OV6, α-Fetoprotein, Hepatocyte Growth Factor and Transforming Growth Factor Beta 1 in Patients with Chronic Hepatitis, Cirrhosis and Hepato Cellular Carcinoma.

Nawal El Badrawy[1], Olfat A. Hammam[2]*, Maged El Ghanam[1], Mahmoud Al Ansary[1], Moataz Hassan[1] Abdel Aziz Ali Saleem[1]

[1]Hepato-Gasteroenterology, [2]Patholgy Departments, Theodor Bilharz Research Institute, Giza, Egypt.
mansary2@gmail.com

Abstract: The aim of this work is to study the relation of OV6 (oval cells), AFP and hepatocyte growth factor (HGF) and transforming growth factor beta 1 (TGFB1) to repopulation of the liver in patients with chronic hepatitis C, liver cirrhosis and hepatocellular carcinoma (HCC). Ninety patients with chronic liver disease and HCC were subjected to clinical examination, laboratory investigations for hepatitis C, liver function tests and abdominal ultrasonography. Liver biopsy was performed for histopathological examination. They were 3 groups; positive for hepatitis C: chronic hepatitis (35), liver cirrhosis (25) and HCC (30) and ten control patients with negative serological markers for hepatitis (C&B). Immunohistochemical staining for tissue α-fetoprotein (AFP), OV6, HGF and TGFB1 was done. In normal liver specimens, tissue AFP, OV6, HGF and TGFB1 showed no expression. In chronic hepatitis, tissue AFP was negative, OV6 showed insignificant increase while HGF and TGFB1showed a significant increase compared to control group $p<0.05$, which may indicate repopulation of the liver by proliferation of hepatocytes rather than oval cells at this stage. In cirrhosis with disturbed liver architecture, fibrosis and impaired liver functions, there was an insignificant increase in OV6 and tissue AFP and a significant increase in HGF and TGFB1 relative to control ($p<0.001$), which may be related to the bi-potential property of oval cells differentiating into both hepatocytes and bile ductules, and to the anti proliferative effect of TGFB1on hepatocytes. In HCC there was significant increase in tissue expression of OV6, AFP and TGFB1 relative to control, to CH and to LC groups ($p<0.001$), while HGF was significantly increase ($p<0.001$) relative to control & CH. In conclusion, repopulation of the liver in chronic hepatitis may be more related to hepatocytes proliferation rather than to oval cells. In liver cirrhosis, the increase in oval cells is not significant to restore liver functions. In HCC; OV6, AFP, HGF and TGFB1were significantly increased. Further studies are needed in search for factors that may hinder differentiation of oval cells to functional hepatocytes in liver cirrhosis and may lead to malignant transformation.

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1. Introduction

Chronic Hepatitis C Virus (HCV) is recognized as a major threat to global public health. An estimated 170 million people worldwide are infected and 3 to 4 million new cases per year, most of them are chronically infected and at risk for liver cirrhosis and hepatocellular carcinoma. In Egypt, 10%–20% of the general populations are infected and HCV is the leading cause of HCC and chronic liver disease in the country(1-2). Liver cirrhosis in humans represents the end stage of chronic liver injury and it is characterized by the disorganized proliferation of hepatocytes and biliary cells, excessive scarring, and loss of the three-dimensional architecture of the hepatic lobule, which leads to chronic liver failure eventually requiring transplantation (3).

The ability of the liver to regenerate is a property that is unique among solid organs in mammalian species. Extensive research has been conducted to elucidate cellular & molecular events occurring during liver regeneration (4). Two cell lineages have been considered as candidates; the first is hepatic stem cell and the second is mature hepatocyte (5). Oval cells are small oval shaped epithelial cells identified as hepatic stem cells in the adult liver following severe repetitive liver injury (6) and they are bi-potential capable of differentiating into either hepatocytes or cholangiocytes. Oval cells might give rise to HCC as a result of the arrest of stem cell maturation (7). OV6 and AFP are of the many markers which have been used to identify oval cells and OV6 is widely chosen as the oval cell marker of choice (8).

According to Yamagami *et al.* (9) and Lee *et al.* (8) HGF controls the growth of hepatocytes, it is produced principally in mesenchymal cells. Suzuki *et al.* (10) showed that HGF induced early transition of albumin –ve to albumin +ve cells and was an effective mitogen for differentiating cells. Also HGF and its receptor system c-met play an important role

in regulating growth and metastasis of liver tumors (11). Recent studies have focused on the relationship of HGF to the development and chronic nature of viral hepatitis and the progression of chronic hepatitis and development of HCC **(9).**

TGFB1 plays an important role in regulation of cell growth and differentiation, angigenesis, extracellular matrix formation, immunosupression and cancer development (12). Mammals produce 3 TGFB1ligand. TGFB1,TGFB2 and TGFB3. TGFB1 is the major form in adults (13).

TGFB produced by the stelette cells inhibits hepatocytes replication (14). The signaling mechanisms activated by TGFB family proteins have emerged as important players in the self renewal and maintenance of stem cells in their undifferentiated state (15). When TGFB signaling is interrupted, the inbalance can result in an undifferentiated phenotype and cancer may ensue (16). TGFB represents a potentially important link between fibrosis and neoplasia (17).

Aim of the work: To study the relation of oval cells (OV6), tissue AFP, hepatocytes growth factor (HGF) and transforming growth factor beta1 (TGFB1) to repopulation of the liver in patients with chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.

2.Material and Methods

Patients

Ninety patients (positive for hepatitis C),72 males and 18 females; mean age 40.3 ± 2.4, range 25-65 years were the subject of this study. Patients were admitted to the Department of Gastroenterology and Hepatology, Theodor Bilharz Research Institute, Giza, Egypt. They included 35 cases of chronic hepatitis C virus infection (CH), 25 cases with cirrhosis and 30 cases of HCC. The presence of HCV-RNA in patients sera was detected by real-time polymerase chain reaction. They were subjected to thorough clinical examination, urine and stool analysis, liver function tests, ultrasonography and liver biopsy for histopathologic and immuno-histochemical (IHC) studies. The study protocol was approved by the Ethics Committee of TBRI according to the Institutional Committee for the Protection of Human Subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland.

Ten control liver biopsies were from individuals subjected to laparoscopic cholecystectomy after their consent. They were 4 males and 6 females with a mean age of 48.3 ± 2.3 years. Their liver function tests were normal and had no serologic evidence of hepatitis B and/or C viruses.

