Detection of Stem Cell Populations in Post gestating Mice Liver: Histo-immune Study

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ABSTRACT: Cellular therapy with liver stem cells and their progeny is a promising new approach which will contribute to gene therapy of liver diseases .So, the aim of this study was to identify normal structures and cell types within the liver and biliary systems of postgestating liver by light and electron microscope with particular reference to especial type of stem cells (oval cells).Also, the current study was performed to determine whether the hematopoietic cell markers, such as CD^+34,were co-expressed by oval cells by using immunohistochemical stain. Two ages of mice were used: 7- and 60-days after birth. Analyses of section profiles revealed that liver hepatocytes at 7-days were organized into hexagonal plates and at 60-days hepatocytes were organized into hepatic lobules with complete portal area .At postgestation, hepatocytes seemed provided with long parallel cisternae of rough endoplasmic reticulum, developed mitochondria and noticeable amount of glycogen. It was possible to identify large amounts of liver oval cells by using CD^+34 antibody and phenotypically define them. It was also possible through this study to prove that the amount of these oval cells decreased with age increase. The present results had led to a reasonably detailed picture derived the presence of oval cells as well as differentiation of the different hepatic cell types.

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INTRODUCTION

Adult stem cells are found in and around different organs and tissues of the body in a developing embryo. It is now possible to grow pure populations of specific cell types from these adult stem cells (Ahuja *et al*., 2006). They are key embryonic development present in many adult tissues, where they are responsible for regeneration and maintenance. Adult stem cells may not be as versatile in developing in various types of tissue as embryonic stem cells. The location and rarity of the cells in the body might rule out safe and easy access (Johnson and Williams, 2006). Adult stem/progenitors cells are classified based on their capability of self-renewal and clonogenic properties, and should be able to give rise to fully differentiated/mature phenotype with characteristic morphology displaying tissue specific surface markers (Jones *et al*., 2004).

The liver regenerates primarily by the proliferation of mature hepatocytes (Michalopoulos, 2007). However, the adult liver also contains hepatic progenitor cells that are activated when hepatocyte proliferation is inhibited, such as in severe cirrhosis. The hepatic progenitors appear to reside in the small terminal bile ducts and when activated they proliferate giving rise to a cell population called “oval cells”, which can differentiate into both hepatocytes and Biliary epithelial cells (Oertel and Shafritz, 2008). Oval cells can be regarded as “bipotential precursors” for the two hepatic parenchymal cell lineages. The bipotential nature of oval cells suggests that they originate from fetal hepatoblasts that remain undifferentiated in a stem cell niche within the ducts (Schmelzer et al., 2006). Duncan et al. (2009) defined the liver stem cell as cells that give rise to regeneration after partial hepatectomy and transplantable liver-repopulating cells.

In all models of liver regeneration, when accumulation of oval cells was observed, an inflammatory component (acute or chronic) was evident (Gordon et al., 2000). Oval cells share some phenotypic characteristics with hematopoietic progenitor cells, namely, the receptor for stem cell factor (c-kit), and its ligand stem cell factor and the related proteins flt-3 and flt-3 ligand, considered to define early embryonic hematopoietic precursors (Kamija and Gonzalez,2004).

So, the goal of this present study was to illustrate the main developmental events including structures and cell morphology seen at E7 and E60 which may lead to the understanding of hepatic stem cell biology in each age and to determine whether the hematopoietic cell markers, such as CD^+34, are co-expressed by oval cells which are considered as a type of hepatic stem cell.

MATERIAL AND METHODS

Materials

a-Normal buffered Formalin-Fresh were obtained from Fisher Scientific (Pittsburgh, PA).

b-Eosin-Y was purchased from Richard Allan Scientific (Kalamazoo, MI).
c-Hematoxylin was purchased from Anatech Ltd. (Battle Creek, MI).
d-CONFIRM™anti-CD34 (QBEnd/10) Primary Antibody Catalog Number 790-2927 were purchased from PharMingen Inc. (San Diego, CA).

Methods

Animals and tissue preparation

Comparable age (2-3 months) and weight (20–25 g) of pathogen-free mice were used in the present study .They were purchased from the animal house of King Fahd Center for Medical Research (KFMRC.) for Research in King Abdul-Aziz University where mice were easy to obtain, maintain, and breed. Five mature males and twenty females white MF1albino mice were housed as groups (one male & four females) in temperature- and light-controlled conditions (14 h. light and 10 h. dark) at 25 ± 3 C° with constant humidity 40 -60 %, with free access to food and water. The maintenance of the animals was in full compliance with the standard laboratory animals care protocols approved by KFMRC. Animal care and use committee. After mating, ten neonates at 7 days old and ten adult female at 60 days old were obtained , the height and weight of each were measured. The animals were sacrificed by cervical dislocation, livers were transferred into 10% phosphate-buffered formalin for 24 hr. to process light microscope technique or into glutaraldehyde to process electron microscope technique.

