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Immunohistochemistry - Parenchymal and nonparenchymal cells in the liver of prenatal and postnatal groups of guinea pigs

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Immunohistochemistry is a valuable and inexpensive tool used to identify the normal and abnormal parenchymal and nonparenchymal cells of the liver. The immunohistochemical identification of the liver cells of guinea pig from prenatal and postnatal age groups was conducted as per the ethical committee approval and this study would form the base line approach for disease diagnosis. The collected liver were processed for immunohistochemical staining protocol using antibodies namely CD68, CD34, CK 7, GFAP, αSMA, vimentin and insulin. The guinea pig liver of postnatal ages showed moderate positive immune reaction to CD34 antibody in the sinusoidal endothelial cells and decreased with age. CD68 positive immunoreactive granules showed strong reaction for the Kupffer cells and reaction decreased with age. Moderate CK7 positive immunoreaction was found in the lining epithelium of bile ducts. Hepatic stellate cells showed moderate GFAP positive reaction and decreased with age. HSCs were demonstrated as dense brown coloured alpha SMA immune positive cells in the hepatic sinusoids. The HSCs were demonstrated in the sinusoids by showing strong vimentin positive dark brown coloured cytoplasmic granules which decreased as age advanced. By Insulin immunohistochemical technique, beta cells were identified within the liver parenchyma in the sinusoidal space.

Key words: Alpha SMA, CD34, CD 68, CK7, GFAP, IHC, Insulin, vimentin

Introduction

Immunohistochemistry (IHC) is a tool/technique used for investigating the cellular components within tissue sections placed on glass slides. The strong affinity between antibodies and antigens is the fundamental idea behind this technique. The information produced by IHC allows us to detect both the presence/absence of the antigen and its specific cellular localization. The IHC is the only technique capable of examining proteins at anatomical levels. It is applied in clinical practice and pathology research to pinpoint specific proteins linked to a particular disease state in humans[1].

The CD34 expression in the liver would help to diagnose and grade vascular tumours and estimate microvascular invasion[2]. The CD 68 antibody staining reaction was used to identify the presence, location and density of liver macrophages, which was crucial for diagnosing liver diseases and to study the role of macrophages in inflammation and in various diseases like hepatitis and hepatocellular carcinoma[3].

The CK7 is an intermediate filament protein and is a special marker of bile duct epithelial cells in distinguishing cancers such as intrahepatic cholangiocarcinoma and hepatocellular carcinoma. In normal human liver tissues, it is not difficult to differentiate hepatocytes from biliary epithelial cells with CK 7. But in cholestatic diseases, CK7 positive hepatocyte shaped cells were also identified and were identified as abnormal in diseases like alcoholic liver disease, primary biliary cholangitis[4].

The GFAP was the new specific marker for quiescent hepatic stellate cells or Ito cellsand it allows differentiation between Ito cells and other fibroblastic liver cells. Desmin was considered previously as a marker for Ito cells, but it also showed positive reaction to cells other than Ito cells like endothelial cells and Kupffer cells but GFAP only detects Ito cells in humans[5]. The cellular localization of insulin degrading enzyme immunoreactivity mainly exists in parenchymal cells in the rat liver and insulin receptors were found on the cytoplasmic membranes and

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Table 1 — Table showing the number of guinea pigs used for research work

Age groups	Prenatal groups		Postnatal Groups				Total
	Pregnant mom with 0-30 days foetus	Pregnant mom with 30 days to birth foetus	0-2 weeks (Male + Female)	2-8 weeks (Male + Female)	8-16 weeks (Male + Female)	16-32 weeks (Male + Female)	
No of animals	3	3	3+3	3+3	3+3	3+3	30

granular endoplasmic reticulum of hepatocytes and endothelial cells[6].

Vimentin is a marker for mesenchymal cells and the increased presence of this antibody was deducted in liver diseases like fibrosis and cancer in mouse liver[7]. Alpha SMA antibody is commonly used as a marker in liver immunohistochemistry to identify activated hepatic stellate cells which are myofi broblast like cells that can proliferate and produce fibrous tissue at the time of liver injury or fibrosis in rat[8].

The purpose of this study is to investigate the immunohistochemical cellular characteristics of liver and its specific location in the prenatal and postnatal age groups of guinea pigs.

Materials and methods

The immunohistochemical identification of the cells of the liver of guinea pig from prenatal and postnatal age groups (Table.1) was conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai-7. Guinea pigs were procured from the Department of Laboratory Animal Medicine, Madhavaram Milk Colony, TANUVAS, Chennai -51 as per the ethical committee approval of Tamilnadu Veterinary and Animal Sciences University (Lr. No. 1467/ DFAB/IAEC/2018 dated 13.07.2018). After collection, guinea pigs were euthanized as per the standard operating procedure by using the Carbon dioxide asphyxiations as per CPCSEA norms and they were subjected for the dissection. Three samples were collected from each prenatal group and six samples were collected from each postnatal group and were proceeded to the immunohistochemistry staining protocol.

The collected liver were washed in the normal saline and fixed in 10% neutral buffered formalin for immunohistochemical studies. Then the tissues were dehydrated in the ascending grades of the alcohol cleared in xylene and embedded in paraffin[9].

