobule of neonatal liver was hexagonal in form. And at the six angles of the lobules, there are portal triads, which is consistent with Zhang S et al (21) and Guido M et al (17) who mentioned that the lobule is a roughly hexagonal structure, and it consists of plates of hepatocytes lined by sinusoidal spaces that radiate toward a central efferent vein, and that each of the six hexagon corners is demarcated by a portal triad.

Morphometric histology:

A-capsule thickness:

In the current research, the average thickness of the liver capsule in neonates was 20.95 ± 2.85 μm, Glisson's capsule is composed of multilayered capsular fibroblasts in a collagen matrix. The capsule is around 30–50 μm thick in adult, and there is a substantial correlation between liver thickness and age. Balog S et al (22).

B-Diameter of the classical lobule;

The current investigation revealed that the diameter of classical lobule was 0.75–1mm, with means SD of 0.75 0.84, which is consistent with Crawford AR et al (23) statement that the diameter of a lobule in a typical human liver is between 0.8 and 1.5mm. and with Ho H, Zhang EJFiP (24).

C- The diameter of the portal triad:

Regarding to this study, the diameter of portal triad was <100 μm, and the means ±SD were 2.1 ± 0.4, It agreed with Sherstiuk SO et al (25)

D- The Numbers of portal triads in each classical lobule;

Regarding to this study there are from 3-5 portal triads in each classical lobule in the neonate liver, this diss agreed with Crawford AR et al (19), who mentioned there are 6 portal triads in each classical lobule

Table 1: Means ± standard deviations of the histological morphometrically result of the neonatal liver.

Baby age (1-28)day	**capsule thickness**	**Diameter of the classical lobule**	**Diameter of the portal triad**	**Numbers of portal triads in each classical lobule**
Baby no	μm	μm	mm	NO
1	18.5	720	1.6	3
2	17.5	710	1.2	4
3	16.5	670	1.5	3
4	15.5	650	1.3	2
5	14.5	630	1.1	2
6	19.5	740	1.8	4
7	20.3	780	1.10	4
8	20.4	790	1.30	5
9	20.5	760	1.40	5
10	20.6	740	1.40	5
11	20.10	770	2.10	5
12	20.8	680	2.15	5
13	20.7	690	1.20	5
14	21.30	814	1.50	5
15	21.5	813	2.12	6
16	22.70	820	3.16	6
17	22.9	830	2.57	6
18	22.20	870	2.67	6
19	22.10	800	3.55	4
20	23.6	890	3.89	4
21	24.6	880	3.97	6
22	24.8	886	4.10	6
23	25.3	895	4.14	6
24	25.7	887	3.78	6

25	20.10	730	3.18	2
26	20.15	760	3.32	2
27	20.13	710	3.28	2
28	20.20	750	3.37	3
29	23.5	784	4.45	3
30	18.5	680	2.12	2
Mean ± SD	20.95±2.85	750 ±0.84	2.4±0.4	4.1±1.3

5- Conclusion

The liver is located in the upper part of the abdominal cavity, occupies right hypochondrium, and covered by connective tissue layer called Glisson capsule.

the gross appearance and measurements of neonatal liver, is different from adult liver, dimension, weight, and volume. Neonatal capsule thickness, diameter of the classical lobule and diameter of the portal triad were 20.95 μm , 0.75 mm , 2.1 mm respectively.

Acknowledgement

In the name of ALLAH, the Most Merciful and Gracious. This study was carried out with the blessings of the Lord of the Worlds and with the assistance of a large numbers of people who gave generously their advice and directions in order to make this quest easier to complete, and it is difficult to list them all here. Therefore, I express my gratitude and appreciation to everyone who contributed to complete the scientific aspects of this work.

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