Micro Vascular Density MVD-CD34 and VEGF Expression in the Liver of Patients with Chronic Hepatitis, Cirrhosis and Hepatocellular Carcinoma

Olfat Ali Hammam[*1], Nawal El Badrawy[2], Maged El Ghanam[2], Moataz Hassan[2], Wael Safwat[2], Amgad Anas[2]

Departments of Pathology[1] and Hepato-Gasteroenterology[2], Theodor Bilharz Research Institute, Ministry of Scientific Research, El-Nil Street, Warrak El-Hadar, P.O. Box 30, Imbaba 12411, Cairo, Egypt.
[*]totoali1@hotmail.com

Abstract: *Purpose:* The aim of this work is to study angiogenesis of the liver of patients with chronic hepatitis, liver cirrhosis and hepatocellular carcinoma by assessment of mirovessel density MVD-CD34 and VEGF in liver tissue. *Method*: Sixty patients with chronic liver disease and 30 patients with hepatocellular carcinoma (HCC) were subjected to clinical examination, laboratory investigations for hepatitis C, liver function tests and ultrasonography. Liver biopsy was performed for histopathological examination. They were 3 groups: chronic hepatitis [CH] (35), liver cirrhosis [LC] (25) and HCC (30) and ten healthy volunteers as controls with negative serological markers for hepatitis (C&B), HCC were 7 with grade I, 14 with grade II and 9 with grade III. Immunohistochemical staining for tissue vascular endothelial growth factor (VEGF) was done and MVD-CD34 was assessed by Image Analysis System. *Results*: In normal liver tissue specimen, both CD34 & VEGF were negative. MVD-CD34 was increased significantly from CH to LC to HCC and increased significantly in grade I to II to III HCC. VEGF showed a significant increase in LC compared to CH & insignificant in mean HCC compared to LC. With differentiation of HCC, MVD-CD34 increased for grade I, to II to GIII ($p< 0.01$). VEGF showed a higher expression in grade I and decreased in grade II& III. *Conclusion*: MVD-CD34 is more responsible of angiogenesis than VEGF with progression of chronic liver disease to HCC. Further research for other factors mediating and cells contributing to angiogenesis is needed, which may have therapeutic implications in the control of chronic liver disease and HCC.
[Olfat Ali Hammam[*1], Nawal El Badrawy[2], Maged El Ghanam[2], Moataz Hassan[2], Wael Safwat[2], Amgad Anas[2]. Micro Vascular Density MVD-CD34 and VEGF Expression in the Liver in Patients with Chronic Hepatitis, Cirrhosis and Hepatocellular Carcinoma. Journal of American Science 2011;7(6):808-815].(ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Vascular endothelial growth factor (VEGF) - Immunohistochemistry (IHC)- Hepatocellular carcinoma (HCC)-Liver cirrhosis (LC)-Hepatitis C virus (HCV)-Hepatitis B virus (HBV).

1. Introduction:

Chronic viral Hepatitis infections including the hepatitis B virus (HBV) and hepatitis C Virus (HCV) are major risk factors contributing to HCC development [1].

Whatever the etiologic 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 one compromised and there is also an increased risk of developing liver cancer [2]. With progression of the scarring process, the endothelial lining of the sinusoids undergoes conversion to a non fenestrated cell leading to an appearance which has been termed" capillarization" of sinusoids [3]. In liver, resting endothelial cells rarely proliferate under physiologic conditions. When they undergo a process called "sinusoidal capillarization", these endothelial cells form tight junctions along with deposition of extracellular matrix followed by a new vessel formation [4].

According to Tosh and Strain [5], there are two reasonably well defined types of stem cells in the bone marrow. The hematopoeitic stem cells (HSC) and the mesenchymal stem cells. The HSC is the precursor for the lymphoid and the myeloid cells of the blood and they are quite well characterized and have been isolated from humans as cells with a CD34 +ve phenotype. According to Fausto [6] oval cells can be induced to proliferate under different pathological conditions. Herrera et al. [7] isolated and characterized a population of human liver stem cells (HLSCs) which in culture supplements with VEGF, expressed the endothelial marker CD34 and Lee et al. [8] stated oval cells express some of the antigens of hematopoeitic cells such as CD34.