Immunohistochemistry staining protocol[9]

1. 3 µm paraffin sections were cut and mounted on charged slides and incubated at 60 -70˚C for 30 minutes.
2. Sections were deparaffinized by two changes in xylene.
3. Sections were dehydrated with absolute alcohol two changes and washed twice in distilled water.
4. Heat mediated antigen retrieval was done using TRIS-EDTA, buffer (pH 8.5 -9.0).
5. Sections were washed twice in distilled water for two minutes.
6. Blocking of endogenous peroxidase was done with 3 per cent hydrogen peroxide for ten minutes.
7. Sections were incubated in the following antibodies (Ready to use) primary antibody in a moist chamber for one hour.
   - CD68 for Kupffer cell of liver.
   - CD34 for peribiliary capillaries and periportal and perilobular sinusoids.
   - CK 7 for Biliary epithelium.
   - GFAP for hepatic stellate cells.
   - αSMA for stellate and smooth muscle cells.
   - Vimentin for type III Intermediate filament proteins.
   - Insulin for Beta cells.
8. Poly Excel HRP (Ready to use) secondary antibody was added and incubated for twelve minutes and sections were washed three times in PBS.
9. Diaminobenzidine (DAB) chromogen solution (1 ml DAB buffer + 1 drop DAB chromogen) was added and kept for two to five minutes and washed in distilled water.
10. The sections were counterstained with Gill's haematoxylin for one minute. The sections were treated with running tap water for five minutes. Sections were dehydrated through graded series of alcohol and xylene and mounted in synthetic mountant.

The immunohistochemical images were captured using the Leica microscope (CH9345 Heerbrugg).

Results

The CD34 immuno reactivity was identified in the vascular endothelial cells in the prenatal liver. The

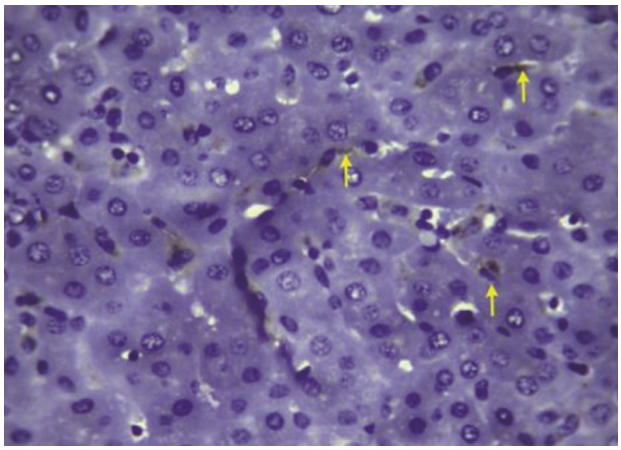

Fig. 1 — Photomicrograph of 20 week-old guinea pig liver showing moderate CD34 positive immuno reaction (arrow) in the sinusoidal endothelial cells x 400

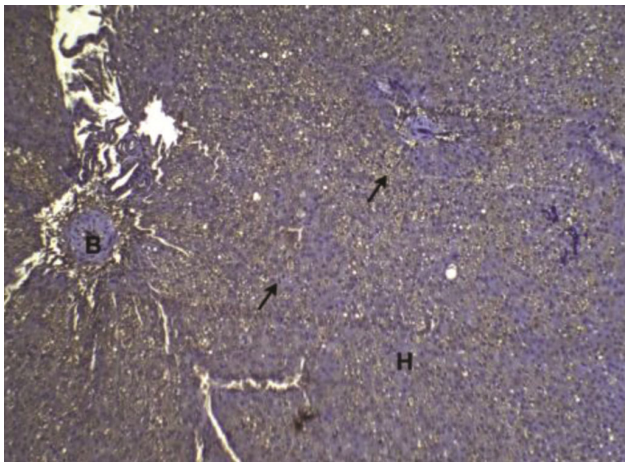

Fig. 2 — Photomicrograph of 45 day-old guinea pig foetal liver showing CD68 positive immuno granules (arrow) in the cytoplasm of sinusoidal cells of the liver except hepatocytes (H) and smooth muscle fibres surrounding the blood vessels (B) x 100

endothelial cells lining the central vein and peribiliary capillaries showed mild positive reaction to CD34 antibody while the sinusoidal endothelial cells showed very mild reaction to CD 34 antibody which signifies the developing stage of the organ. The guinea pig liver of 2-8 weeks, 8-16 weeks and 16-32 weeks of age showed moderate to mild positive immune reaction to CD34 antibody in the sinusoidal endothelial cells near central vein and portal areas and peribiliary capillary endothelial cells in decreasing phase from 0-2 weeks showing maximum to almost minimal expression of CD34 in hepatic sinusoidal endothelial cells of 16-32 weeks of age (Fig. 1). Mild positive reaction was found in the sinusoids near the central vein and portal areas. Sinusoids found in the middle of the lobule did not exhibit any positive

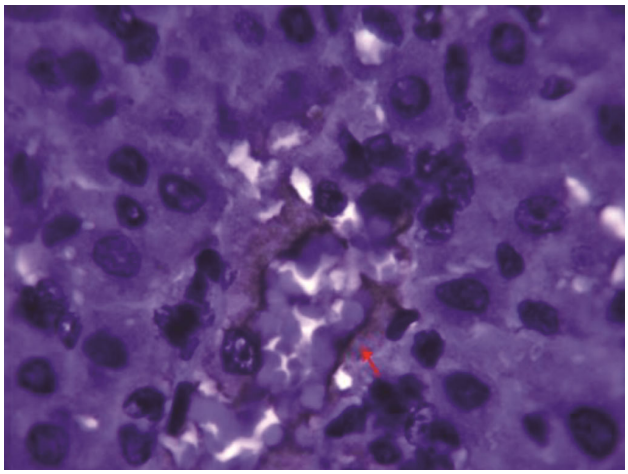

Fig. 3 — Photomicrograph of two week-old guinea pig liver showing mild CD68 positive immunoreactive granules in the cytoplasm of the vascular endothelial cells (arrow) x 1000

reaction towards CD34 antibody. CD34 positive granules were found in the cytoplasm of the sinusoidal endothelial cells and vascular endothelial cells. As age advanced, the CD 34 immunopostivity to sinusoidal endothelial cells decreased.