Liver biopsies taken were fixed in 10% buffered formalin for 24 hours, and then processed in ascending grades of ethyl alcohol, xylene, wax and paraffin blocks. Sections (4 μm) were cut on albuminized glass slides and stained with Hematoxylin & eosin and Masson trichrome stains. All sections were subjected to light microscopic examination for histopathological classification of cases; chronic hepatitis, cirrhosis according to Desmet *et al.,* and Knodell *et al.* (18,19),and HCC cases according to Edmondson and Steiner (20). Other liver sections (4 μm) were cut on slides, which were treated with TESPA (3-aminopropyl-triethoxysilane, Sigma) for IHC.

Immunohistochemistry for Detection of OV6, tissue AFP, HGF and TGFB1tissue antigens:

Immunohistochemical reaction was performed using an avidin biotin complex (ABC) immunoperoxidase technique according to Hsu and Reine (21) using anti human OV6, tissue AFP, HGF and TGFB1 on paraffin sections; dewaxed in xylene and hydrated in descending grades of ethanol. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide in 100% methanol for 20 min. Antigen retrieval was performed by microwaving the sections in citrate buffer (pH 6.0) for 15 min at 700 W. Sections were incubated overnight at 4^{o}C with the anti-human primary antibodies against OV6 (purchased from R&D, USA), HGF, TGFB1 and tissue AFP (purchased from Santa Cruz Biotechnology Inc.; Santa Cruz, USA) monoclonal antibody, diluted at 1:50,1:50,1:150, 1:100 respectively in BPS. Next day, after thorough washing in PBS, the sections were incubated with streptavidin-biotin-peroxidase preformed complex and evidentiated using a peroxidase/DAB (diaminobenzidine) enzymatic reaction for OV6 & HGF& TGFB1 and AFP. Staining is completed by 5-10 minutes incubation with 3, 3'-diaminobenzidine (DAB) + substrate - chromogen which results in a brown-colored precipitate at the antigen site for OV6 & HGF& TGFB1and tissue AFP (cytoplasmic stain). Slides were washed in PBS for 5 minutes. Slides were placed in 70%, 95% and then 100% alcohol each for 5 minutes. The cell nuclei were counterstained with Mayer's hematoxylin. The cover slips were mounted using Dpx.

Positive and negative control slides for each marker were included within each session. As a negative control, liver tissue section was processed in the above mentioned sequences but the omission of the primary antibody and PBS was replaced.

Evaluation of immunostaining:

Sections were examined under light microscopy at x 400. The number of positively

stained cells was recorded in ten microscopic fields with the highest expression and the percentage was calculated from their mean. Zero% was given to unstained sections. Pattern of all markers were cytoplasmic staining.

Immunohistochemical scoring of OV6

OV6 was expressed as cytoplasmic stain, brown color; the number of positive cells counted in 10 microscopic fields, with power of magnification (x 400) according to Crosby *et al.* (22).

Immunohistochemical scoring of tissue alpha fetoprotein:

Tissue alpha fetoprotein was expressed as cytoplasmic brown color and counting the number of positive cells in 10 microscopic fields, with power of magnification (x 400) according to Hasib *et al.* (23).

Immunohistochemical scoring of HGF:

Immunohistochemical reactions for HGF was expressed as cytoplasmic stain, brown color, counted as the number of positive cells in 10 microscopic fields, with power of magnification (x 400) according to Inoue et al.(24).

Immunohistochemical scoring of TGFB1:

TGFB1 was expressed as cytoplasmic brownish color and counting positive cells in 10 microscopic fields with highest expression, with power of magnification(x 400), according to (Kim *et al.* (25).

Statistical analysis:

The Statistical Package for Social Sciences (SPSS) for Windows (version 10) computer program was used for statistical analysis. For comparison of more than 3 group's means, one-way ANOVA test, Post Hoc test was used. Comparison between percent positive cases was calculated by Chi-square test. A P value < 0.05 was considered statistically significant.

3. Results:

Patients were 66 males (73.3 %) and 24 females (26.7 %), their age ranged 23-72 years with a mean of 46.6±4.5 years, as well as 10 patients with normal liver as control group. They were 4 males and 6 females, their age ranged (30-45 years) with a mean of 39.4±3.7years, PCR was positive for HCV, serum biochemical tests are shown in table 1.

Table 1. Liver biochemical profile of studied groups

Variables	CH (n=35) Mean ± SD	LC(n=25) Mean ± SD	HCC(n=30) Mean ± SD	Control (n=10) Mean ± SD
ALT (0-41) U/L	53.95 ± 29.16 [a,b]	45.8 ± 31.91 [a]	36.87 ± 21.5	19.8 ± 5.59
AST (0-38) U/L	38.3 ± 17.33 [b]	69.8 ± 16.15 [a,c]	72.9 ± 34.75 [a]	20.2 ± 5.87
Tot. protein (6-8) gm/dl	7.76 ± 1.17	8.02 ± 0.64 [a]	7.74 ± 1.14	7.24 ± 0.39
S. albumin (3.5-5) gm/dl	4.46 ± 0.4 [b]	3.62 ± 0.22 [a,b,c]	3.28 ± 0.54 [a]	4.22 ± 0.18
Total bilirubin (0.1-1.2)mg/dl	0.9 ± 0.5 [b]	1.2 ± 0.45 [b]	1.7 ± 1.02 [a]	0.9 ± 0.15
Direct bilirubin (0-0.25)mg/dl	0.46 ± 0.35 [a]	0.55 ± 0.5 [a]	1.09 ± 0.81 [a]	0.21 ± 0.06
Prothrombin Concentration (70-100)%	90.2 ± 10.43 [a,b]	83.2 ± 10.6 [a,b,c]	76.0 ± 11.29 [a]	98.8 ± 2.53

[a]: p value <0.05 relative to the control group; [a']: p value <0.001 relative to the control group
[b]: p value <0.05 relative to the HCC group; [b']: p value <0.001 relative to the HCC group
[c]: p value <0.001 relative to the CH group

The number of positive cases for OV6, AFP, HGF, and TGF1expression in liver biopsies is shown in table 2. The number of positive cases for OV6 cases expression was significantly increased in LC and HCC compared to CH cases.

There is no tissue Ov6 expression in the control group. Expression of OV6 is cytoplasmic, in the cells lining bile ducts, hepatocytes at the periphery of portal tracts and at the periphery of cirrhotic nodules, whereas there is a high expression in the HCC group. There is no statistical significant difference in tissue Ov6 expression between the CH & LC groups relative to the control group, while in HCC group; it is statistically significant relative to the control group at $p < 0.001$. Tissue Ov6 expression in HCC group is statistically higher in compared to &LC groups at $p < 0.001$ (Tables 2 and 3; Figs. 1a, b, c, d).

