Original Article

Post Mortem Study on the Diameter of Hepatocyte of North-East Bangladeshi people

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Abstract:
Background: *Bangladesh, a developing country, where the advancement of modern treatment facilities are facing the alarming threat in liver disease. Disease pattern as well as diagnostic and treatment options may be helped by examining the diameter of hepatocyte, as they change with age. It seems that there is a research vacuum in this area and demands more studies, including gross anatomical studies, with data available in Bangladesh.* ***Objective:*** *To find out the relation of diameter of hepatocyte with the proper diagnosis and treatment of parenchymal liver disease in the North-East Bangladeshi people.* ***Methods:*** *A Cross-sectional study was conducted in the Department of Anatomy, Sylhet M.A.G. Osmani Medical College, Sylhet on 50 human liver that were collected form unclaimed and examined dead bodies from the Department of Forensic Medicine, Sylhet M.A.G. Osmani Medical College, Sylhet, during the period from January 2013 to December 2013. The collected samples were divided into 4 groups upon age. Histological study was carried out on relatively 24 fresh samples. Then statistical analysis was done by SPSS.* ***Results:*** *The mean diameter of hepatocyte was 11.8 (SD±1.6) µm, 13.6 (SD±2.1) µm, 17.0 (SD±1.9) µm, 11.58 (SD±1.0) µm, 13.6 (SD±2.7) µm in the age group of 2 to 15 years, 16 to 30 years, 31 to 45 years and 46 to 75 years respectively.* ***Conclusion:*** *Significant difference was observed between age and diameter of hepatocyte ($p<0.001$).*

Keywords: Liver, Hepatocyte, Cadaver

Introduction:
The liver is the largest internal organ in the human body known as "the custodian of the milieu interieur"[1]. It is an accessory gland of the gastrointestinal tract but it has a remarkable diversity of other functions unrelated to alimentation[2,3]. The liver is reddish brown in colour and usually soft to firm in texture[4] and the size and shape of the liver may be long and lean or squat and square which is variable and generally match with the general body shape[5].

The Liver is a mixed gland and has a wide variety of functions. Three of its basic functions are production and secretion of bile and bile salts; production of insulin like growth factor (IGF-I), production of various clotting factors, inactivation of various toxins, steroids and other hormones, production of urea, filtration of the blood, removing bacteria and other foreign particles. The liver synthesizes heparin, an anticoagulant substance, and has an important detoxicating function. It produces bile pigments from the hemoglobin of red blood corpuscles[6].

The liver, like the lungs, has a dual blood supply "The liver receives 80% of its blood supply via the hepatic artery and the 20% via the portal vein" [7]. But in case of nutritional demand portal vein provides 66-75% whereas hepatic artery provides 25-33% [8].

The lymphatics of the liver drain into three or four nodes that lie in the porta hepatis (hepatic nodes), 'these nodes also receive the lymphatics of the gallbladder, they drain to pyloric nodes as well as directly to coeliac nodes'. Lymphatics from the bare area drain into the posterior mediastinal nodes[9]. The liver is innervated by parasympathetic fibers from both vagi and sympathetic fibers from spinal segments T_7 to T_{10} [10].

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Like the other glands liver is made up of parenchyma and stroma. The parenchyma is made up of liver cells or hepatocytes which are arranged into thousands of small (~0.7 x 2 mm), polyhedral hepatic lobules which are the classic structural and functional units of the liver. These lobules are separated by connective tissue stroma, derived from glissons capsule which after complete investment of liver enters in the interior and separate lobule from each other.

Each lobule has three to six portal areas at its periphery and central vein in its center. The portal zones consist of connective tissue in which a venule, an arteriole and a ductule are embedded and these three structures are called the portal triad[11].

Hepatocytes make up approximately 80% of the cell population of the liver. The nucleus varies somewhat in size, with 40-60% polyploid. The majority of hepatocytes have a single nucleus, but as many as 25% are bi-nucleate[3].

Hepatocytes are linked by numerous gap junctions and desmosomes. Lateral plasma membranes of adjacent hepatocytes form microscopic channels, the bile canaliculi. These canaliculi form the origins of the biliary tree and their tight junctions prevent bile from entering interstitial fluid or blood plasma: this is the blood–bile barrier[4].

Methods:
Human livers were collected from the unclaimed dead bodies autopsied in the Department of Forensic medicine in Sylhet M.A.G. Osmani Medical College, Sylhet during study period from January 2013 to December 2013 meeting the inclusion and exclusion criteria included in the study. The collected samples were divided according to age into 4 groups, Group-A: 2-15 years; Group-B: 16-30 years; Group-C: 31-45 years Group-D: 46-75 years. Again each group (Group – A, B, C, D) was subdivided into Male and Female depending upon their sex.