In prenatal animals, granules of CD68 positive immune reactivity was found in the vascular endothelial cells, sinusoidal endothelial cells, in the cytoplasm of the connective tissue fibres surrounding the hepatic artery (Fig. 2). In the present study, CD68 immunopositive cells were not found in the Kupffer cells of prenatal and preweaning group of animals of 0-2 weeks of age. The bile duct epithelial cells also showed positive reaction to CD68 antibody. In preweaning group of animals, mild CD68 positive immunoreactive granules were found only in the cytoplasm of the vascular endothelial cell (Fig. 3). Strong CD68 positive immunoreactivity was found in the vascular endothelial cells but showed only moderately positive immunoreactive granules in the cytoplasm of the bile duct epithelial cells in the portal areas of guinea pig of 2-8 weeks, 8-16 weeks and 16-32 weeks of age. The CD68 positive immunoreactive granules were found as strong reaction in the cytoplasm of the Kupffer cells of guinea pig of 2-8 weeks, 8-16 weeks and 16-32 weeks of age (Fig. 4). Kupffer cells were found more in the sinusoids near the central vein than in the sinusoids of periportal areas. Hepatocytes did not show any positive immunoreactivity to CD68 in all the ages studied.

In the present study, CK7 positive immune reactive cells were not identified in prenatal and preweaning group (0-2 weeks) of animals. Moderate CK7

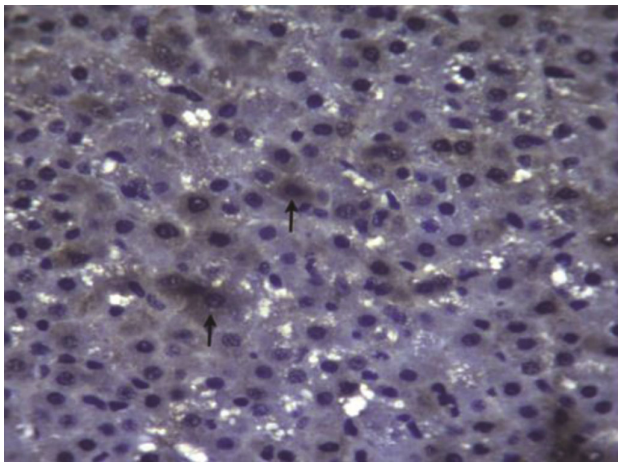

Fig. 4 — Photomicrograph of 12 week-old guinea pig liver showing CD68 positive immunoreactive granules (arrow) in the cytoplasm of the Kupffer cells x 400

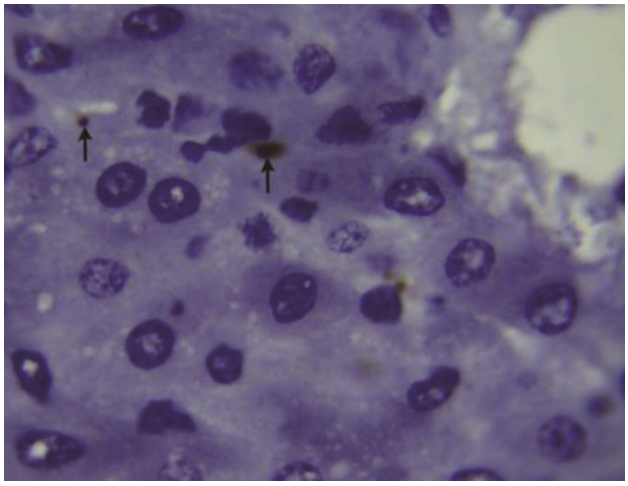

Fig. 5 — Photomicrograph of 10 week-old guinea pig liver showing moderate GFAP immunopositive cytoplasmic granules (arrow) in the hepatic stellate cells x 1000

immunopositivereaction was found in the cytoplasmic granules of cells which were found within the lining epithelium of bile ducts and in the periphery of the bile ducts of guinea pigs of 2-8 weeks, 8-16 weeks and 16-32 weeks of age. But, mild positive CK7 immunoreaction in the cytoplasmic granules of some cells of the sinusoids was found in the hepatic lobules of 2-8 weeks, 8-16 weeks and 16-32 weeks of age. Only some cells of the sinusoids showed moderate brown coloured cytoplasmic granules in the sinusoidal non-parenchymal cells and were considered as a stem cells for bile duct epithelium.

Hepatic stellate cells were not demonstrated in the prenatal and preweaning groups (0-2 weeks) of animals by using GFAP immune staining technique.

