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AN ARCHITECTURE OF MICROSCOPIC ANATOMY OF HUMAN LIVER.

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ABSTRACT

This research work was conducted to study the microscopic Anatomy of Liver inorder to identify the normal histological features of liver in low and high magnifications with aim of establishing a basic understanding, which will help recognise pattern of injury in liver diseases. Liver tissue was preserved in formalin and embedded in paraffin wax to obtain translucent sections. Paraffiin sections were stained with Haemotoxylin and Eosin which had observed under 10X magnification delineated hepatic lobule demarcation had been evidencing by Glisson capsule which condenses at portal triad at six angles with single central vein at centre. Under 40X magnification hexagonal lobule had polyhedral hepatocytes were radially arranged with two cell thickness encloses sinusoids, connective tissue stroma, the portal triad contains branches of Portal vein, hepatic artery and bile ductile. Based on bile secretion three adjacent hepatic lobule delivered into an axial hepatic ductile of triangular portal lobule formed from imaginary line connecting three central veins. Diamond shaped parenchymal acinus provides the best association between the perfusion of blood, metabolic activity and liver derangement made by imaginary line connecting two central veins and two portal triad. Alteration on the normal histological architecture being suggestive of liver disorders which can be identified in histopathological sections and to be reflected in the functions of liver.

KEYWORDS: Hexogonal lobule, Portal lobule, Liver acinus, Histopathology, Liver Function Test.

INTRODUCTION

The hepar has exocrine and endocrine functions being required for haemostasis. It has been synthesising and releasing 500 to 1200 ml bile into the bile canaliculi per day. The liver stores fats, vitamins and carbohydrate as glycogen. When the cells of the body needs glucose, the glycogen stored in the liver is converted back into glucose and released into the blood stream thus furnishing of energy. Bile salt by emulsification of fat into fatty acids and triglycerides by the involvement of pancreatic lipase which is absorbed by lacteals. It excretes bilirubin, which is formed in the body after the degradation worn-out RBC by the macrophage called, Kupffer cells. Hepatocytes also have important role in immune system. Endocrine functions of liver hepatocytes involve synthesis of numerous plasma proteins including albumin, globulin, prothrombin, fibrinogen. Hepatocytes also detoxify drugs and harmful substances as blood percolates through synusoids. It has vital role during foetal life as it produces blood cells.[1,2,3,4]

The microscopic Anatomy of Liver conceptualized in several ways the most common being Hepatic lobule, Portal lobule and Liver acinus. A classical anatomical lobule is hexagonal in shape and forms the structural unit of liver with a central vein at centre (Fig1A). Though the demarcation is by the presence of glisson capsule but it is not well defined in human. Neverthless the hexagonal shape of the lobule can be defined by imaginary lines connecting the portal tracts which are present at the six angles of the lobule. The portal canal is made by condensation of Glisson's capsule keeping three structures portal venule, hepatic arteriole and biliary ductile hence the name Portal triad / canals. Though hexagonal lobule is the structural unit of liver but hepatocytes are the structural and functional unit of hepar which are arranged in one cell or two cell thickness radiating from central vein to the periphery and it forms spongy and labrinthine anastomosing hepatic cords or plates encloses irregular spaces is the sinusoids. The space between sinusoids and hepatocytes is the perisinusoidal space of Disse which facilitates exchange of materials between blood and hepatocytes. Blood in portal venule and hepatic arteriole mixes and irrigated into the sinusoids which flows towards the central vein. The sinusoids contains endothelial cells, Kupfer cells and Ito cells. The endothelial cells are discontinuous and fenestrated without basement membrane. Kupffer cells are phagocytic cells with several irregular or stellate process project into the sinusoids which forms part of

mononuclear phagocytic system responsible for destroy pathogens and worn out RBC.[1,2,3,6,7,8] Ito cells or hepatic lipocytes are stellate cells present in the perisinusoidal pace which containing Vitamin A and collagen. Once liver injured these cells are responsible for the increased production of collagen causing fibrosis which is a characteristic of liver Cirrhosis.[14]