Poon et al. [9] used CD34 as an endothelial cell marker and Amarapurkar et al. [10] used it in normal liver, cirrhosis and HCC. CD34 has been widely used for the assessment of sinusoidal like neo-angiogenesis in HCC. Amongst, viral infection, HCV has been demonstrated to show more angiogenesis and has been suggested to represent a risk factor for HCC in patients with chronic HCV [11]. Furthermore high expression of CD34 positive sinusoidal endothelial cells is a risk factor for HCC in patients with HCV associated chronic liver disease [12].

VEGF is a known marker of angiogenesis [13]. It is thought to be a selective mitogen for endothelial cells. It acts as a link between angiogenesis, immune system and tissue re-modeling

[14]. It was found that VEGF secreted by replicating hepatocytes induces sinousoial endothelial cell proliferation during regeneration after partial hepatectomy in rates [15]. Greene et al. [16] in their studies on hepatic regenerative process suggested that the regulation of angiogenesis controls the regenerative process. According to Amarapurkar et al. [17] understanding the process of angiogenesis is of great help in developing new therapeutic approach for the chronic liver disease patients; and interfering with angiogenesis may be a potential target to avoid progression of liver disease.

This paper aims to study angiogenesis of the liver assessed by MVD-CD34 and VEGF expression in liver biopsy in patients with chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.

Materials and Methods

Patients

Ninety patients (66 males and 24 females); were the subject of this study. Patients were admitted to the Department of Gastroenterology and Hepatology, Theodor Bilharz Research Institute (TBRI), Cairo, Egypt. They included 35 cases of chronic hepatitis C virus infection (CH), 25 cases with cirrhosis and 30 cases of HCC, all with HCV infection. Of the HCC patients seven patients were grade I, 14 were grade II and nine were grade III. The presence of HCV-RNA in patient's sera was detected by real-time polymerase chain reaction. All patients were subjected to thorough clinical examination, urine and stool analysis, liver function tests, ultrasonography and liver biopsy for histopathologic and immuno-histochemical 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 taken 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 or C viruses.

Liver biopsies were fixed in 10% buffered formalin for 24 hours, and then processed in ascending grades of ethyl alcohol; xylene, wax and paraffin blocks were prepared. 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 evaluating the histopathological and basic classification of cases. Five histological features have been observed to be relatively characteristic of (although not pathognomonic for) chronic hepatitis: (1) lymphoid aggregates in portal tracts, (2) degenerative injury type changes of bile ducts, (3) large droplet steatosis, (4) Mallory body-like material within injured hepatocytes, and (5) Lymphocytic aggregates within the lobules [18-19].They were evaluated on a five point scale, using 20 random fields at x100 and x400 magnification per slide. Architectural changes, fibrosis and cirrhosis were evaluated on a seven point scale according to Knodell score system [19]. HCC cases were classified into 3 grades (I, II, III) well, moderately, and poorly differentiated tumors [20]. Other liver sections (4µm) were cut on slides, which were treated with TESPA (3-aminopropyl-triethoxysilane, Sigma) for immunohistochemistry (IHC).

Immunohistochemistry for Detection of tissue CD34 and VEGF antigens

Immuno-histochemical reaction was performed using an avidin biotin complex (ABC) immunoperoxidase technique according to Hsu and Raine [21] using anti human CD34, and VEGF antibodies on paraffin sections, which were 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 CD34, (purchased from R&D, USA), and VEGF (purchased from Santa Cruz Biotechnology Inc.; Santa Cruz, USA) monoclonal antibody, diluted at 1:50,150 respectively in BPS. Next day, after thorough washing in PBS, the sections were incubated with alkaline phosphatase for both VEGF and tissue CD34. Staining is completed by 5-10 minutes incubation with alkaline phosphatase + substrate - chromogen which results in a pink-red colored precipitate at the antigen site for VEGF and tissue CD34 (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.