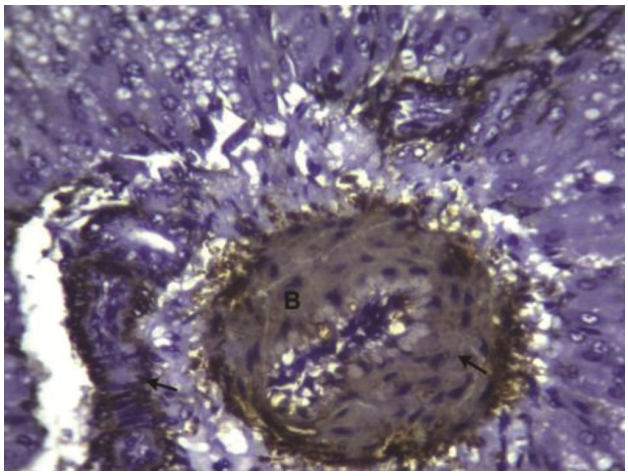

Fig. 6 — Photomicrograph of 45 day-old guinea pig foetal liver showing alpha SMA immuno positive granules (arrow) in the cytoplasm of smooth muscle cells around the blood vessels (B) x 400

Hepatic stellate cells in the 2-8 weeks, 8-16 weeks and 16-32 weeks of age groups of animals showed moderate GFAP positive cytoplasmic granules (Fig. 5). The hepatic stellate cells were found within the sinusoids. However, GFAP positive hepatic stellate cells were mostly located in the sinusoids near the portal areas and surrounding the central vein. Hepatic stellate cells were smaller in size. It was observed with elongated cytoplasm with round nucleus.

Beta cells were not observed in the prenatal and preweaning liver using insulin immunohistochemical reaction. In prenatal animals, smooth muscle fibres were not found in the capsule but smooth muscle cells were found surrounding the blood vessels as brown coloured cytoplasm, as central vein and portal vein differentiation was not appreciable in prenatal liver (Fig. 6). The HSCs were not found in the prenatal liver. Vimentin positive immunoreactivity was not observed in the cells of prenatal liver.

In the present study of preweaning animals (0-2 weeks), smooth muscle fibres surrounding the blood vessels of the portal and periportal areas showed strong immunopositivereaction to alpha SMA. Mild reaction was noticed in the central vein as it was surrounded by only a thin layer of smooth muscle fibres. HSCs were demonstrated as dense brown coloured alpha SMA immunopositive cells in the sinusoids of the hepatic lobule (Fig. 7). But, HSCs were large in size with cytoplasmic branching. In capsule of neonatal animals, smooth muscle fibres were arranged in single thin layer. In all the postnatal animals from 2-8 weeks, 8-16 weeks

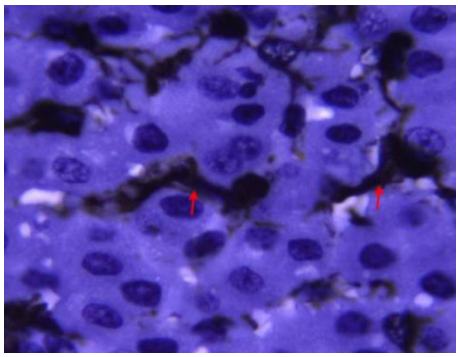

Fig. 7 — Photomicrograph of two week-old guinea pig liver showing immuno positive reaction in HSCs as dense brown coloured alpha SMA immune positive cells (arrow) in the sinusoids of the hepatic lobule x 1000

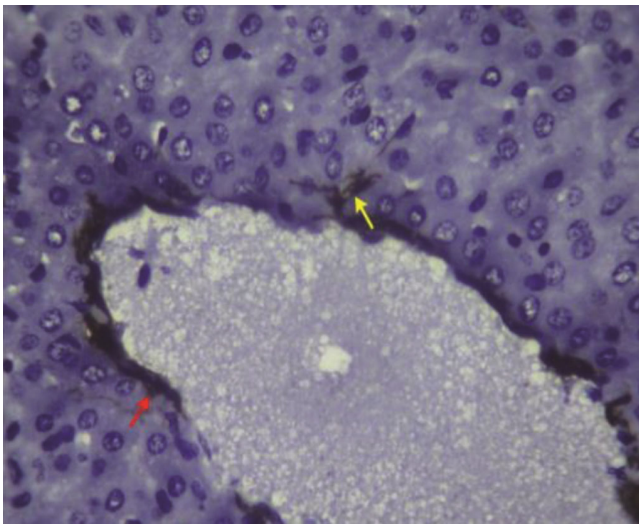

Fig. 8 — Photomicrograph of 28 week-old guinea pig liver showing hepatic stellate cells (yellow arrow) immuno positive alpha SMA cytoplasmic granules within the sinusoidal of the hepatic lobule and in the smooth muscle fibres (red arrow) surrounding the central vein x 1000

to 16-32 weeks of ages studied, strong immunoreactive alpha SMA cytoplasmic granules were found in the smooth muscle cells of the capsule. Hepatic stellate cells in quiescent state were demonstrated by immunopositive alpha SMA cytoplasmic granules within the sinusoids of the hepatic lobule in a scattered fashion (Fig. 8). The hepatic stellate cells demonstrated by alpha SMA immunoreactivity was small and found adjacent to the Kupffer cells. Mild alpha positive immunoreaction was found in the HSC cells in the sinusoids surrounded the central vein (Fig. 8) whereas strong immunoreaction was found in the HSC cells in the sinusoids that surrounded the portal areas. The central vein had only a thin layer of smooth muscle fibres (Fig. 8) whereas portal vein, hepatic artery and bile ducts were