Table 2. Number of positive cases with OV6, tissue AFP, HGF and TGFB1 in studied groups in studied groups

Variable	OV6		Tissue AFP		HGF		TGFB1	
	No of positive cases %		No of positive cases %		No of positive cases %		No of positive cases %	
Control (n=10)	0	0	0	0	0	0	0	0
CH (n=35)	8	22.9%*	0	0	7	20%#	10	29.9%*
LC (n=25)	18	72%	5	20%^	9	36%^	15	60%
HCC (n=30)	25	83.3%	21	70%	18	60%	24	80%

*Highly significant increase in number of positive cases in LC and HCC compared to CH at p <0.001
^Highly significant increase in number of positive cases in HCC compared LC to at p <0.001
#Highly significant increase in number of positive cases in HCC compared to CH at p <0.001

Table 3. Tissue expression of OV6, tissue AFP, HGF and TGFB1 in studied groups

Variable	Control (n=10) Mean ± SD	CH (n=35) Mean ± SD	LC (n=25) Mean ± SD	HCC (n=30) Mean ± SD
OV6	0.0±0.0	4.3±1.01 [c]	10. 3 ± 3.07 [c]	25.8 ± 11.2 [b]
Tissue AFP	0.0±0.0	0.0±0.0 [c]	12.5 ± 1.07 [c]	55.8 ± 15.2 [b]
HGF	0.0±0.0	16.3±1.21 [a, c]	40. 3 ± 7.02 [b]	47.5 ± 13.2 [b]
TGFB1	0.0±0.0	12.1±3.41 [a, c]	38. 3 ± 7.4 [b, c]	62.52 ± 15.2 [b]

[a]: p value <0.05 relative to the control group
[b]: p value <0.001 relative to the control group
[c]: p value <0.001 relative to the HCC group

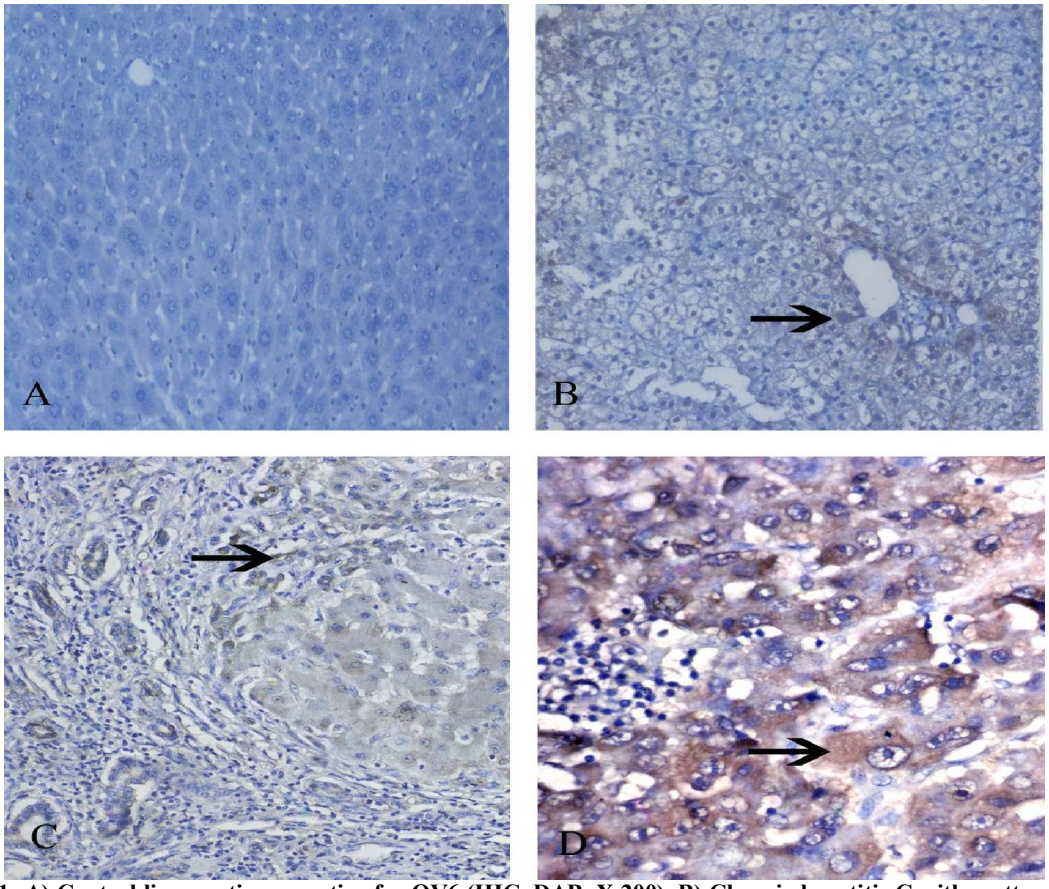

Figure 1: A) Control liver section, negative for OV6 (IHC, DAB, X 200). B) Chronic hepatitis C with scattered bile duct lining cells stained with OV6 as cytoplasmic stain, arrow (IHC, DAB, X 200). C) Chronic hepatitis C merging into cirrhosis with scattered bile duct lining cells stained with OV6, and positive hepatocytes at the periphery of the cirrhotic nodules, arrow (IHC, DAB, X 200). D) HCC, showing hepatocytes positively stained with OV6 as brownish cytoplasmic stain, arrow (IHC, DAB, X 400).

The number of cases Positive for AFP was significantly increased in HCC compared to LC cases. There is no tissue alpha fetoprotein expression in the control and in CH groups. Expression of AFP is cytoplasmic in hepatocytes. There is no statistically significant difference in tissue alpha fetoprotein expression between the LC grouprelative to the control group, while expression in HCC group is statistically highly significant relative to the LC group at $p < 0.001$ (Tables 2 and 3; Figs. 2a, b, c, d).