Determination of microvascular density (MVD)

MVD in tissue sections was evaluated according to Gasparini's criteria [22] by independent observer who was blinded to the patients'

clinicopathologic data. At low power field (x 40), the tissue sections were screened and five areas with the most intense neovascularization (hot spots) were selected.

Microvessel counts of these areas were performed at high power field (x 200). To reduce observer-related variation, counting of the microvessels was performed with a Computer Image Analyzer (Kontrone Image Analysis System, Germany).This essentially consists of a computer controlled microscope (Zeiss axioskope), video camera, two monitors, video printer, computer unit (P.C., IBM compatible ,486Dx100) and a desk jet colored printer 560C. Image analysis was performed using the soft ware program; KS 400. The image analyzer is an integrated system of Windows-based software specially designed for immunohistochemical analysis. Any brown stained endothelial cell or endothelial cell cluster that was clearly separated from adjacent microvessels, tumor cells, and connective tissue elements was counted as one microvessel, irrespective of the presence of a vessel lumen. The image analyzer allowed the operator to select stained microvessels and make a subtraction of the background; an automated microvessel count per field was computed in each hot spot. The mean microvessel count of the five most vascular areas was taken as the MVD, which was expressed as the absolute number of microvessels per 0.74 mm2 (x 200 field). Zero% was given to unstained sections.

Immunohistochemical scoring of VEGF

VEGF was expressed as cytoplasmic pink red color of hepatocytes and endothelial cells lining blood vessels and counting the number of positive cells in 5 microscopic fields, with power of magnification (x 400) according to Shimizu et al. and Takahashi [15, 23].

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.

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 without liver disease as control group. They were (4) males and (6) females, their age ranged (30-45 years) with a mean of 39.4±3.7years. Clinical and biochemical data of the studied groups are shown in Table 1.

Table 1 Clinical and biochemical data of studied groups

Variables	CH (n=35) No. of positive cases %	LC (n=25) No. of positive cases %	HCC(n=30) No. of positive cases%	Control (n=10) No. of positive cases %
Jaundice	10 29..9	15 60	10 33.3	0 00
Lower limb edema	5 14.3	16 64	5 16.6	0 00
Hepatomegaly	12 34.2	0 00	5 16.6	0 00
Splenomegaly	6 17.1	11 44	0 00	0 00
ALT (0-41) U/L **Mean ± SD**	53.95 ± 29.16 [a,b]	45.8 ± 31.91 [a]	36.87 ± 21.5	19.8 ± 5.59
AST (0-38) U/L **Mean ± SD**	38.3 ± 17.33 [b]	69.8 ± 16.15 [a,c]	72.9 ± 34.75 [a]	20.2 ± 5.87
S. albumin (3.5-5) gm/dl **Mean ± SD**	4.46 ± 0.4 [b]	3.62 ± 0.22 [a,b,c]	3.28 ± 0.54 [a]	4.22 ± 0.18

CH: Chronic hepatitis LC: Liver cirrhosis HCC: Hepatocellular carcinoma

[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 <0.01 relative to the HCC group
[c]: p value <0.01 relative to the CH group

In cases of CH & LC CD 34 staining were cofind to large vessels in the portal tracts. In cases of HCC on top of cirrhosis the density of microvessels was higher in the peripheral tumor tissue close to the margin than in the central areas.