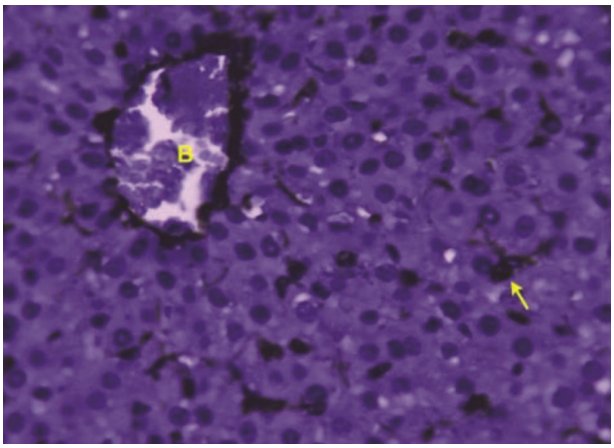

Fig. 9 — Photomicrograph of two week-old guinea pig liver showing vimentin positive immunostaining reaction (arrow) in the cells surrounding the blood vessels (B) and sinusoidal endothelial cells x 400

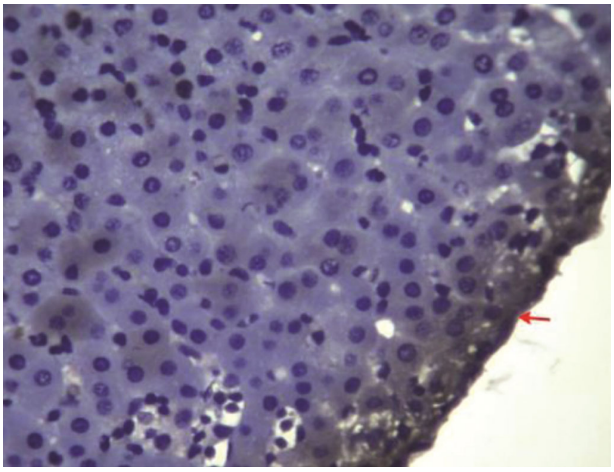

Fig. 10 — Photomicrograph of 12 week-old guinea pig liver showing strong positive immuno reactive vimentin cytoplasmic granules (arrow) in the connective tissue cells of the capsule x 400

surrounded by thick layer of smooth muscle fibres. The smooth muscle fibres of the portal areas also extended some distance into the lobules. Smooth muscle fibres of capsule were found in two layers namely outer continuous layer and an inner discontinuous layer.

Liver of 0-2 weeks of age showed mild vimentin positive immune reaction with brown coloured cytoplasmic granules in the hepatic stellate cells (Fig. 9). Capsule showed only a mild immune positive reaction to vimentin antibody. Vimentin positive immunostaining was also strongly identified in the cells surrounding the blood vessels, bile duct and sinusoidal endothelial cells (Fig. 9). Strong positive immunoreactive vimentin cytoplasmic granules were found in the connective tissue cells of the capsule (Fig. 10). The HSCs were demonstrated

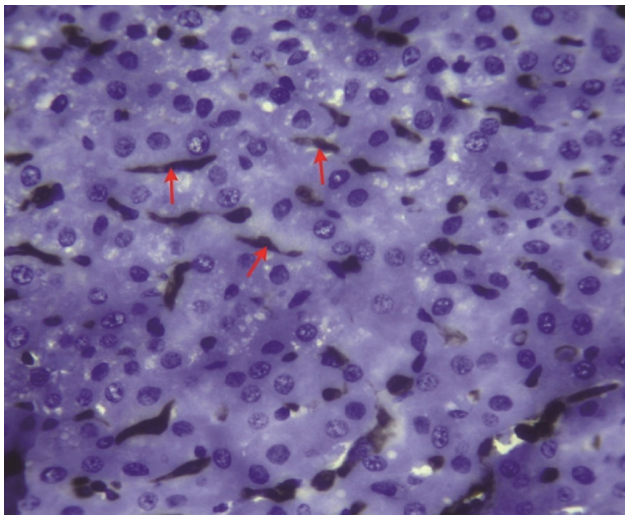

Fig. 11 — Photomicrograph of 10 week-old guinea pig liver showing strong vimentin positive dark brown coloured cytoplasmic granules in the hepatic stellate cells (arrow) of the sinusoids x 400

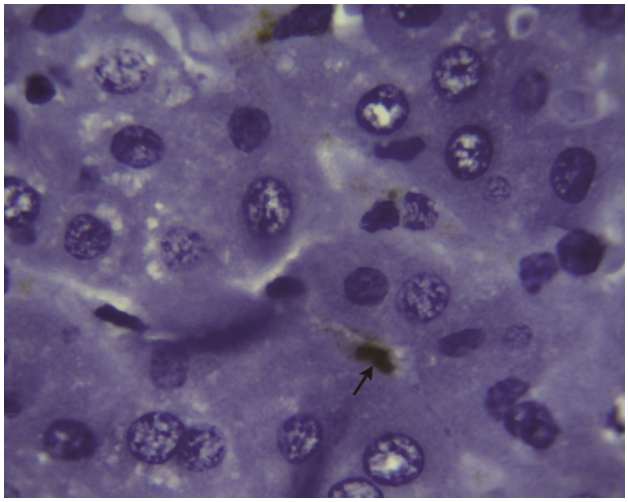

Fig. 12 — Photomicrograph of 24 week-old guinea pig liver showing insulin immunopositive cytoplasmic beta cells (arrow) in the sinusoidal space x 1000