Hepatocytes are polyhedral cells with multiple surfaces at least two surfaces of each hepatocytes are contact with wall of sinusoids through space of Disse. The other surfaces which are contact with adjacent hepatocytes delimit a tubular intercellular space known as bile canaliculi which is the first part of the bile duct system which merge to form Herring canal and terminate in the hepatic ductile in the portal triad.[1] There is potential space between the glisson capsule of portal canal and hepatic plates around it known as Space of Mall in which lymphatics of the liver starts to appear as blind radicles due to excess accumulation of plasma reabsorbs by Space of Disse, thus there is communication between space of Mall and space of Disse which are interlobular and periportal and intralobular and perisinusoidal respectivel.[8]

Portal lobule is the functional unit of liver based on the bile drainage for which bile secreted from three adjacent heapatic lobule drains into the hepatic ductile at the centre of the triangular parenchyma made by imaginary line connecting three central veins (Fig-1B). It is nourished by portal venule and hepatic arteriole from the portal triad at centre hence it is a nutritional lobule. It is not visualised under microscope because the pressure of central vein is less than that of portal vein but it would visible whilst hepatic venous pressure rises.[1,2,3,4,6,7,8]

Portal acinus is another functional unit of liver based on the oxygenation of blood irrigated by two portal venules and two hepatic arterioles into the axis of a diamond shaped area formed by connecting two central veins and two portal canal of the two adjacent hepatic lobule (Fig-1C). There are around 100000 portal acinus in human liver. According to the oxygen gradient of blood supply, it is subdivided into three zones viz. Zone-1/inner zone , Zone-2 /middle zone and Zone-3/Outer zone with well oxygenated, moderately oxygenated and least oxygenated respectively.[1,2,3,4,6,7,8]

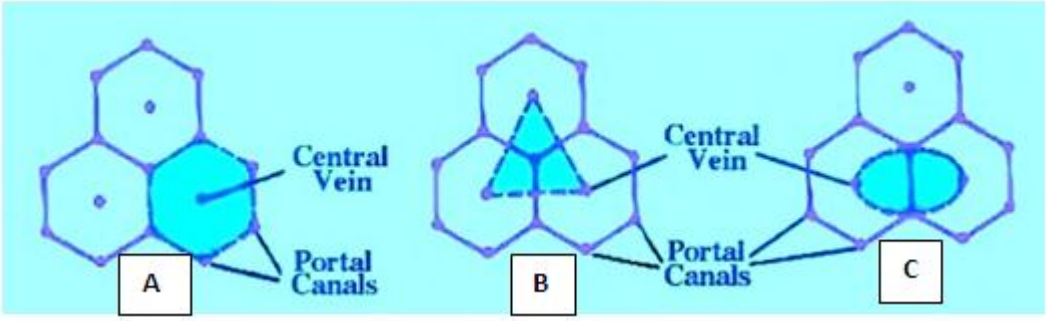

Figure 1: Schematic delineation of Hepatic lobule (A), Portal lobule (B) and Portal acinus (C).

Electron microscopic structure of hepatocytes has to have one or two central euchromatic nucleus with occasional polyploidy chromosomes. The cytoplasm containing Rough Endoplasmic Reticulam (RER), smooth Endoplasmic Reticulam (SER), numerous mitochondria, well developed Golgi apparatus, Lysosomes, Perioxysomes and membrane bound vacuoles. The surfaces of the hepatocytes posses plenty of microvilli which projects into the space of Disse and into bile canaliculi and rest of plasma membrane near caniliculi firmly fused by tight junctions.[1] RER synthesis most of the plasma proteins such as albumin, globulin , fibrinogen, prothrombin and angiotensinogen. SER synthesis lipo-proteins/ Cholestrol which can be liberated by four classes High density lipoprotein (HDL), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) and Chylomicrons. The proportion of HDL is high in normal individuals. Persistent elevation of plasma cholesterol is associated with atherosclerosis of arterial wall and predisposes myocardial infarction and cerebral infraction. Incidence of myocardial infarction is high in individual with elevated plasma LDL, and low with elevated HDL. The SER synthesis enzymes such as Aminotransferase which include Aspartate aminotranferase (AST) or Serum glutamic Oxaloacetic transaminase (SGOT) and Alanine aminotranferase (ALT) or Serum Glutamic Pyruvic transaminase (SGPT). An increase in the level of AST/SGOT and ALT/SGPT are indications of damage of hapatocytes.[12]