By estimating MVD-CD34 by image analysis system (Table 2) positivity was not found in normal control specimens. Twenty percent of CH , 40 % of LC and 100% of HCC were positive, with grades I,II,III. In CH, the mean MVD-CD34 was (15.66±3.44/0.74 mm2), LC (33.63±7.60/0.74 mm2). Specific staining of capillary-like vessels by anti-CD34 antibody was observed in all tumor specimens and between cancer cells (166.77/0.74 mm2). MVD-CD34 in HCC cases was higher than CH and LC groups (P < 0.01). There was significant difference in MVD-CD34 between LC and CH liver (P < 0.05) (Table 2, Fig. 1A- B).

There is statistically significant difference between MVD-CD34 expression levels in HCC grades III (mean MVD-CD34, 114.7±46.83 /0.74 mm2) and HCC grade I (mean MVD-CD34, 64.62±24.86/0.74 mm2) and II (mean MVD-CD34, 91.71±18.29 /0.74 mm2) (at a p <0.01) and no significant difference in HCC grade III relative to HCC grade II at p = 0.129, There was a positive correlation between mean MVD-CD34 and grades of HCC (r=0.325, (p< 0.01) (Table 2, Fig. 2A- B).

The results of VEGF expression are shown in table 2 , all control were negative, 42.8% of CH cases were positive, 80% of LC cases were positive , 100% of HCC were positive .VEGF was mainly seen in the cytoplasm of hepatocytes as intracytoplasmic granules in all tumor cells or periportal hepatocytes, VEGF showed a significant increase (p< 0.05) in CH group relative to control, a significant increase (p< 0.01) in LC & HCC groups related to control and (p< 0.01) compared to CH (Table 3, Fig. 3 A-B).

There is statistically significant difference in VEGF expression levels in HCC grade I (65± 14.4) compared to grade II (25.2±10.5) and grade III (35± 3.9) at a p < 0.01, being lower in grade II &III than grade I HCC. (Table 3, Fig. 4A- B).

There was an inverse correlation between mean VEGF and grades of HCC (r=0.295, (p< 0.01).

Table 2 MVD-CD34 expression levels in studied groups and in different grades of HCC.

Variable	CD34	
	Number of Positive cases for CD34	MVD μ^2/ five microscopic fields Mean ± SD
Control (n=10)	0/10	0.0±0.0
CH (n=35)	7/35	15.66±3.44 [a]
LC (n=25)	10/25	33.63±7.60 [a c]
HCC (n=30)	30/30	166.77±77.29 [b c d]
Grade I (n=7)	7/7	64.62±24.86
Grade II (n=14)	14/14	91.71±18.29 [e]
Grade III (n=9)	9/9	114.7±46.83 [e, f]:

CH: Chronic hepatitis LC: Liver cirrhosis HCC: Hepatocellular carcinoma

[a]: p value <0.05, [b]: p value <0.001 relative to the control group respectively.

[c]: p value <0.05 , [d]: p value <0.01 relative to the CH group. [d]: p value <0.01 relative to the LC group.

[e]: p value <0.01 relative to grade I [f]: p value <0.01 relative to grade II

Table 3 VEGF expression levels in studied groups and in different grades of HCC.

Variable	VEGF	
	Number of Positive cases for VEGF	Percentage of positive area/ five microscopic fields Mean ± SD
Control (n=10)	0/10	0.0±0.0
CH (n=35)	15/35	16.3±1.21[a]
LC (n=25)	20/25	40. 3 ± 7.02 [b, c]
HCC (n=30)	30/30	47.5 ± 13.2 [b, c]
Grade I (n=7)	4/7	65± 14.4
Grade II (n=14)	14/14	25.2±10.5 [e]
Grade III (n=9)	9/9	35± 3.9 [e, f]

CH: Chronic hepatitis LC: Liver cirrhosis HCC: Hepatocellular carcinoma

[a]: p value <0.05, [b]: p value <0.001 relative to the control group respectively.

[c]: p value <0.01 relative to the CH group. [d]: p value <0.01 relative to the LC group.