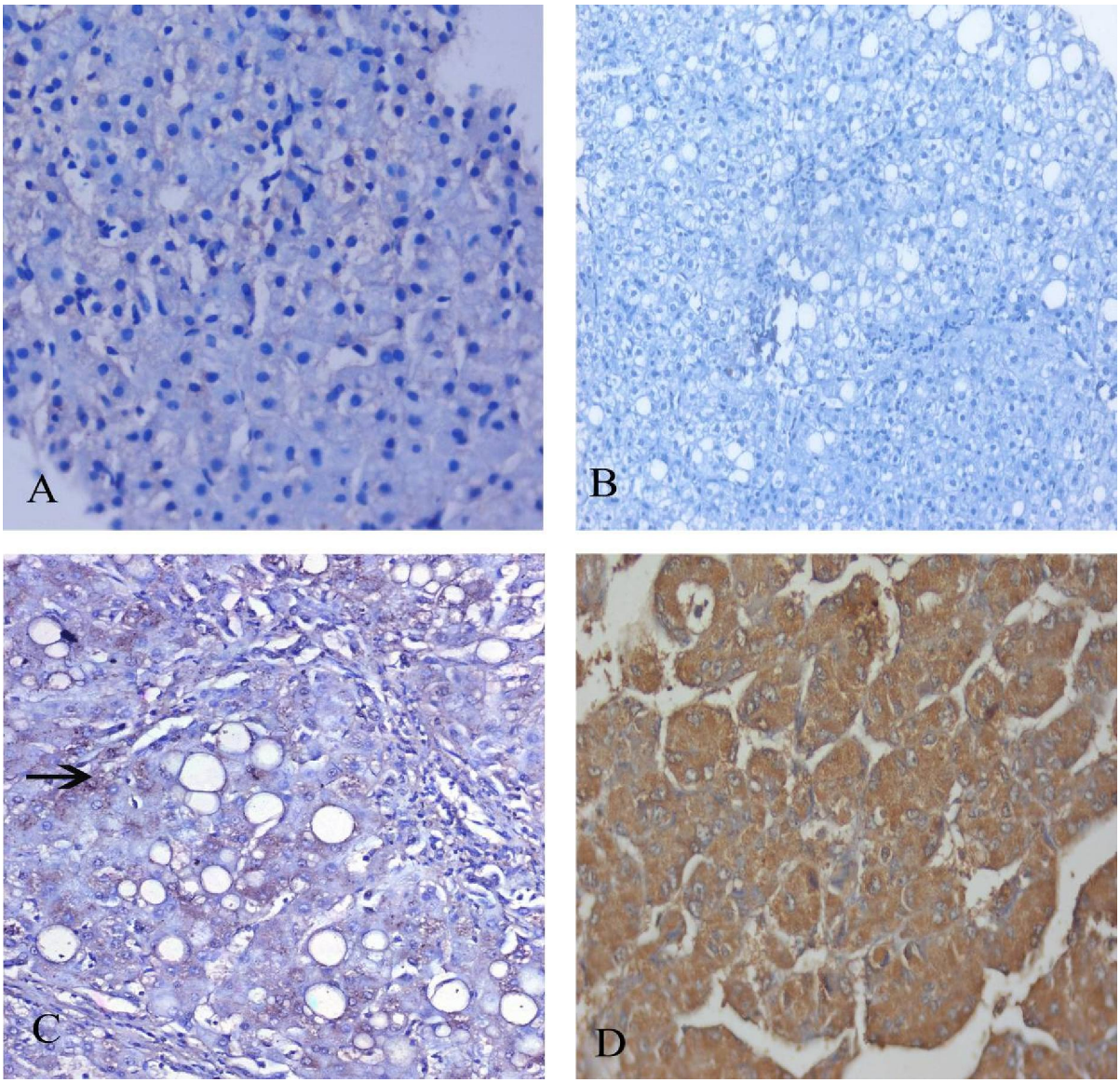

Figure 2: A) Control liver section, negative for AFP (IHC, DAB, X 200). B) Chronic hepatitis, negative for AFP (IHC, DAB, X 100). C) A case of liver cirrhosis showing scattered hepatocytes positive for alpha fetoprotein (cytoplasmic brownish color) monoclonal antibody, arrow (IHC, DAB, X 200). D) A case of HCC showing many hepatocytes showing positive cytoplasmic, arrow stain for alpha fetoprotein monoclonal antibody (IHC, DAB, X 400).

Cases with positive HGF expression were significantly increased in HCC compared to CH and LC groups. There is no tissue HGF expression in the control group. Expression of HGF is cytoplasmic, in the sinusoidal cell lining (Kupffer cells & endothelial cells), in the hepatocytes and bile ducts lining cells. There is statistically significant difference between the CH group relative to the control group at $p < 0.05$ and in LC at $p < 0.001$ relative to control. In HCC group there is statistically significant increase relative to the control at $p < 0.001$(Tables 2 and 3; Figs. 3a, b, c,d).