MATERIALS AND METHODS

The present study was conducted at the department of Anatomy School of Medical Education of Mahatma Gandhi University Kottyam, Kerala from 1995 to 1997 as the dissertation work required for the partial fulfilment of the Masters Degree of Medical Anatomy affiliated at MG University Kottyam (Accredited by NAAC with A-Grade). In-order to study the microscopic Anatomy of Liver the author had carried out the histology techniques and staining of the paraffin section of the liver tissue at the Histology laboratory as per the recommendations of JD Bancroft et al.[10] Fixation was done by formalin to preserve the morphology and prevent autolysis and

facilitates hardening. Water was removed by immersing the tissue in ascending grades of alcohol at 50%, 70%, 90% for 30 to 60 minutes. Dehydration was done by clearing agent Xylene for 2-3 hours. Inorder to get thin sections, tissue was embedded in Paraffin wax by impregnation and casting. Paraffin sections were cut at 5-7mm thickness by a rotator microtome, which was affixed on an albuminised microcope slide and dried in an incubator at 37°C for an overnight. De-paraffinization was done by treating with xylol for 3-5 minutes. Hydration was done by treating the paraffin section in descending grades of absolute alcohol at 90%, 70%, 50% and washed with distilled water for 3 minutes. Staining was done by treating with Haematoxylin and Eosin dyes for 5 minutes and 1 minute respectively. The basic dye haematoxylin had affinity to acid component /nucleus appeared as blue whereas acid dye Eosin tends to combined with basic component/ cytoplasm as pink. Dehydration of the stained section was done by ascending grades of alcohol at 50%, 70%, 90% for 5 minutes. The sections were cleared by Xylene and mounted in Distyrene Plasticizer and Xylene (DPX). We used artificial light compound optical microscope with 10X, 40X and 100X magnifications.

RESULTS

In our study we observed the paraffin sections of liver treated with haematoxylin and eosin stains by low and high magnifications. The surface of liver had an external covering of visceral peritoneum which firmly connected by connective tissue layer, Glisson capsule. Its fibers were collagen and elastic which were pink colour with Haematoxylin and Eosin dyes. It extends into the interior as speta demarcated the liver parenchyma into polygonal hepatic lobule which had been condenses around the portal venule, hepatic arteriole and bile ductile as portal canal at the meeting place of three adjacent hepatic lobules. Therefore six portal canals were found at the angles of a hexagonal lobule. The central vein was seen at the axis of the hepatic lobule which contains blood stains and it was surrounded by connective tissue. The lumen of the Portal venule, bile ductule and hepatic arteriole were large, intermediate and small repectively. Hepatocytes were arranged in cords of plates/ lamina radiating outwards from the central vein to the periphery where it abutting around the portal canal (Fig-2A,B). Each hepatic lamina was made up of twenty hepatic cells from the central vein to periphery which encloses sinusoids.The triangular functional lobule were found around an axial portal canal and three central vein at the periphery of three adjacent hepatic lobule. The diamond shaped liver parenchyma / Portal acinus were observed at two adjacent hepatic lobule which was demarcated by connecting two portal canals and two central veins.