RESULTS

The morphological examination:

Liver weight

In this investigation, it was recorded that there were gradual increases in the liver weight among the two developing ages where the mean value for 10-specimens liver weight at 7-days = 0.9 ± 0.087 g. and 60-days = 1.983 ± 0.4481 g. (Table1).

Body weight

By comparing body weight in the two groups, there were directly proportional relationship between body weight and the age where the mean body weight value for 10-speciemens at 7-days =4.92 ±0.697 g. and 60-days = 28.53 ± 4.230 g. (Table 1).

Relative weight

When the relative weight in the two groups was calculated, it was noticed that the higher ratio at 7-days (8.344 ±0.615 %) and the ratio decrease at 60-days (6.908 ± 0.874 %) (Table1).

Body length

Concerning the body length in the two groups, there were directly proportional relationship between body length and the age (Table 1) where the body length at 7-days =4.166 ±0.288 cm. and 60-days =10.1 ± 0.099 cm.

The histological studies:

By light microscope:

Normal development of the liver at age 7-days after birth

The liver tissue appeared as dark or pale hexagonal or polygonal false lobule by the presence of portal area near by oval cells. The portal area was formed with a terminal branch of large portal vein had thin-wall, a branch of thick -wall hepatic artery and bile duct lined with low -height epithelial cells **.** The individual hepatocyte had a large contact with each other **,**since the size of the intrahepatic hematopoietic compartment were smaller and several cellular mitotic figures had been observed (Figure1).The polygonal parenchyma with round vesicular nuclei and undifferentiated vacuolated one arranged in single cell sheets separated by sinusoid spaces. The sinusoid spaces lined by two types of cells: flat endothelial cells and large Kupffer cells.The hematopoietic cells with intense, hyperchromatic nuclei were observed with presence of megakaryocyte (Figure 2).

The hepatocytes at this age had an increased abundance of cytoplasm and a significantly reduced nuclear to cytoplasmic ratio.The intravascular RBCs had extruded their nuclei and the macrophages were much fewer.

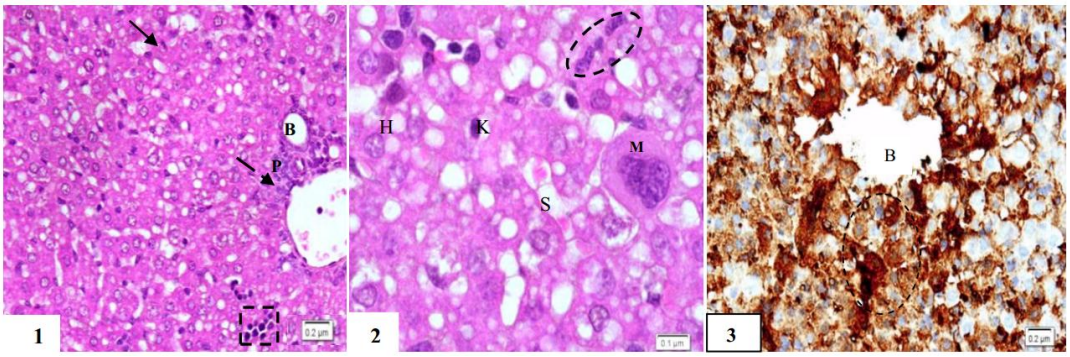
In this age , the oval cells detected with light microscope and evaluated with $CD^{+}34$ – stain were distributed throughout the liver and around the blood vessel ,the reaction appeared as dense brown granules and hepatocytes nuclei with negative-reaction in blue color and faint cytoplasm (Figure 3).

Normal development of the liver at age 60- days after birth:

Low-magnification images of the liver at 60-days revealed a typical liver architecture where the hepatocytes were linked in such a manner that they formed lobule . The lobule consisted of cords separated from each other by the hepatic sinusoids. In compare with 7-days age, there were an increase in cell density as compared to blood sinusoids where the blood sinusoids were markedly decreased and became slit -like in shape .The central vein was located in the center of the lobule and portal triads were located at the lobule periphery, separated by arcs of about 120 (Figure 4). The portal triads contained branches of hepatic artery, portal vein and intrahepatic bile ducts . The sinusoids were lined with endothelium, and contained specialized phagocytic cells, the Kupffer cells .The hepatic parenchymal cells were large polygonal with large central nuclei (sometimes two in a cell) and one or more nucleoli. The cell outline was often indistinct and the cytoplasm extremely variable in appearance. The mouse liver sections stained with

hematoxylin-eosin showed small oval periportal cells with scant cytoplasm and ovoid nuclei (Figure 5).