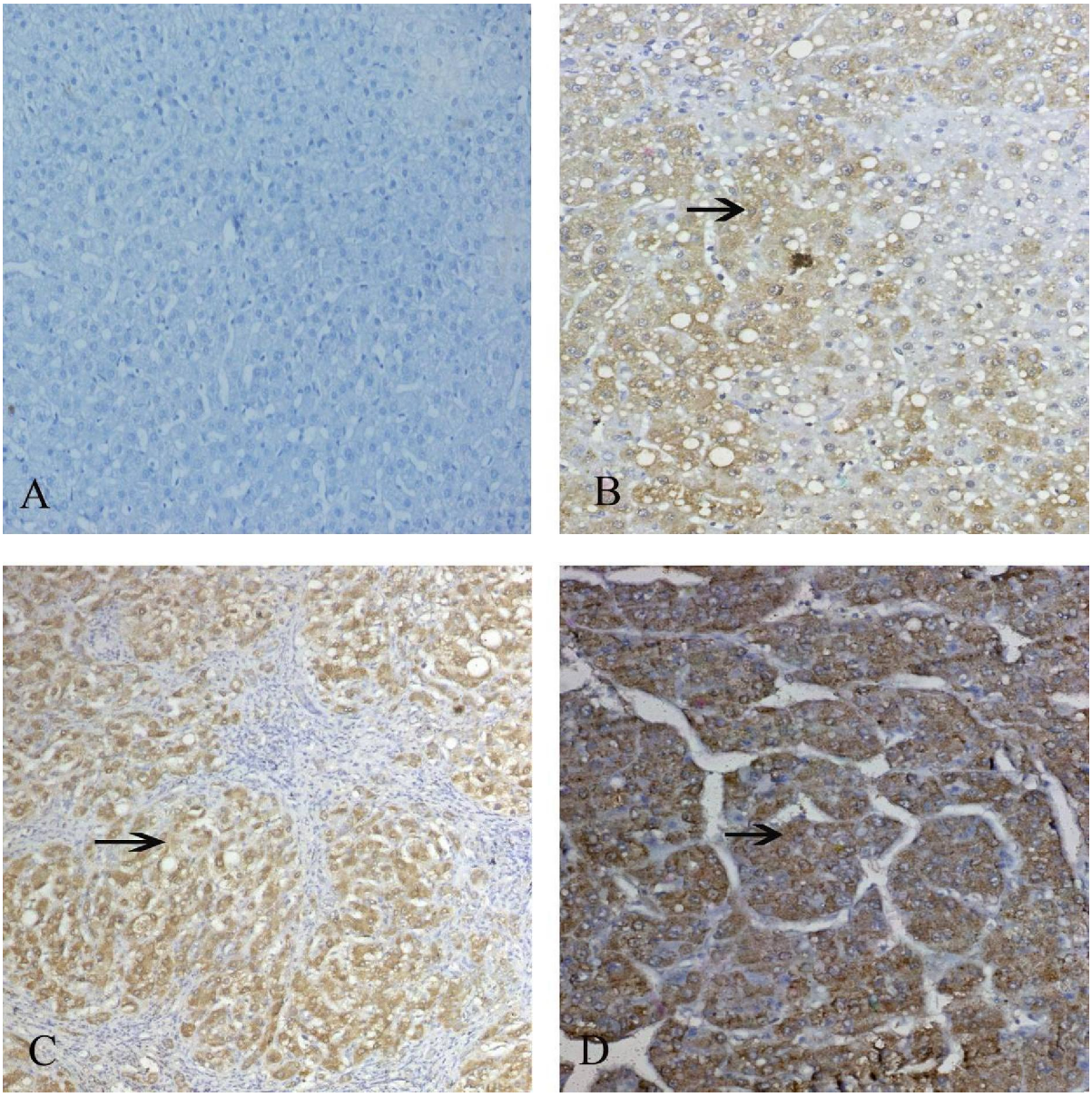

Figure 3): A) Control liver section, negative for HGF (IHC, DAB, X 200). B) Chronic hepatitis C, showing mild HGF expression, (cytoplasmic), arrow (IHC, DAB, X 200). C) Chronic hepatitis C, with cirrhotic nodule, showing increase HGF expression, arrow (IHC, DAB, X 200). D) A case of HCC showing intense cytoplasmic staining for HGF, arrow (IHC, DAB, X 200).

The number of cases positive for TGFB1 was significantly higher in LC and HCC compared to CH group. There is no tissue expression of TGFB1 in the control group. There is statistical significant difference between the CH & LC groups relative to the control group at $p < 0.05$ & $p < 0.00$ 1 respectively. In HCC group there is a statistically significant increase relative to the control and CH & LC groups at $p < 0.001$(Tables 2 and 3; Figs. 4a,b,c,d).

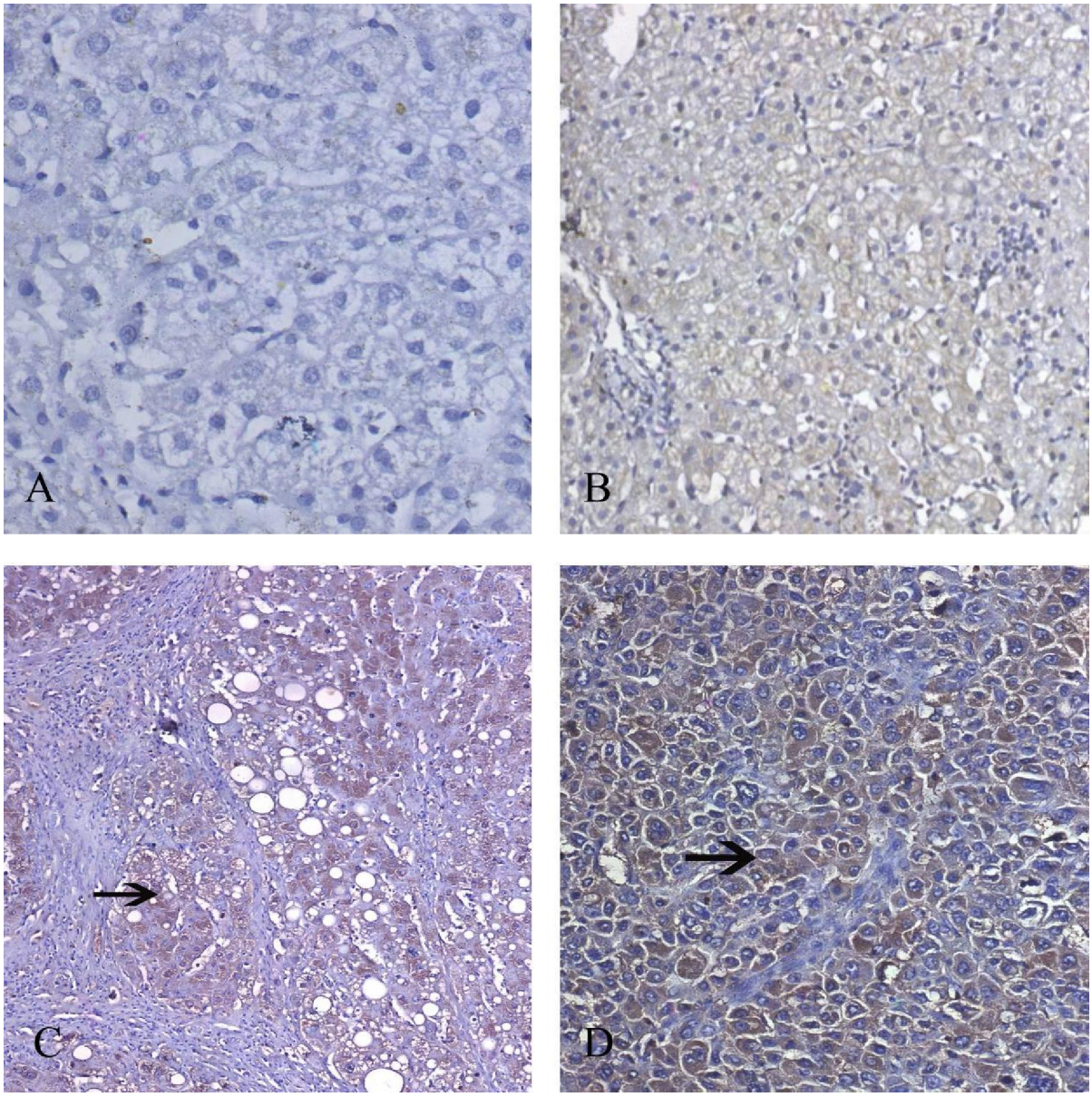

Figure 4: A) Control liver section, negative for TGFB (IHC, DAB, X 200). B) Chronic hepatitis, showing mild TGFB1 expression, arrow (IHC, DAB, X 200). C) A case of chronic hepatitis with cirrhotic nodule, showing increased TGFB1 expression, arrow (IHC, DAB, X 200). D) A case of HCC showing intense cytoplasmic staining for TGFB1, arrow (IHC, DAB, X400).