[e]: p value <0.01 relative to grade I [f]: p value <0.01 relative to grade II

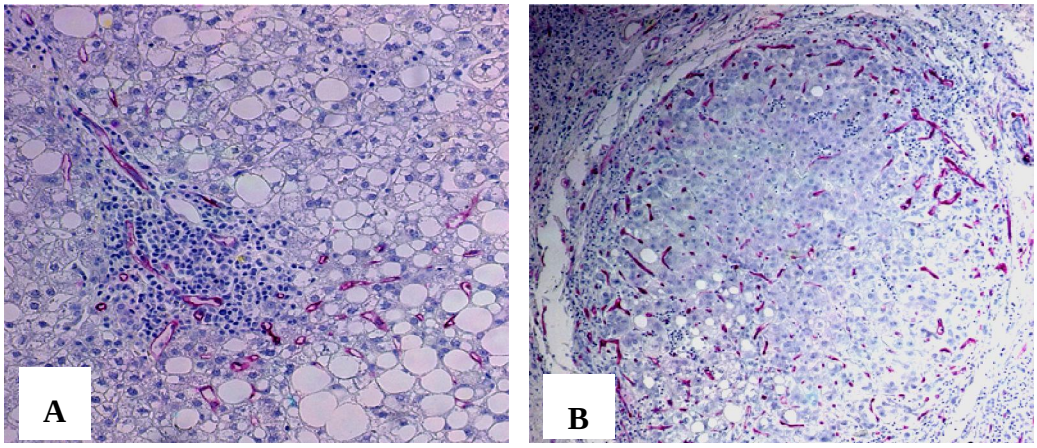

Fig. 1A) A case of chronic HCV, showing few blood vessels stained with CD34 antibody. B) A case of HCV with cirrhosis (cirrhotic nodule) showing moderate number of blood vessels stained with CD34 (IHC for CD34, alkaline phosphatase, X200, X100 respectively).

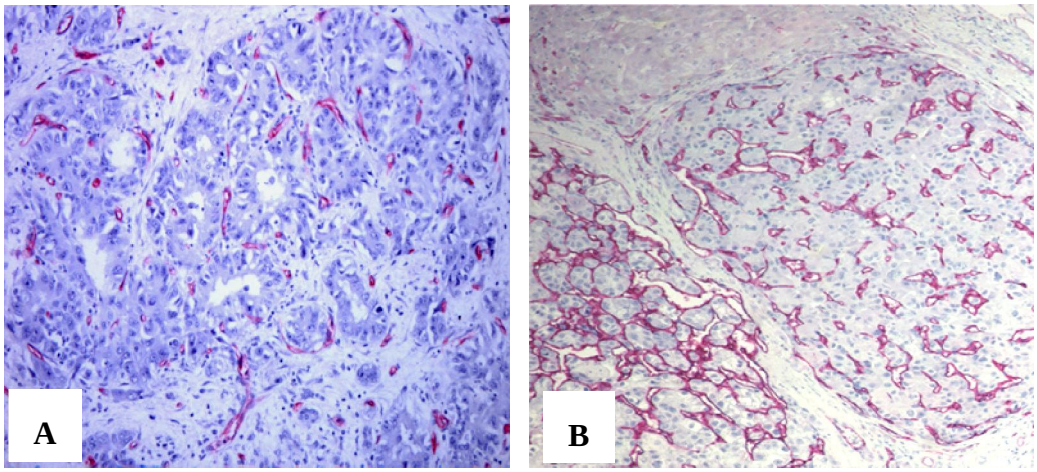

Fig. 2 A) A case of well differentiated HCC showing moderate number of blood vessls expressing CD34 antibody. B) A case of poorly differentiated HCC showing increased (intense) expression for CD34 antibody (IHC for CD34, alkaline phosphatase X 200).