The number of oval cells evaluated with $CD^{+}34$ – stain was lower and oval cells were only located in the smaller interlobular bile ducts area (Figure 6).The use of immunohistochemical markers Ventana Medical Systems' (Ventana) CONFIRM anti-$CD^{+}34$ (QBEnd/10) used in this study showed pure population of mouse hepatic oval cells which expressed the $CD^{+}34$ antigens .

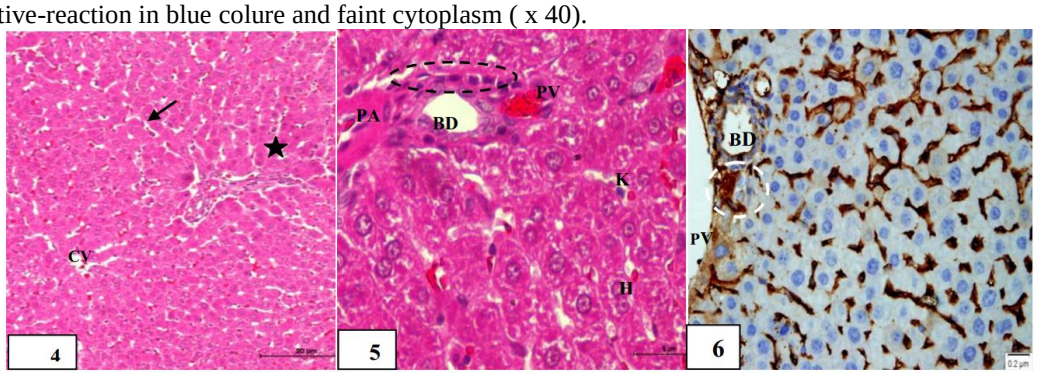


Light micrograph of liver section (7 -days) postnatal fetus of mouse showing:

Figure (1) :Hepatocyte forming plate , small size of intrahepatic hematopoietic cells(dashed-line) and dividing cell (arrow) .Note ,oval cells (dashed-arrow) near portal vein ,portal artery(P) and bile duct (B) (H&E x 40).

Figure (2) :Rare megakaryocytes (M) , solitary dense hematopoietic foci (dashed-line), hepatocytes with round nuclei (H) and narrow tortuous blood sinusoids (S) contain large Kupffer cells(K) (H&E x 100).

Figure (3):Oval cells with high numbers which evaluated with $CD^{+}34$ – stain (dashed line) distributed throughout the liver and around the blood vessel (B) , the reaction appeared as dense brown granules .Note, hepatocytes nuclei with negative-reaction in blue colure and faint cytoplasm (x 40).



Light micrograph of liver section (60 -days) of adult mouse showing:

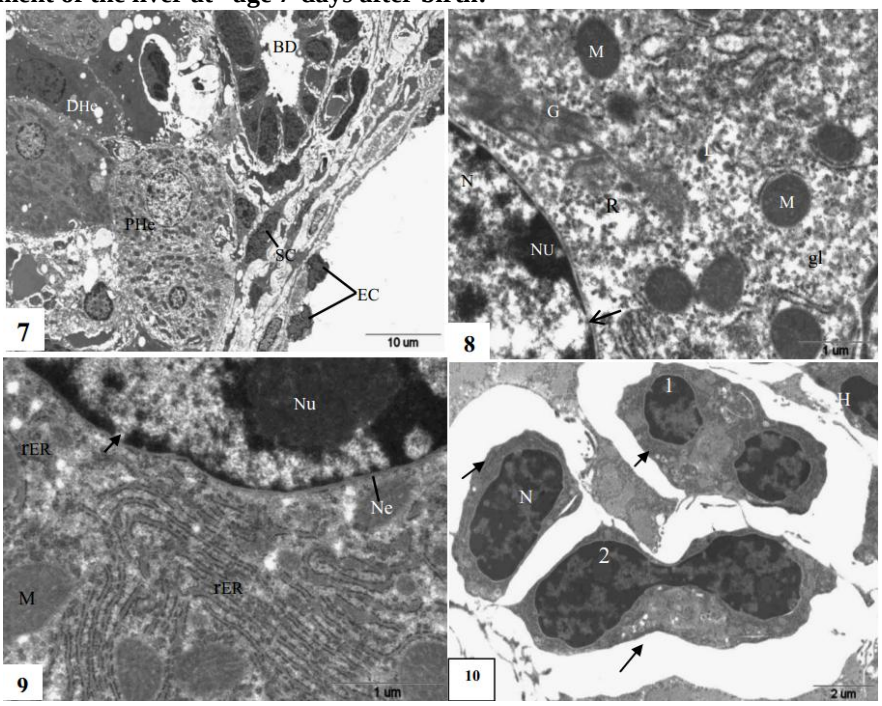
Figure (4): Increase cell density forming cords separated from by slit - like hepatic sinusoids (arrows). Note, central vein (CV) in lobule center, portal triads (star) at lobule periphery (H&E x10) .