4. Discussion

Hepatic progenitor cells are small cells with an oval nucleus and scant cytoplasm which is strongly immunoreactive for rat oval cells (OV6). Oval cells are one of the important origins of liver stem cells (26). These facultative progenitor cells commonly referred to as oval cells are located within the terminal bile ducts and are able to give rise to both hepatocytes and cholangiocytes (27). In our study, there was no tissue expression of OV6 in the liver in normal control group. In CH group there was an insignificant increase in OV6 expression. In LC, there was an increase but still insignificant compared to control. Tissue AFP expression was negative in control cases, and in CH group. In LC, tissue AFP showed an insignificant increase compared to control. The study by Roskmans *et al.* (28) showed that in very early stages of human liver regeneration, small oval like cells (putative progenitor cells) appear and are immunoreactive for OV6. Fausto *et al.* (29)

revealed a very low number of AFP+ve cells in the normal adult liver, predominantly in the periportal region. Most of these cells have the morphologic appearance of oval cells. The AFP +ve cells are located either within the bile ducts or in the hepatic parenchyma immediately adjacent to the ducts. Fausto and Campbell (27) showed that in more sustained liver injury, when hepatocytes are unable to enter the cell cycle, a second compartment of cells actively proliferates and is capable of restoring damaged liver mass. These facultative progenitor cells, commonly referred to as oval cells, are located within the terminal bile ducts and are able to give rise to both cholangiocytes and hepatocytes. Differentiation of oval cells can contribute the hepatocytes population usually when hepatocytes proliferation is impeded and hepatic destruction is severe (30). Lowes *et al.* (31) stated that oval cells were not observed in normal liver control. Their number increased significantly ($p<0.01$) as disease severity increased from mild through moderate to severe chronic hepatitis. Oval cells were located predominantly in the periportal region and were occasionally observed to form ductular structures. According to Chen *et al.* (32), oval cells emerge when hepatocytes regeneration is impaired suggesting that they are the progeny of hepatic stem cells. Clouston *et al.* (33) reported that hepatic progenitor cells (HPCs) numbers correlated with fibrosis in chronic hepatitis C patients.

Shafritz and Dabeva (34) studies revealed a very low number of AFP +ve cells in the normal adult liver. Most of the cells have the morphologic appearance of oval cells rather than hepatocytes. According to Zheng and Taniguchi (35). AFP expression can be used as an indicator for an early hepatic lineage and has also served as an important marker for the activation of stem cell compartment. In our study, the insignificant increase in OV6 expression in CH may denote repopulating of the liver at this stage by hepatocytes more than oval cells. Tissue AFP positivity may indicate cirrhotic changes According to Folkwski *et al.* (39), hepatocytes proliferation rate increased in hepatitis C with increasing histological damage until cirrhosis is reached when the proliferation rate falls. Overwhelming liver injury, chronic liver injury or large scale hepatocytes senesce result in a potential stem cell compartment being activated from the smallest branches of the intrahepatic biliary tree. These reduction in hepatocytes proliferation in chronic hepatitis occurs concurrently with the activation of this potential stem cell compartment (14).

In the cases of the present study HGF showed no expression in the liver in normal control group, a significant increase ($p<0.001$) was found in CH and ($p<0.05$) in LC groups compared to control. HGF has not been found immunocytochemically in the normal liver without cirrhosis or chronic hepatitis (36). Also Ljubimova *et al.* (37) were unable to find HGF expression even by sensitive RT-PCR in normal liver.

HGF is a hepatotropic factor and in the regenerating liver stimulates proliferation of adult hepatocytes (38). According to Yamagami *et al.* (2001) HGF controls the growth of hepatocytes because it is produced principally in mesenchymal cells and is not found in hepatocytes themselves. Inoue *et al.* (24) showed that HGF expression was increased significantly during the course of cirrhosis development but decreased significantly at the appearance of cirrhosis manifestation. Their results suggest that the highly proliferative capability of hepatocytes at an early stage of cirrhosis development is induced by increased intrahepatic HGF expression. They suggested that intrahepatic HGF produced by Kupffer cells, HSCs and endothelial cells in the liver is increased for hepatocytes replication in response to hepatocyte damage. However, the ability to produce intrahepatic HGF is exhausted probably due to over-loaded production resulting in the manifestation of liver cirrhosis. However Okano *et al.* (40) reported intense staining of HGF in patients from AH to CH and LC.
In our study TGFB1 showed no expression in the liver in normal control group. There was a significant increase ($p<0.05$) in CH group & in liver cirrhosis group ($p<0.001$) relative to control.

Nguyen *et al.* (13) found that TGFB1 induces growth inhibition by arresting cells in the G1 phase of the cell cycle, and that oval cells are less sensitive to TGFB1 induced growth inhibition than hepatocytes. They suggested a reciprocal relationship between oval cells and hepatocytes proliferation and in the presence of TGFB1, inhibition of hepatocytes proliferation could impair the regenerative response to liver injury. Thenappen *et al.* (41) concluded that TGFB1signaling plays a critical role in hepatic proliferation and transitional phenotype and its loss is associated with activation of hepatic progenitor cells.

In the present study, in CH, there was insignificant increase in OV6, tissue AFP was negative, while HGF & TGFB1 showed a significant increase, which may indicate repopulation of the liver by proliferation of hepatocytes rather than oval cells at this stage. In LC, the rising of OV6 & tissue AFP was insignificant with the significant increase in HGF and TGFB1, concomitant with disturbed liver architecture, fibrosis and impaired liver functions which; may be related among other factors to the bipotential property of oval cells differentiating into

both hepatocytes and bile ductules. Lysy *et al.* (42) stated that there was no evidence of strong liver repopulation level by stem cells in animal modules.According to Chen *et al.* (32), although hepatic stem cells are activated and can differentiate into hepatic oval cells and transient hepatocyte-like cells, ultimately, it is not clear why the diseased liver can not be regenerated completely. They speculate that the toxins released from hepatocytes necrosis inhibit not only the proliferation and regeneration of residual hepatocytes but also the differentiation and evolution of hepatic oval cells toward hepatocytes.
According to Kung and Forbes (43), whatever the etiological cause of chronic liver disease, liver injury usually results in a form of excess scarring termed liver cirrhosis where the liver synthetic and metabolic function is compromised and there is also increased risk of developing liver cancer.