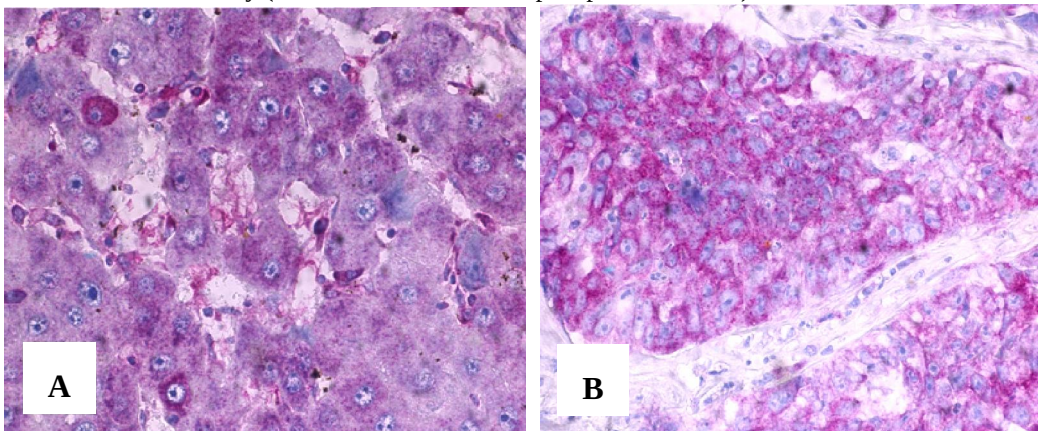

Fig. 3 A) A case of chronic HCV, showing mild VEGF expression, cytoplasmic, stain sparing the nuclei B) A case of chronic HCV with active cirrhotic changes, showing moderate VEGF expression (IHC for VEGF, alkaline phosphatase X 200).

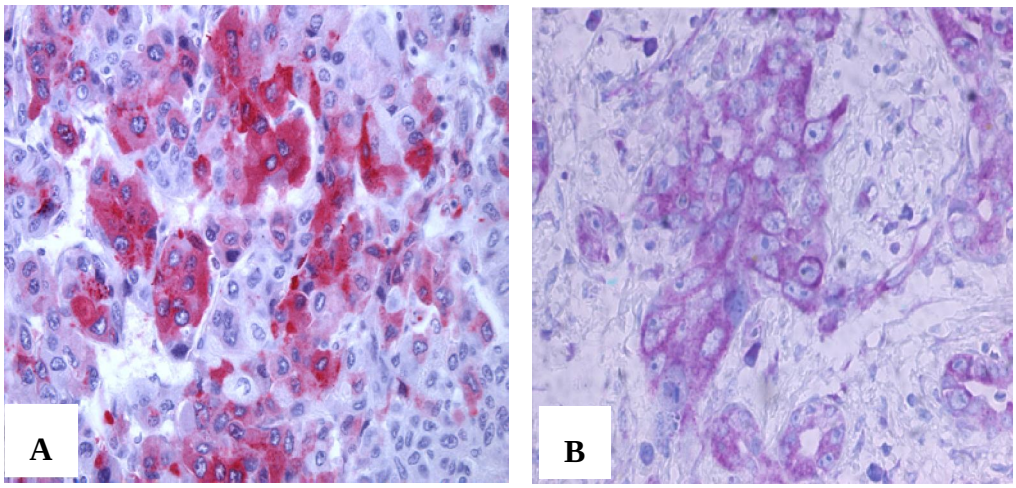

Fig. 4 A) A case of well differentiated HCC showing intense expression of VEGF. B) A case of poorly differentiated HCC showing weak, scattered hepatocytes stained for VEGF (IHC for VEGF, alkaline phosphatase, X 200)

4. Discussion

In our study MVD-CD34 staining was not detected in normal liver biopsies. It was significantly increased ($p<0.05$) in liver cirrhosis (LC) relative to control. In HCC, a highly significant increase ($p<0.01$) was found relative to control and to LC cases. VEGF, showed negative staining in control cases, a significant increase in CH ($p<0.05$) relative to control, a significant increase ($p<0.01$) in LC and HCC groups relative to control.