Procedure for histological study: For the measurement of diameter of hepatocytes six slides were selected from each group (Group- A, B, C, D). Preparation of the slide: Tissue blocks ($2cm^2$) were fixed in 10% formaldehyde for 12 hours in a plastic container. The tissues were washed in running tap water, dehydration was done with ascending grades of alcohol, cleared with xylene, infiltrated and embedded in paraffin. Paraffin blocks were cut at 5 mm thickness and were stained with routine (H &E) stain. Measurement of diameter of hepatocytes: To get the diameter of each hepatic cell, two special measuring instruments were used, stage micrometer and ocular micro-meter. At first the stage micrometer was set on the microscope stage. Then the ocular micrometer was placed at the eye piece. On the stage micrometer there was a straight lines which was one millimeter in length was divided into 100 small divisions. Thus each small division measured 0.01mm. The ocular micrometer also had a line, calibrated into small divisions.

It was to be noted that during this procedure, the objective to see the hepatocyte and the eye piece lens used were those as would be used to see the hepatocyte. After that standardization, the stage micrometer was removed. Then the slide was placed one by one and the greatest transverse diameter and the perpendicular diameter of mid transverse diameter of the hepatocytes were measured and expressed in the term of µm.

For example, if the transverse diameter of hepatocyte was equal to 5 small divisions on the ocular micrometer, then the transverse diameter in term of µm would be 5x1.501 µm or 7.505 µm. Then the mean greatest transverse diameter and the mean greatest perpendicular diameter were calculated.

The diameter was then calculated by the following formula:
Diameter = (Transverse diameter + Perpendicular diameter)/ 2

100 divisions of stage micro-meter	=	1000 µm
1 division of stage micrometer	=	10 µm
In medium magnification (40X)		
3 stager micrometer division	=	20 ocular micrometer division
1 stager micrometer division	=	20/3 ocular micrometer division
6.6 ocular micrometer division	=	10 µm
1 ocular micrometer division	=	10/6.66 = 1.501 µm

Results:
In the present study, the mean diameter of hepatocyte was 11.08 (SD±1.6) µm in the age group of 2-15 years; 13.6 (SD±2.1) µm in the age group of 16-30 years, 17.0 (SD±1.9) µm in the age group of 31-45 years and 11.58 (SD±1.0) µm in the age group of 46-75 years. The differences among the groups were statistically significant ($f=13.47$; $p<0.001$). Distribution of diameter of hepatocyte by different age group was shown in Table I.

Table I: Distribution of diameter of hepatocyte by different age group.

Diameter of hepatocyte (µm)	Age Group				*p-value
	Group-A (n=6)	Group-B (n=6)	Group-C (n=6)	Group-D (n=6)	
Mean ± SD	11.8 ± 1.6	13.6 ± 2.1	17.0 ± 1.9	11.58 ± 1.0	p<0.001 f=13.47
Range	9.3-13.3	11.3-16.1	14.4-19.4	10.4-12.9	

*One way ANOVA test was applied to analysed the data.

Discussion:

In this study diameter of hepatocyte ranged from 9.3-19.4 µm with the mean of 13.4 µm (SD ± 2.7). Arey measured the diameter of hepatocyte and it was about 22×30 µm, but he described the hepatocyte diameter varies with storage and secretory activity[13]. Leeson and Leeson stated that hepatic cells are polygonal with six or more surfaces usually 20-35 µm in size[14]. Sharlock and Dooley stated that liver cells (hepatocytes) are polygonal and approximately 30 µm in diameter[15]. Gartner and Hiatt mentioned that the hepatocytes are polygonal cells and approximately 20-30 µm in diameter[16]. Mescher stated that the hepatocytes have a diameter of 20-30 µm[11]. Borley stated that hepatocytes are polyhedral with 5-12 sides and are from 20-30 µm across[17]. Ross stated that hepatocytes are large polygonal cells measuring between 20-30 µm in diameter[18].

This study showed that the mean diameter of hepatocyte was 11.8 µm (SD ± 1.6) in the age group of 2-15 years, 13.6 µm (SD ± 2.1) in the age group of 16-30 years, 17.0 µm (SD ± 1.9) in the age group of 31-45 years and 11.58 µm (SD ± 1.0) in the age group of 46-75 years. The difference among the groups were statistically significant (p<0.001) found that the mean diameter of hepatocyte was 12.42 ± 0.28 µm in the age group of 15 years; 14.42 ± 0.53 µm in the age group of 16-30 years, 16.31 ± 0.54 µm in the age group of 31-45 years and 12.55 ± 0.11 µm in the age group of 46-75 years[12].

In the current study the diameter of the hepatocytes were increased up to the age of 45 years then decreased. In this regards Robbins et al. described that there is relatively little variation in liver cell size and structure in normal adult in middle life. But in the later life atrophy and dropping out of isolated cells followed by compensatory hypertrophy and regeneration, induces some variation in size of the cells and nuclei and the appearance of binucleate forms as well as rare mitotic figures. Such changes before later life strongly suggest previous parenchymal injury and regeneration activity[1].

Conclusion:

In the current study the diameter of the hepatocytes were increased up to the age of 45 years then decreased. There exist some similarities and some variations in the findings of different parameters. In cases of variations the findings of the present study were less than the findings of other countries. The variations may be due to racial difference of the study populations. In this study the specimen were preserved in 10% formaldehyde that may be caused some amount of shrinkage of the specimens and subsequent lower value in some of the parameters in comparison to others reports in western books where the parameter were supposed to be from fresh specimen. Further studies on larger populations and different sex and ethnicity may be done to establish a complete data of Bangladeshi population.

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