Figure (5) : Small oval periportal cells (dashed line) with scant cytoplasm and ovoid nuclei, bile ductule (BD), portal vein (PV) and portal artery (PA).Note, sinusoid containing Kupffer cells (K) and binucleated hepatic cells (H) (H&E x 100).

Figure (6): Low number cells evaluated with $CD^{+}34$ – stain only located in the smaller interlobular bile ducts area (dashed line), the reaction appeared as dense brown granules and hepatocytes nuclei with negative-reaction in blue colure. Note, bile duct (BD) and portal vein (PV) (x 40).

By electron microscope:

Normal development of the liver at age 7-days after birth:



Transmission electron micrographs of the mouse liver parenchyma at 7-days showing:

Figure (7): Portal area with portal vein lined with endothelial cells (Ec) separated from hepatocytes by a space contained satellite cells (SC) , well defined bile duct (BD) , more contacted hepatocytes differentiated into dark (DHe) and pale (PHe) hepatocytes (Uranayl acetate –lead citrate - X1450).

Figure (8): Euchromatic nucleus (N) with peripheral nucleolus (Nu) enclosed by double nuclear envelop with nuclear pore (arrow), small round mitochondria (M) ,polar view of Golgi complex with parallel row of cisternae (G),polysomes (R), primary lysosomes (L) and glycogen granules (gl) in pale cytoplasm (Uranayl acetate –lead citrate – X13500).

Figure (9): Dark hepatocyte with heterochromatic nucleus have nucleolus (Nu), nuclear envelop (Ne) with nuclear pores (arrows),long rough endoplasmic reticulum (rER)) in close association with mitochondria (M) (Uranayl acetate –lead citrate – X19000).

Figure(10): Solitary hematopoietic cells (arrows) with intense hyperchromatic nuclei (N) and scant cytoplasm inbetween hepatocytes (H), (1) telophase- stage ,(2) cytokinasis stage with abnormal chromatin connection (Uranayl acetate –lead citrate – X5800).

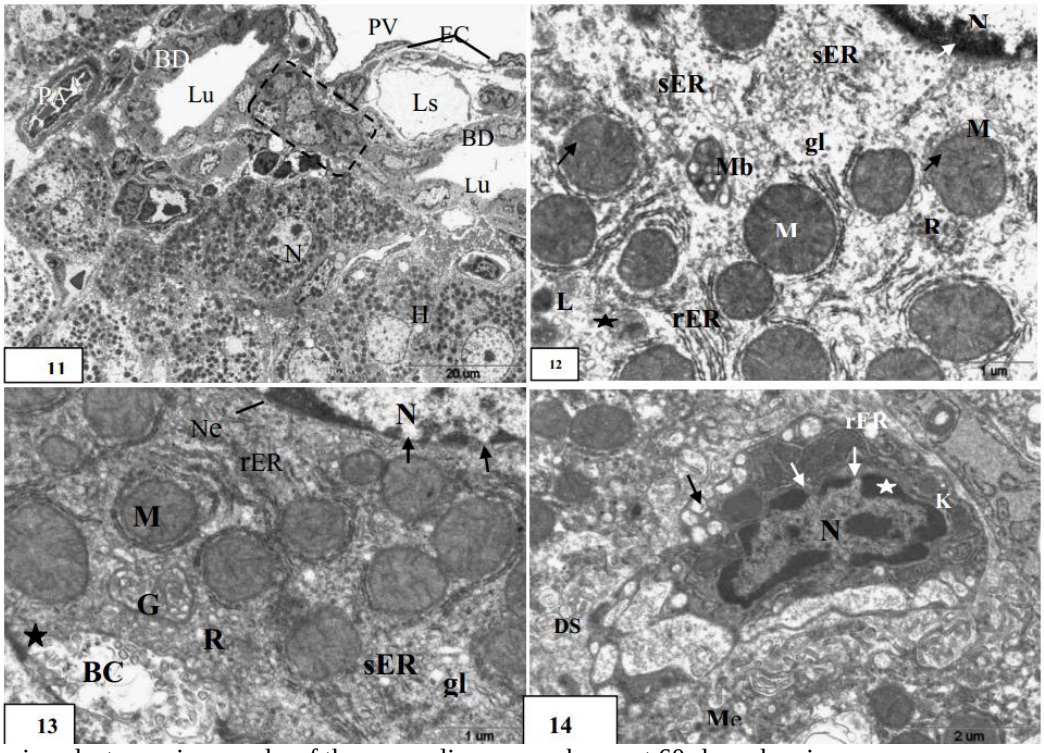
The mouse fetus liver of (7-days) old had portal area with only portal vein lined with endothelial cells and well defined bile ductule .The bile ductule constituted of 8-15 aligned cells rested on basement membrane and their apical surface had numerous short microvilli that extended into the lumen. Under electron microscope hepatocytes in the liver exhibited polarity characterized by structurally distinct apical and basolateral domains. Large hepatocytes differentiated into two types of cells : the pale and dark one .The pale hepatocyte had round pale nucleus in pale cytoplasm crowded with ovoid mitochondria and the dark hepatocyte with abundance cytoplasm characterized by highly stained mitochondria (Figure 7).With high magnification ,the pale hepatocyte contained active pale nucleus (3472±417.19 nm) (Table 2) with peripheral small nucleolus enclosed by two parallel proliferated membranes ,round large mitochondria (597.45±51.62 nm) (Table 2) filled with mitochondrial matrix, rough endoplasmic reticulum took the shape of vesicles and short profiles ,polar view of Golgi complex with parallel rows of cisternae had large vesicles, solitary ribosomes, primary lysosomes with dense content and less glycogen granules in comparison with dark hepatocyte (Figure 8).