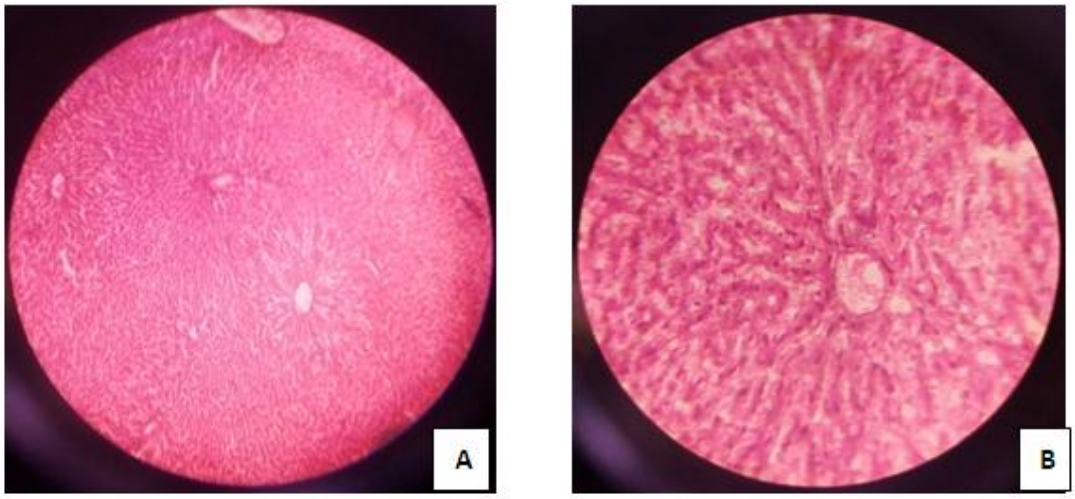

Figure 2: Depicting captures of 10X (A) and 40 (X) of Microscopy of Human Liver.

DISCUSSION

Microscopic anatomy of liver aimed to reveal the normal architecture of liver parenchyma and its connective tissue stroma in low and high magnifications which provides the structural basis for functional correlation and is required for the determination of pathological disorders of liver. In our study we found the hepatocytes being organised into three cell clusters viz. Classical hepatic lobule, Portal lobule and Hepatic acinus. Among them Hepatic lobule/Structural/ Anatomical lobule have delineated by interlobular septa which extends from the glisson capsule and each had centred by central vein (Fig-2A). However Portal lobule and Portal acinus have not been depicted by connective tissue stroma. Portal lobule being centred around portal triad and Portal acinus centred by preterminal transverse vessels and bile ductile derived from adjacent portal triad.

Reports of H.Greys et al, Romanes et al Dutta et al RMH McMin et al stated that the Hepatic lobule is six sided parenchymal unit of the liver with an axial central vein at the centre and its peripheral angles has to have portal traid in which hepatocytes arranged in two cell thickness.

Table 1: Exhibiting Analysis of the Histological Perspective of Other Authors with Current Study.