in the sinusoids of the age groups from 2-8 weeks, 8-16 weeks to 16-32 weeks by showing strong vimentin positive dark brown coloured cytoplasmic granules and the proportion of HSCs decreased with age (Fig. 11). The HSCs identified by vimentin immunostaining were small with round to oval nucleus, elongated cytoplasm showing stellate appearance (Fig. 11). The HSCs cells were found in the sinusoids near the central vein and portal areas. Vimentin positive cells were also found surrounding the vascular endothelium namely in the central vein, portal vein, bile duct, hepatic artery. Sinusoidal endothelial cells also showed positive reaction toward vimentin antibody (Fig. 11). All the hepatic stellate cells in the sinusoids of the liver were demonstrated by vimentin immune staining method.

By Insulin immunohisto chemical technique, beta cells were identified within the liver parenchyma adjacent to the hepatocytes in the sinusoidal space (Fig. 12). Beta cells in the liver were found as single cells. These cells were elongated in shape with dense brown coloured immunoreactive granules were found in the cytoplasm with dark round nucleus (Fig. 12). Beta cells were also found in the sinusoidal space near the central vein and portal areas. In the postnatal ages from 2-8 weeks, 8-16 weeks to 16-32 weeks, the beta cells of liver were also found near the Kupffer cells (Fig. 12).

Discussion

Prenatal Liver

Asahina *et al*. (2009) in mice explained that hepatic stellate cells precursors originated from posterior mesoderm and reached the liver through septeum transversum. They also explained that liver was the main site for haemopoiesis in midgestation, so mesenchymal stem cells from mesoderm migrated to the liver to establish haemopoietic stromal network. But, hepatic stellate cells were not demonstrated in the prenatal and preweaning groups (0-2 weeks) of animals by using GFAP immunostaining technique. In contrast to the present study, Loo *et al.*[10] identified hepatic stellate cells by using GFAP and alpha SMA antibody in human foetus. This might be due to species difference. Beta cells were not observed in the prenatal and preweaning liver using insulin immunohistochemical reaction. In prenatal animals, smooth muscle fibres were not found in the capsule but smooth muscle cells were found surrounding the blood vessels as brown coloured cytoplasm, as central vein and portal vein differentiation was not appreciable in prenatal liver. Beall and Rosenquist[11] tasted the presence of smooth muscle fibres by alpha SMA positive reaction in avian embryo coincides with the present study. HSCs were not found in the prenatal liver as vimentin positive immunoreactivity was not observed in the cells of prenatal liver.

Preweaning guinea pig liver

The preweaning liver of 0-2 weeks of age showed mild positive reaction to CD34 antibody in the endothelial cells lining the central vein and peribiliary capillaries with very mild reaction in the sinusoidal endothelial cells but in human foetus, the sinusoidal

endothelial cells showed strong expression to CD 34 antibody[12], which might be due to species differences and indicated the development stage of the organ in the present study.

In preweaning group of animals, mild CD68 positive immunoreactive granules were found only in the cytoplasm of the vascular endothelial cell. In the present study, CD68 immunopositive cells were not found in the Kupffer cells of preweaning group of animals of 0-2 weeks of age. Carolla *et al.*[13] Identified that none of the ruminant liver showed positive reaction for Kupffer cells by CD68 immuno stainingsimilar to our present observation.

In the present study of preweaning animals (0-2 weeks), smooth muscle fibres surrounding the blood vessels of the portal and periportal areas showed strong positive immunoreaction to alpha SMA. Similar results were also reported by Huaxin *et al.*[14] in humans. Mild reaction was noticed in the central vein as it was surrounded by only a thin layer of smooth muscle fibres. The HSCs were demonstrated as dense brown coloured alpha SMA immunopositive cells in the sinusoids of the hepatic lobule. But in human and rat liver during prenatal development, alpha SMA expression was limited to the walls of the blood vessels and HSCs which were in quiescent state and did not express significant amount of alpha SMA[8]. But in our present observations normal expression of HSCs as in adult was expressed and similar results were also noticed in canines[15]. HSCs were large in size with cytoplasmic branching and contained cytoskeletal proteins such as vimentin, alpha SMA, desmin and GFAP in human[16]. In capsule of preweaning animals, smooth muscle fibres were arranged in single thin layer.

Preweaning liver of 0-2 weeks of age showed mild vimentin positive immunoreaction with brown coloured cytoplasmic granules in the hepatic stellate cells as stated by Tang[17] that vimentin also appeared in non-mesenchymal cells like epithelial and myogenic cells in humans as vimentin was normally marker for mesenchymal cells. The presence of vimentin immunoreactivity in the liver of a preweaning animal was a normal feature of development in mesenchymal cells, showing the organ's active growth and differentiation processes. Mesenchymal to epithelial transition events were related to embryonic development, tissue construction, and wound healing in mouse preweaning status and vimentin antibody was to mark the events of mesenchymal to epithelial transition[18]. Capsule showed only a mild immuno positive reaction to vimentin antibody. Vimentin positive immunostaining was also strongly identified in the mesenchymal cells surrounding the blood vessels, bile duct and sinusoidal endothelial cells. Similar observations were also recorded by Ko*et al.*[19] in humans. But Ko*et al.*[19] found that the oval cells or liver progenitor cells of rodent which was an intermediate stage of hepatic differentiation of stellate cells showed the expression of vimentin.