In our study of cases with HCC, tissue AFP & OV6 showed a highly significant increase (p<0.001) relative to control, to CH and to LC groups. Lowes *et al.* (31) suggested that oval cells proliferation is not disease specific but occurs in response to progressive liver injury and fibrosis. The association between severity of liver disease and increasing number of oval cells is consistent with the hypothesis that oval cells proliferation is associated with increasing risk of or development of HCC with advancing liver disease. Dumble *et al.* (7) stated that oval cells might give rise to HCC as a result of the arrest of stem cell maturation. There are two major nonexclusive hypothesis of the cellular origin of cancer from stem cells; due to maturation arrest or from de-differentiation of mature cells (5). Liver progenitor cells (LPCs) are activated in severe or chronic liver injury, particularly when mature hepatocytes replication is impaired. These LPCs have also been implicated in the development of liver tumors (42). According to Leverero (44) increasing experimental evidence suggests that HCV contributes to HCC by directly modulating pathways that promote the malignant transformation of hepatocytes. In a number of animal modules of hepatocarcinogenesis, the development of HCC is preceded by oval cell activation and the HCC in these animal modules express oval cell markers such as OV6 & AFP. This indicates that oval cells represent a potential target cell population for hepatocarcinogenesis (26). Reya *et al.* (45) stated that stem cells have the highest potential for proliferation and a much longer life span compared with their progeny and therefore have a greater opportunity to accumulate genetic mutation. According to Fujioka *et al.* (46), AFP expression was not found in well differentiated HCCs and tissue AFP positive cases are suggested to be biologically more malignant than HCCs that are AFP negative.

In the present study, HGF showed significant increase in HCC compared to control cases and to CH group. Yoshinaga *et al.* (36) stated that HGF has not been found immunocytochemically in normal liver without cirrhosis or chronic hepatitis.

According to Ljubimova *et al.* (37), immunohistochemical data correlated with PCR results regarding the overexpression of HGF/c-met system in HCC. Immuno flourescence data were compatible with the existence of two mechanisms of HGF in HCC, the first one is normal or paracrine HGF production by sinusoidal, Ito and Kupffer cells. The second one is abnormal "additional" or autocrine HGF production, possibly by epithelial (bile duct) and parenchymal (hepatocytes) in some cirrhotic cases and especially in HCC.

Yamagami *et al.* (9) showed that HGF in patients with HCC showed little localization in cancer cells, but was noted in infiltrating mesenchymal cells in both cancerous and non cancerous regions. Their results suggest that high serum levels of HGF revealed higher carcinogenic states in liver in patients with type C, chronic hepatitis and liver cirrhosis.

TGFB1 family protein have also emerged as key players in promoting the growth of stem cells in their un-differentiated state (47). Amin and Mishra (15) hypothesized that the interruption of TGFB1 pathway resulted in HCC through disruption of a normal pattern of cellular differentiation by hepatic progenitor /stem cells. Dong *et al.* (12) reported that the positive rate of TGFB1 expression in HCC samples was significantly higher than that in the surrounding tissue. There is also growing evidence that TGFB signaling proteins play a role in both maintenance of progenitor cells in their undifferentiated state and in the initiation of differentiation (41). According to Bissell (17); it is well established that TGFb drives fibrosis, however its impact on the initiation or progression of neoplasia is controversial.

5. Conclusion:

In the present, there was an insignificant increase in OV6 expression from CH to LC to be significant in HCC. Tissue AFP showed positivity in cirrhosis and high expression in HCC. The increase in HGF was more in LC and HCC than CH.TGFB1 showed progressive high expression from CH to LC to HCC. Further studies are needed in search of factors that may hinder differentiation of oval cells to functional hepatocytes in liver cirrhosis and may lead to malignant transformation.

References

1. Abdel-Wahab MF, Zakaria S, Kamel M, Abdel-Khaliq MK, Mabrouk MA, Salama H, Esmat G, Thomas DL, Strickland GT. High seroprevalence of hepatitis C infection among risk groups in Egypt. Am J Trop Med Hyg 1994 51(5):563-567.
2. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Aly Ohn ES, Anwar W, Sallam I. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000 11;355(9207):887-891.
3. Yannaki E, Athanasiou E, Xagorari A, Constantinou V, Batsis I, Kaloyannidis P, Proya E, Anagnostopoulos A, Fassas A. G-CSF-primed hematopoietic stem cells or G-CSF per se accelerate recovery and improve survival after liver injury, predominantly by promoting endogenous repairs programs. Exp Hematol 2005;33(1):108-19.
4. Fausto N. Liver regeneration. J Hepatol 2000;32 (1 Suppl):19-31.
5. Zheng JF, Liang LJ. Transplanted bone marrow stromal cells are not cellular origin of hepatocellular carcinomas in a mouse model of carcinogenesis. World J Gastroenterol 2008 21;14(19):3015-20.
6. Kofman AV, Morgan G, Kirschenbaum A, Osbeck J, Hussain M, Swenson S, Theise ND.Dose- and time-dependent oval cell reaction in acetaminophen-induced murine liver injury. Hepatology 2005;41(6):1252-61.
7. Dumble ML, Knight B, Quail EA, Yeoh GC.Hepatoblast-like cells populate the adult p53 knockout mouse liver: evidence for a hyperproliferative maturation-arrested stem cell compartment. Cell Growth Differ 2001;12 (5):223-31.
8. Lee TK, Castilho A, Ma S, Ng IO. Liver cancer stem cells: implications for a new therapeutic target. Liver Int 2009; 29(7):955-65.
9. Yamagami H, Moriyama M, Tanaka N, Arakawa Y. Detection of serum and intrahepatic human hepatocyte growth factor in patients with type C liver diseases. Intervirology 2001;44(1):36-42.
10. Suzuki A, Iwama A, Miyashita H, Nakauchi H, Taniguchi H.Role for growth factors and extracellular matrix in controlling differentiation of prospectively isolated hepatic stem cells. Development 2003; 130(11):2513-24.
11. Luo YQ, Wu MC, Cong WM. Gene expression of hepatocyte growth factor and its receptor in HCC and non tumorous liver tissues. World J Gastroenterol 1999; 5(2):119-121.
12. Dong ZZ, Yao DF, Yao M, Qiu LW, Zong L, Wu W, Wu XH, Yao DB, Meng XY.Clinical impact of plasma TGF-beta1 and circulating TGF-beta1 mRNA in diagnosis of hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 2008;7(3):288-95.
13. Nguyen LN, Furuya MH, Wolfraim LA, Nguyen AP, Holdren MS, Campbell JS, Knight B, Yeoh GC, Fausto N, Parks WT. Transforming growth factor-beta differentially regulates oval cell and hepatocyte proliferation. Hepatology 2007;45(1):31-41.
14. Alison MR, Islam S, Lim S.Stem cells in liver regeneration, fibrosis and cancer: the good, the bad and the ugly. J Pathol 2009;217(2):282-98.
15. Amin R, Mishra L. Liver stem cells and TGF-Beta in hepatic carcinogenesis. Gastrointest Cancer Res. 2008; 2(4 Suppl):S27-30.
16. Mishra L, Shetty K, Tang Y, Stuart A, Byers SW The role of TGF-beta and Wnt signaling in gastrointestinal stem cells and cancer. Oncogene 2005 29; 24(37):5775-89.
17. Bissell DM. Chronic liver injury, TGF-beta, and cancer. Exp Mol Med 2001 31;33(4):179-90.
18. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ.Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19(6):1513-20.
19. Knodell RG, Ishak KG, Black WC, Chen TS, Criag R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981; 1: 43-435.
20. Edmondson H and Steiner P. Primary carcinoma of the liver: A study of 100 cases among 48,900 necropsies. Cancer 1956; 7:462–503.
21. Hsu SM and Raine L (1981). Protein A, avidin and biotin in immunohistochemistry. J Histochem Cytochem 29: 1349-1353.
22. Crosby HA, Hubscher SG, Joplin RE, Kelly DA, Strain AJ. Immuno-localization of OV-6, a putative progenitor cell marker in human fetal and diseased pediatric liver. Hepatology 1998; 28(4):980-5.
23. Hasib AN, Abaza H and Shawky S. Role of serum and tissue alpha-fetoprotein in differentiation between primary and secondary hepatic malignancy. Egyptian Journal of Biomedical Sciences 1998; 1: 147-153.
24. Inoue H, Yokoyama F, Kita Y, Yoshiji H, Tsujimoto T, Deguchi A, Nakai S, Morishita A, Uchida N, Masaki T, Watanabe S, Kuriyama S. Relationship between the proliferative capability of hepatocytes and the intrahepatic expression of hepatocyte growth factor and c-Met in the course