Dark hepatocyte had dense smaller nucleus (1689 ±285.7 nm) with more heterochromatin and less euchromatin (Table 2) .The nucleus had marignated large nucleolus enclosed by double proliferated nuclear envelop , the rough endoplasmic reticulum was seen divided into two types: first one as long parallel cisternae and the second one inclosed association with

mitochondria . The dark liver cell contained small mitochondria (582.14±39.9 nm) (Table 2) with a variable shape (round or oval). It had outer smooth membrane and inner rebranched one, the ribosomes were of two types : the free and polysomic one and the glycogen granules were well represented (Figure 9).

Solitary hematopoietic cells were still presented with intense hyperchromatic nuclei and scant cytoplasm in different state in between hepatocytes .They might be telophase stage with complete separation of the two daughter nuclei and cytokinesis stage with abnormal chromatin connection between the two daughter nuclei (Figure 10). In this stage, oval cells could not be detected with electron microscope.

Normal development of the liver at age 60- days after birth:



Transmission electron micrographs of the mouse liver parenchyma at 60-days showing:

Figure (11): Portal area with thin wall portal vein (PV) lined by flat endothelial cells (EC) , bile duct (BD))with cuboidal cells around lumen (Lu), lymph space (LS), thick wall portal artery (PA), hepatocytes (H) with one or two central nuclei (N) in dense or pale cytoplasm , small oval cells with large nuclei to cytoplasm ratio radiating from the periportal region (dashed-line) (Uranayl acetate –lead citrate - X1100).

Figure (12): Pale hepatocytes with low density cytoplasm have few polysomes (R), few glycogen granules (gl),few rough endoplasmic reticulum (rER), round mitochondria (M), nucleus (N) with marginated heterochromatin (white arrow), multivesicular body (Mb) , primary lysosome (L) and secondary lysosome (star) (Uranayl acetate –lead citrate - X13500).

Figure (13): Dark hepatocytes with dense mitochondria (M), more rough endoplasmic reticulum (rER), few smooth one (sER), polysomes (R), concentrated glycogen granules(gl), bile canaliculus (BC),desmosome (stars), nucleus (N) surrounded by nuclear envelop (Ne) with nuclear pores (arrows) and Golgi complex (G) (Uranayl acetate –lead citrate - X13500).

Figure (14): Tortuous sinusoid (S) contained phagocytic Kupffer cell (K) have triangular dense nucleus (N) with peripheral chromatin masses (stars), nuclear pores (white arrows), pinocytotic vesicles (black arrows),rough endoplasmic reticulum (rER),Disse space(DS) filled with microvilli(Me) (Uranayl acetate –lead citrate - X10500).