Postnatal guinea pig liver from 2 weeks to 32 weeks of age

The guinea pig liver of adult age groups (2-8 weeks, 8-16 weeks and 16-32 weeks) showed moderate to mild positive immunoreaction to CD34 antibody in the sinusoidal endothelial cells and peribiliary capillary endothelial cells with maximum expression of CD34 antibody in sinusoidal endothelial cells in 2-8 weeks of age and minimal expression of CD34 antibody in sinusoidal endothelial cells of 16-32 weeks of age. Similar results were also recorded by Radu *et al.*[20] in adult human liver where CD34 expression were not observed in the sinusoidal endothelial cells but observed in the portal vessel and central vein endothelial cells. But in our present study, mild positive reaction was found in the sinusoids near the central vein and portal areas whereas CD34 expression was also found in the early lymphocytes to haemopoietic stem progenitor cells in human[20]. But, in the present study, sinusoids found in the middle of the lobule did not exhibit any positive reaction towards CD34 antibody similar to the study of Radu *et al.*[20] in humans. CD34 positive granules were found in the cytoplasm of the sinusoidal endothelial cells and vascular endothelial cells. So, CD34 antibody in the liver was mainly used to identify and ascertain the vascular and neoplastic changes and to differentiate between benign and malignant liver lesions like hepatocellular carcinoma and abnormal capillaries as normal liver expression of CD34 antibody would be focal staining of sinusoidal endothelium in humans[2]. Similar usage can be applied for the present study. As age advanced, the CD 34 immunopositivity to sinusoidal endothelial cells decreased.

Strong CD68 positive immunoreactivity was found in the vascular endothelial cells but showed only moderately positive immunoreactive granules in the cytoplasm of the bile duct epithelial cells in the portal areas of adult guinea pig of 2-8 weeks, 8-16 weeks and 16-32 weeks of age and this indicated the development of the organ showing strong CD68

reaction to vascular endothelial cells in 2-8 weeks of age and mild reaction in 16-32 weeks of age. Similar observation were also recorded by Gottfried *et al.*[21] as mild expression of CD68 was also found in non-myeloid cells like fibroblast, endothelial cells, tumour cells and smooth muscle cells in humans.StrongCD68 positive immunoreactive granules were found in the cytoplasm of the Kupffer cells of 16-32 weeks of age, moderate in 8-16 weeks of age and minimal in 2-8 weeks of age. Similar observations as in adult guinea pigs were also recorded by Liu*et al*.[22] in human liver and Horikawa *et al.*[23] in the liver of guinea pig, monkey, bovine species and pigs as strong labelling of Kupffer cells by CD68 in guinea pig liver. Beljaars *et al*.[24] found a higher number of CD68 positive cells in fibrotic liver significantly when compared to normal liver in mice and humans. Kupffer cells were found more in the sinusoids near the central vein than in the sinusoids of periportal areas. Hepatocytes did not show any positive immunoreactivity to CD68 as CD68,a transmembrane glycoprotein which was used as a marker for monocytes and macropahgesnamely Kupffer cells which will be expressed on inflammatory conditions and minimal expression in normal[24] in mice and human.

Moderate CK7 positive immunoreaction was found in the cytoplasmic granules of cells which were found within the lining epithelium of bile ducts and in the periphery of the bile ducts. The CK7 was a useful tool to identify bile duct structure in tissue section in humans. In chronic cholecystitis conditions, hepatocytes became immunoreactive for CK7 indicating a gradual phenotypic shift towards bile duct type cells[16]. The CK7 was a useful marker for bile duct cells during cell isolation procedure[25] in rats. Bile duct cells proliferated in rat liver following bile duct ligation was immunoreactive for CK7[26]. So the best marker for bile duct epithelial cells was CK7 in all animals and proved in the present study in all ages too. But, mild positive CK7 immunoreaction in the cytoplasmic granules of some cells of the sinusoids was found in the hepatic lobules of 2-8 weeks, 8-16 weeks and 16-32 weeks of age. Not all the sinusoidal spaces were immunostained. Only some cells of the sinusoids showed moderate brown coloured cytoplasmic granules in the sinusoidal non-parenchymal cells. This might be due to the cells lining the canal of Herring or bile ductules.

Hepatic stellate cells in the adult liver (2-8 weeks, 8-16 weeks and 16-32 weeks) showed moderate GFAP positive cytoplasmic granules in the hepatic stellate cells in quiescent state as reported by Carotti *et al.*[5] in rats. The GFAP was an early marker of stellate cells activation in the liver of animals and human[27,28]. GFAP expression was decreased upon HSCs activation as stated by Campbell *et al.*[29] The hepatic stellate cells were found within the sinusoids. HSCs were characterized by high amount of retinoids in their quiescent state. The HSCs were known as collagen producing cells in liver, which was responsible for fibrogenesis in chronic liver diseases in rats[30]. However, GFAP positive hepatic stellate cells were mostly located in the sinusoids near the portal areas and surrounding the central vein. Hepatic stellate cells were smaller in size. It had elongated cytoplasm with round nucleus. GFAP was found as marker for identifying HSCs in rats[31] whereas, GFAP was positive in only a periportal subpopulation of HSCs of humans[14] as per the results of the present study.