Authors	Hepatic Lobule (HL)	Portal Lobule(PL)	Portal Acinus (PA)
H Greys et al	Polyhedral in shape with central vein at its central axis surrounded at its edges by groups of three tubes.	Adjoining part of three HL in which the bile drains into the bile ductile in the portal canal at the meeting place of it.	Unit of liver based on the concept of blood flow, pattern of blood circulations and pathological degeneration.
G.J Romanes et al	Centred with central venule but no clear cut lobular pattern exists	Volume of liver that drains bile into single interlobular ductule	Depends on hepatic circulation oxygen tension highest close to tracts but lowest at central vein but if circulations contains poisons the cells near portal tracts tends to damage.
RMH McMin et al	Hexagonal shape with central vein at central feature and its corners have gathering of bile ductules, hepatic artery and portal vein at PT	Vascular and billiary channels of the portal areas are united by anastomosing connections	Diamond shape area with central veins at one pair of opposite corners and portal canal at the other pair
K. Garg et al	Lobular investment by GC is incomplete at HL which is Hexagonal in shape with portal tract at three to five at its corners	Polygonal shape with portal tract at centre and three neighbouring veins on each side of it.	Liver parenchyma around a preterminal branch of hepatic arteriole between two adjacent HL. Peripheral part get affected by anoxia but central part by toxins
A.K. Dutta et al	Hexagonal mass of liver cell with 1mm width having a central vein at its axis and six portal canal at its periphery.	Polygonal territory of liver tissue centred around a portal triad drawn by joining central veins of three adjacent HL	Diamond shaped area of liver parenchyma forms structural and metabolic units of liver which divided into three zones based on oxygenation.
Ranganathan et al	Polyhedral hepatic lobules having central vein at its centre and portal canal at the periphery.	It is a triangular outline consists of adjoining part of three hepatic lobule and can be visualised by imaginary line connecting three adjoining HL	Unit of liver situated around a preterminal branch of a hepatic arteriolewith maximum oxygenation at inner zone and least oxygenation at outer zone.
IB Singh et al	Hexagonal areas with central vein at centre and its angular interval having PT	The area of liver tissue supplied by one branch of portal vein is regarded as True functional unit	Area of liver tissue supplied by supplied by one hepatic artery.
A Paul et al	Hexagonal Anatomical lobule with central vein at its centre.	Functional lobule called PL which is triangular and are obtained by joining three adjacent central vein	-
Current study	Polygonal shape with central vein at centre and six portal canals were found at angles of HL.	Triangular in outline with an axial portal canal and three central vein at the periphery of three adjacent HL.	Diamond shaped liver parenchyma between two adjacent hepatic lobule demarcated by connecting two portal canals and two central veins

Enclosing liver sinusoids.[1,2,3,4] Blood of Portal vein and hepatic artery mixes and irrigated in the sinusoids to the central vein mean while heaptocytes secretes bile which is collected by bile caniliculus at their adjacent sides brought the bile ductile at the portal triad. Our study agreed the findings of previous reports for which hepatic lobule were polygonal shape with central vein at centre and portal triad at its six angles. K.Garg et al reported that hepatic lobular investments by GC is incomplete for so the peripheral portal canal might be three to four.[5] RMH Romanes et al reported that portal lobule is volume of bile that drains bile into single interlobular ductule situated at centre of three adjacent hepatic lobule.[2] However other authors reported that portal lobule is triangular unit formed based on the bile production from three adjacent hepatic lobule. Since it is a functional unit which is being designed based on the billiary drainage to the bile ductule at the centre of the triangular area formed from three adjacent anatomical lobule. The secretions of the hepatocytes which contains the liver enzymes Serum Glutamic Pyruvic Transaminase (SGPT) or Alanine amino Transferase (ALT) and Serum Glutamic Oxaloacetic Transaminase (SGOT) or Aspartate Aminotransferse (AST).[12] Portal acinus is diamond shaped unit of liver parenchyma connecting two portal triads and two central veins in which preterminal

branches of two hepatic arteries and two portal veins forming the backbone with more oxygenation near the centre and less at the periphery. The level of the SGOT and SGPT elevated in cases with disturbance in normal architecture of hepataocytes which has been more reflected at the non-oxygenic zone of the portal acinus and the enzymes might be spilled into the blood stream.

Difiores reported that the Glisson capsule made boundaries of each hepatic lobule of pig's liver was treated with Mallory-azan appear which stains the connective septa as dark blue. The bile canaliculi being revealed as tiny channels between each hepatocytes by staining the liver sections with osmic acid and staining with hematoxylin and eosin. Collagen Type-4 or Reticular fibres lines the sinusoids and forms a network around the central vein provides supporting connective tissue of liver which stains black and the liver cells stains pale pink /violet in Reticulin method. When treated with Periodic Acid Scchiff the glycogen granules in the hepatocytes stains bright red and irregular distribution.[13] In our study the liver sections were treated with H &E in which hepatocytes stains dark pink but the nuclei appears violet and interlobular septa light pink in colour in low and high magnifications. Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen due to over stimulation of Iato cell or hepatic lipocytes resulting liver cirrhosis.[14]

CONCLUSION

Histological aspects of liver depicts the three basic units of liver in which alteration of the cell architecture leading to disturbance in the functions of liver.

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