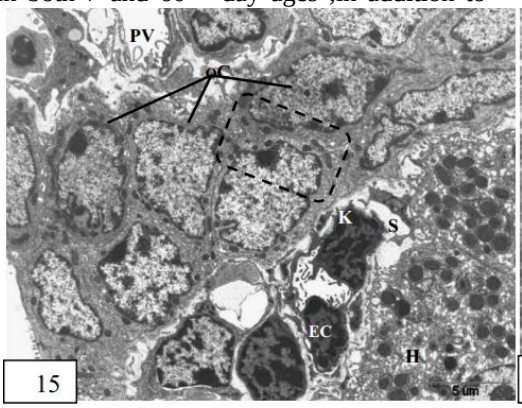
The mouse liver of (60-days) old had portal areas as found in 7-days old in addition to thin wall lymph space and thick wall portal artery .The hepatocytes were large polygonal with one or two central nuclei in deep or pale staining cytoplasm .Also, very small blast-like cells which were small in size with large nuclei to cytoplasm ratio radiating from the periportal region (oval cells) were recorded (Figure 11).The mouse liver of (60-days) old as in 7-days old consisted of two types of cells: the pale and dark one .

Ultrastructural examination showed that the pale hepatocytes were occupied by well-developed ovoid mitochondria (877.55±51.67nm) surrounded by double membrane in which the inner one extended into mitochondrial matrix to form the cristae ,pale nucleus (9740.55±296 nm) (Table 2),low density cytoplasm had few polysomes, few glycogen granules , less rough endoplasmic reticulum in comparison to smooth endoplasmic reticulum, , multivesicular body, primary and secondary lysosomes. The primary lysosomes appeared with dense content and the secondary ones showed only dense concentric area (Figure 12).

The dark cells' cytoplasm contained numerous dense mitochondria (830.96±37.6 nm) (Table 2), more rough endoplasmic reticulum in comparison with few smooth ones , many polysomes , abundant glycogen, small nucleus (5314.46±599 nm) (Table 2) enclosed by profilated nuclear envelop . The full and total development of liver parenchyma had been appeared with bile canaliculi on the side surfaces. Bile canaliculi defined luminal spaces densely decorated with microvilli appeared between the borders of adjacent hepatocytes which association together by desmosomes (Figure 13).

In both 7 and 60 – day ages ,in addition to dark and pale hepatocytes another two types of were cells found in the liver : the flat endothelial cells which lined the blood sinusoids and large phagocytic Kupffer cell which had a triangular nucleus , primary lysosomes and pinocytic vesicles . The flat fenestrated endothelial cells were separated from hepatocytes by Disse space filled with microvilli. Disse space that surrounded the hepatic sinusoid and bounded by hepatocyte membrane from one side and on the other side by endothelial membrane contained stellate cells (Figure 14).

In the periportal area, very small "blast-like cells" identified beneath the bile ductule (oval cells) with a large nucleus to cytoplasmic ratio (Figure 15). The oval cells were very tightly packed with a large number of anchoring junctions making it difficult to appreciate individual cell shape. They had short profiles of rough endoplasmic reticulum, few small mitochondria (300.132±25 nm) (Table 2), active nucleus (1905.8±206 nm) enclosed by double nuclear membranes studded with ribosomes. The oval nucleus had two type of chromatin: electron –dense hetero-chromatin around the nuclear envelope forming irregular clumps and nucleolus, the other electron-lucent euchromatin was seen spreaded in the nucleoplasm (Figure 16).



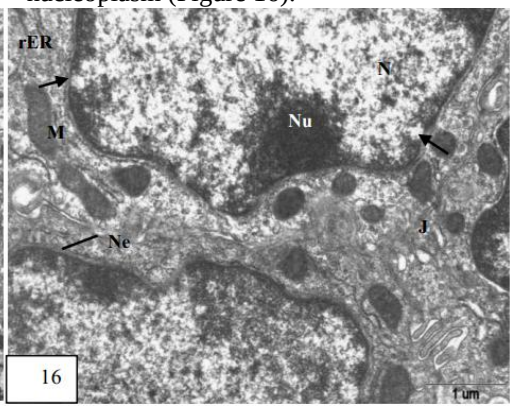


Figure (15): Small oval periportal cells (OC) with scant cytoplasm, ovoid nuclei, few small mitochondria. Note, blood sinusoid (S) lined with endothelial cell (EC) and contained kupffer cell (K). hepatocytes (H)and Portal vein (PV) (Uranayl acetate –lead citrate – X3400).