By Insulin immunohistochemical technique, beta cells were identified within the liver parenchyma adjacent to the hepatocytes in the sinusoidal space as per the findings of Radu *et al.*[20] in diabetic and nondiabetic mice andin adipose tissue, spleen, bone marrow and thymus. The pancreas, liver, stomach and intestine were developmentally related as foregut with endodermal origin. So, the hepatocytes may transdifferentiated into beta-like cells expressing insulin antibody upon progenitor expression but the functionality of these transdifferentiated cells (Phenotypically plastic cells) were incomplete[32]. So, beta cells in the liver were found as single cells and not in groups satisfy current research work. These cells were elongated in shape with dense brown coloured immunoreactive granules were found in the cytoplasm with dark round nucleus. Beta cells were also found in the sinusoidal space near the central vein and portal areas. In the postnatal ages from 2-8 weeks, 8-16 weeks to 16-32 weeks, the beta cells of liver were also found near the Kupffer cells. But, in adult Wistar rats, beta cells were found in the sinusoids[33].Cytoplasmic immunostaining of insulin may also be due to circulating insulin that has been taken up or is part of a complex, not produced endogenously by hepatocytes was also noticed[6]. This might also be the reason for the presence of beta like cells in the liver parenchyma of present study.

In all the postnatal animals from 2-8 weeks, 8-16 weeks to 16-32 weeks of age studied, strong immuno

reactive alpha SMA cytoplasmic granules were found in the smooth muscle cells of the capsule as reported by Huaxin*et al*.[14] in humans. Hepatic stellate cells (HSC) were demonstrated by immuno positive alpha SMA cytoplasmic granules within the sinusoids of the hepatic lobule. The hepatic stellate cells demonstrated by alpha SMA immuno reactivity was small and found adjacent to the Kupffer cells as recorded by Beall and Rosenquist[11] in avian embryo. Mild alpha SMA positive immunoreaction was found in the HSC cells in the sinusoids surrounded the central vein whereas strong immunoreaction was found in the HSC cells in the sinusoids that surrounded the portal areas and the reaction decreased with age as alpha SMA was the ideal marker for activated HSCs in young and adult animals but in healthy animals, only quiescent HSCs were present so the expression was mild to moderate[34] in human liver were as per the results of our present study. The central vein had only a thin layer of smooth muscle fibres whereas portal vein, hepatic artery and bile ducts were surrounded by thick layer of smooth muscle fibres. The smooth muscle fibres of the portal areas also extended some distance into the lobules. Smooth muscle fibres of capsule were found in two layers namely outer continuous layer and an inner discontinuous layer. Similar observations were also recorded in avian embryo[11].

Strong positive immunoreactive vimentin cytoplasmic granules were found in the connective tissue cells of the capsule. The HSCs were demonstrated in the sinusoids of the adult liver of ages from 2-8 weeks, 8-16 weeks to 16-32 weeks by showing strong vimentin positive dark brown coloured cytoplasmic granules. The HSCs identified by vimentin immunostaining were small with round to oval nucleus, elongated cytoplasm showing stellate appearance. The HSCs cells were found in the sinusoids near the central vein and portal areas. But the expression of vimentin positive quiescent HSCs were strong but the proportion was found reducing from 2-8 weeks to 16-32 weeks of age and similar results were also observed by Yousaf *et al.*[35] in rats. Vimentin positive cells were also found surrounding the vascular endothelium namely in the central vein, portal vein, bile duct, hepatic artery. Sinusoidal endothelial cells also showed positive reaction toward vimentin antibody. All the hepatic stellate cells in the sinusoids of the liver were demonstrated by vimentin immunostaining method. Similar results were also found in rodents[19].

Conclusion

By Immunohistochemistry, prenatal liver showed the presence of CD68 immunoreactive granules in most of the vascular endothelial cells and bile duct epithelial cells but showed decreased reaction as age advanced. HSCs and beta cells were not observed in prenatal and preweaning groups of guinea pigs and were noticed in the other postnatal groups indicated the same embryological origin, insulin receptor in cells and functionality of beta cells was incomplete. Mild positive CD34 reaction was found in sinusoidal endothelial cells and peribiliary capillary endothelial cells in the preweaning group and moderate reaction as age advanced in postnatal groups. The CK7immunopositive cells were identified in the bile duct epithelial cells and in the space of Disse of the sinusoids in the postnatal age group of guinea pigs. The GFAP positive hepatic stellate cells were mostly located in the sinusoids near the portal areas and surrounding the central vein and were quiescent in nature. Postnatal group of animals showed strong immunopositive reaction to alpha SMA antibody in smooth muscle cells and HSCs. Mild alpha SMA positive immunoreaction was found in the HSC cells in the sinusoids surrounded the central vein whereas strong immunoreaction was found in the HSC cells in the sinusoids that surrounded the portal areas. Mild vimentin positivity was found in adult animals. All the hepatic stellate cells in the sinusoids of the liver were demonstrated by vimentin immunostaining method. HSCs showed mild to moderate GFAP reaction in adult guinea pig liver.These antibodies were used to identify the parenchymal cells like hepatocytes, bile duct epithelial cells and sinusoidal and vascular endothelial cells and also non-parenchymal cells like Kupffer cells, hepatic stellate cells and beta-like cells in guinea pigs of different prenatal and post natal age groups. These antibodies will show aberrant reaction upon diseased condition of the liver.

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Conflict of Interest

All authors declare that they have no conflict of interest.

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