of cirrhosis development in rats. J Mol Med 2006; 17:857-64.
25. Kim CK, Lim JH, Lee WJ. Detection of hepatocellular carcinomas and dysplastic nodules in cirrhotic liver: accuracy of ultrasonography in transplant patients. J Ultrasound Med 2001;20(2):99-104.
26. Libbrecht L, Roskams T. Hepatic progenitor cells in human liver diseases. Semin Cell Dev Biol 2002;13(6):389-96.
27. Fausto N, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. Mech Dev 2003;120 (1):117-30.
28. Roskams T, De Vos R, Van Eyken P, Myazaki H, Van Damme B, Desmet V.Hepatic OV-6 expression in human liver disease and rat experiments: evidence for hepatic progenitor cells in man. J Hepatol 1998;29(3):455-63.
29. Fausto N, Lemire JM, Shiojiri N. Cell lineages in hepatic development and the identification of progenitor cells in normal and injured liver. Proc Soc Exp Biol Med 1993 ; 204(3):237-41.
30. Fausto N. Liver regeneration and repair: Hepatocytes, progenitor cells, and stem cells. Hepatology 2004; 39 (6): 1477-1487.
31. Lowes KN, Brennan BA, Yeoh GC, Olynyk JK. Oval cell numbers in human chronic liver diseases are directly related to disease severity. Am J Pathol 1999; 154(2):537-41.
32. Chen YK, Zhao XX, Li JG, Lang S, Wang YM Ductular proliferation in liver tissues with severe chronic hepatitis B: an immunohistochemical study. World J Gastroenterol 2006 7; 12(9):1443-6.
33. Clouston AD, Powell EE, Walsh MJ, Richardson MM, Demetris AJ, Jonsson JR. Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. Hepatology 2005;41(4):809-1
34. Shafritz DA, Dabeva MD. Liver stem cells and model systems for liver repopulation. J Hepatol 2002; 36(4):552-64.
35. Zheng YW, Taniguchi H. Diversity of hepatic stem cells in the fetal and adult liver. Semin Liver Dis 2003; 23(4):337-48.
36. Yoshinaga Y, Matsuno Y, Fujita S, Nakamura T, Kikuchi M, Shimosato Y, Hirohashi S. Immunohistochemical detection of hepatocyte growth factor/scatter factor in human cancerous and inflammatory lesions of various organs. Jpn J Cancer Res 1993;84(11):1150-8.
37. Ljubimova JY, Petrovic LM, Wilson SE, Geller SA, Demetriou AA. Expression of HGF, its receptor c-met, c-myc, and albumin in cirrhotic and neoplastic human liver tissue. J Histochem Cytochem. 1997;45(1):79-87.
38. Ishiki Y, Ohnishi H, Muto Y, Matsumoto K, Nakamura T. Direct evidence that hepatocyte growth factor is a hepatotrophic factor for liver regeneration and has a potent antihepatitis effect in vivo. Hepatology 1992;16(5):1227-35.
39. Falkowski O, An HJ, Ianus IA, Chiriboga L, Yee H, West AB, Theise ND. Regeneration of hepatocyte 'buds' in cirrhosis from intrabiliary stem cells. J Hepatol 2003;39(3):357-64.
40. Okano GI, Shiota G, Kawasaki H. Expression of hepatocytes growth factor HGF &HGF receptor (C-met)protein in liver diseases; an immuno-histochemical study. Liver international; 19(2).
41. Thenappan A, Li Y, Kitisin K, Rashid A, Shetty K, Johnson L, Mishra L. Role of transforming growth factor beta signaling and expansion of progenitor cells in regenerating liver. Hepatology 2010;51(4):1373-82.
42. Lysy PA, Campard D, Smets F, Najimi M, Sokal EM. Stem cells for liver tissue repair: current knowledge and perspectives. World J Gastroenterol 2008 14;14(6):864-75.
43. Kung JW, Forbes SJ. Stem cells and liver repair. Curr Opin Biotechnol 2009 ;20(5):568-74.
44. Levrero M. Viral hepatitis and liver cancer: the case of hepatitis C. Oncogene 2006 26;25(27):3834-47.
45. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001 1;414(6859):105-11.
46. Fujioka M, Nakashima Y, Nakashima O, Kojiro M. Immunohistologic study on the expressions of alpha-fetoprotein and protein induced by vitamin K absence or antagonist II in surgically resected small hepatocellular carcinoma. Hepatology 2001;34 (6):1128-34.
47. Lee TK, Castilho A, Ma S, Ng IO. Liver cancer stem cell, Implications for a new therapeutic target. Liver cancer stem cells: implications for a new therapeutic target. Liver Int 2009;29(7):955-65.