Figure (16): Oval cells with active nucleus (N) enclosed by double nuclear envelop (Ne) and nuclear pores (arrows), peripheral heterochromatin and nucleolus (Nu).Note, few small fetal mitochondria (M), short profiles of rough endoplasmic reticulum (rER) and anchoring junctions (J) (Uranayl acetate –lead citrate – X7900).

Table (1): Analysis of body, liver, relative weight and body length of the different groups

Variables	Groups	7- Days	60- Days
Body weight (g.)	Mean ± S.D.	4.92 ± 0.697	28.53 ± 4.230
Body Length(cm)	Mean ± S.D	4.166 ± 0.288	10.1 ± 0.099
Liver weight (g.)	Mean ± S.D	0.9 ± 0.087	1.983 ± 0.448
Relative weight (%)	Mean ± S.D	8.344 ± 0.615	6.908 ± 0.874

Table (2): Analysis of the mean diameter of mitochondria (M) and nucleus (N) in the different cells of the examined groups.

Variables	Groups	7 -days		60 -days		
		PHe	DHe	PHe	DHe	OC
M (nm)	Mean±S.D.	597.45±51.62	582.14±39.79	877.55±51.67	830.96±37.65	300.132±25.75
N (µm)	Mean±S.D.	34.727±4.10	16.899±2.80	32.468±9.60	26.574±5.90	9.529±2.60

DISCUSION

In this study, mouse maintained a variable liver: body mass ratio in the chosen ages which disagree with (Hata *et al*., 2007) who found a constant ratio .This fact supported by (Apte *et a*l. 2007) as, during histological examination of the postnatal liver, it was important to remember that this characteristic liver architecture was not fully present until 2 to 3 weeks after birth and liver mass increased by several folds in the first 3 weeks of postnatal life but declined in the fourth postnatal week when the liver: body weight ratio approached adult levels and this supported the present results where the higher ratio was at 7-days and the ratio decreased at 60-days after birth.

Beginning around one week postnatally, liver parenchymal cells began to organize into well-defined hepatic plates (Grossi et al., 1985) separated by sinusoid spaces (Lemaigre, 2003) as seen in this study at 7-days,in addition to portal triad.Proliferation of biliary cells, along with an increase in the formation of portal triads, could be observed between ten and twenty days after birth (Apte et al. 2007).

The higher magnification of 7-days sections revealed that the small, solitary hematopoietic foci were forming as indicated by (Sasaki and Sonoda, 2000) and the macrophages were much smaller as mentioned by (Sasaki et al., 1993). Also, megakaryocytes could also be found as isolated cells surrounded by hepatocytes (Grossi et al., 1985).

There was a higher presence of hepatocellular cytoplasmic glycogen stores in 7-days of age than in 60-days of age , glycogen liver storage during late development is critical for the maintenance of glucose homeostasis during the first few days of postnatal life (Crawford *et al*., 2010).

In this study the liver at 60-days of age had a characteristic histological architecture that agrees with (Apte *et al*. 2007) where the hepatocytes were linked in such a manner to form units known as hepatic lobules . The central vein was located in the center of the lobule, whereas portal triads were located at the lobule periphery (Samuelson 2007). The portal triads contained branches of the hepatic artery and portal vein as well as intrahepatic bile ducts.

In the present study at 7-days age and 60-days age hepatocytes exhibited polarity ,the establishment of hepatocyte cell polarity was crucial in generating a mature, functioning organ (Parviz et al. 2003).The liver's role in postnatal endocrine and exocrine processed necessitate its complex cell polarity. The basal surface of the hepatocyte faced the sinusoidal capillaries. The hepatocytes and sinusoidal epithelium were separated by the space of Disse, where exchange occurred between extravasated serum and hepatocytes (Parviz et al. 2003).The exocrine function of the liver involved the secretion of bile at the hepatocyte's apical surface (Roskams and Desmet, 2008).

In both 60-days and 7-days after birth groups, the sinusoidal capillaries consisted of phagocytic Kupffer cells, which scavenge spent cell debris from the circulation and fenestrated endothelial cells that were highly specialized to facilitate selective transport between hepatocytes and blood (Braet, 2001) .

The transcription factors that controlled hepatocyte maturation illustrated the concept of a "dynamic transcriptional network." A set of transcription factors, commonly referred to as liver-enriched factors, organize to form a network of autoregulatory and cross-regulatory loops. The binding of each individual liver-enriched factor to the gene regulatory regions of all other liver-enriched factors was tested by in *vivo* analyses of protein-DNA interactions (Kyrmizi et al., 2006).This revealed that the number and complexity of interactions increased when hepatocyte maturation proceeds. This increased correlated with the progressive rise in concentrations of most liver-enriched factors and with the increased stability of the network (Kyrmizi et al., 2006).

During postnatal hematopoietic involution, hepatocyte volume rapidly increased, and four types of specialized junctions :adheres junction, desmosomes, tight junctions and gap junctions, appeared to be fully developed in liver cells as found by (Sonoda, et al.,2001) with agree with our results at 7-days and adult liver.

In a normal adult liver, mature differentiated hepatocytes were quiescent (in the G0 stage of the cell cycle) and exhibit minimal turnover (Overturf et al. 1997) as, the average life span of adult hepatocytes ranges from 200 to 300 days (Bucher and Malt, 1971). A number of different hypotheses had been proposed to explain hepatocyte turnover. In "streaming liver" model, normal liver turnover was proposed to be similar to intestinal regeneration, with young hepatocytes originating in the portal zone and then migrating toward the central vein (Zajicek, et al., 1985). Differential gene expression by periportal and pericentral hepatocytes was believed to arise during the

hepatocyte maturation process, which represented a typical lineage progression. The data from Fellous (2009) et al supported the streaming liver hypothesis in human tissue (Fellous, *et.al*., 2009). However, strong evidence against the streaming liver hypothesis also existed. First, retroviral marking studies provided clear evidence against any hepatocyte migration during normal turnover (Braet , et,al.,2001). Analysis of the X-inactivation pattern in livers from female mice also argues against hepatocyte migration within the lobule (Shiojiri and Sugigama, 2004).

It has been generally accepted that the liver contained cells with stem-like properties and that these cells could be activated to proliferate and differentiate into mature hepatocytes and cholangiocytes under certain pathophysiologic circumstances (Shen, 2007) recapitulating hepatoblasts differentiation during fetal development (Dabeva and Shafritz, 2003).These cells might be related to the so-called "oval cells", originally identified by Farber (1984) as immature (Farber ,1984). In the normal liver, hepatocytes seemed to be the main progenitors of new hepatocytes, although a role for oval cells could not be discounted. Thus, there were two types of stem cell responses, from biliary-derived oval cells and from differentiated hepatocytes (Alison et al., 2001). Oval cells were heterogeneous, and may display features of both bile duct cells and hepatocytes (Zhang et al., 2003). Regardless of the final nomenclature, the precursors to oval cells are not mature hepatocytes (Wang et al., 2003).

In the present study, the adult mouse liver sections (stained with hematoxylin-eosin) showed complete lobular structure without oval cell proliferation with agree with (Qin et al., 2004).Also, with electron microscope ,these cells appeared small in size (approximately 10 µm), with a large nucleus to cytoplasm ratio, radiating from the periportal region (Petersen et al., 2003) .

Appropriate antibodies can be used to highlight the presence of oval cells in histological sections and further had utility in cell sorting (Alison *et al*.,1997) where oval cells express $CD^{+}34$ (Omori et al., 1997) a marker of early hematopoietic progenitor cells (Krause, 2002) and vascular endothelium, implicated in signal transduction and cell adhesion . Avital *et al*. (2001) found that very rare cells CD+34 , could repopulate irradiated hosts for the long term and can differentiated and engrafted with high efficiency into epithelial cells of the liver . Also, Suzuki et al.,(2000) found oval cells in adult liver and marked for $CD^{+}34$ which had been considered to be close descendants of stem cells.

The use of immunohistochemical markers Ventana Medical Systems' (Ventana) CONFIRM anti-CD34 (QBEnd/10) presented in this study permitted us to obtain a relatively pure population of mouse hepatic oval cells at 7-days they were only restricted in periportal area as groups and finally at 60-days represented as individual cells at the ductular area with agree with (Petersen et al.,2003) who reported that mouse oval cells located in the periportal region and spreading outward expressed high levels of $CD^{+}34$. The CD34 molecule was a highly conserved protein presented in the hematopoietic system of mice and humans , it has been used in both species for the identification of hematopoietic stem cell (HSCs) (Kruse et al., 1994). It has been reported that about 200 of the highest CD34 expressing cells could radioprotect irradiated mice with both long- and short-term capabilities (Weissman et al., 2001).

Using stem cell lines, are being made to standardize the protocols, which will not only be useful in testing the toxicity of a chemical or a physical agent, but also in the field of drug development, environmental mutagenesis, biomonitoring and other studies (Ahuja et al.,2006).

The immunocytochemically detection would be very useful in future studies in understanding the uniqueness of this intriguing hepatic cell type.

This study described the morphology and ultrastructure of the oval cells present in the bile ducts of 7 and 60 day old mice. This study demonstrated also clearly that these oval cells were identified by the stem cell marker CD+34.

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