Park et al. [24] found that there was a gradual increase in CD34 expression from cirrhotic nodules to dysplastic nodules to HCC. Kim & Hu [14] by immunohistochemistry staining using anti CD34 and anti VEGF antibodies; found that CD34 was reactive throughout the neoplastic tissue, albeit it was confined to a few periportal sinusoids and vessels in fibrous septa of adjacent cirrhotic liver. Expression of VEGF which was localized to the cytoplasm was not correlated with all clinicopathological parameters. CD34 was closely associated with neo-vascular process in cirrhosis and hepatocellular carcinoma. Di Carlo et al. [25], studied by immunohistochemistry, the expression and distribution of CD34 in chronic liver diseases and in HCC. They found that the sinusoids of the liver showed no or focal immunoreactivity for CD34, an increased immunoreactivity was observed in the periportal sinusoids of cirrhotic nodules, whereas diffuse and strong staining was in overall HCC. They concluded that immunoreactivity for CD34 represents an effective method to evaluate angiogenesis. Ma Jee et al. [26] concluded that CD34 is a useful marker for distinguishing HCC from non cancerous liver tissue; as CD34 was not present along the sinusoidal wall in normal human liver, was weakly present in the perinodules in few cases of cirrhosis, and HCC showed a diffuse capillarization with over expression. Cui et al. [27] found enhanced CD34 expression of sinusoidal like vascular endothelial cells in HCC.

In our study, VEGF showed higher expression in CH and LC but no significant difference in HCC group from LC groups. Yang et al. [13], by Immunohistochemical staining , found that CD34 was expressed in the vascular endothelial cells of normal liver , paracarcinomatous tissue and HCC tissue in the following proportions of specimen; 86.7% , 93.8% and 100 % respectively . However, they found expression of CD34 in both normal and tumor tissue indicating that it was not a reliable marker for HCC.

According to Saaristo et al. [28] VEGF is a cell specific mitogen and is a major inducer of angiogenesis in human cancers. It was found by Shimizu et al. [15] that VEGF served by replicating hepatocytes induces sinusoidal endothelial cell proliferation and that a peak of hepatocytes production of VEGF, an endothelial mitogen, correspond to an increase of VEGF receptor expression on endothelial cells after partial hepatectomy in rats. Yoshiji et al. [29] have also shown that VEGF expression increases significantly during fibrogenesis and carcinogenesis and that the combined effect of VEGF & its receptor reflect the combined effect of both on hepatic stellate cells and endothelial cells. Also in the study by Amarapukar et al. [17] none of cases with normal histology was CD34 or VEGF positive. Angiogenesis was positive in 45.5% of cases of chronic liver diseases and was proportional to the stage of fibrosis. VEGF expression was commonly found in early stages of fibrosis and increased significantly during

fibrogenesis and carcinogenesis. They also found a significant high expression of CD34 in HCC as compared to chronic liver diseases.

In the study by Amarapukar et al. [10] by Immunohistochemical staining; CD34 positive staining was taking as any cell that stained brown with a dotty, linear, semi-circular or circular pattern and was clearly separate from an adjacent one.They found over expression of endothelial marker CD34 with gradual progression from normal liver to cirrhosis to HCC& metastasis.

Brdosky et al. [30] found that fibrotic stroma surrounding cirrhotic nodules has an increased number of vessels not only compared to the cirrhotic nodule but to the normal liver parenchyma as well. They also found that vascular density in the areas of HCC and internodular fibrotic tissue in cirrhotic liver was significantly higher than in non cirrhotic parenchyma. Additionally cirrhotic nodules were characterized by significantly lower vascularization compared with normal liver which may lead to diminished cell function.

The study by Namashima et al. [31] using CD34 staining for MVD-CD34, concluded that MVD representing tumor angiogenesis offers a new candidate prognostic factor in HCC to predict tumor recurrence and patient survival, in addition to traditional biological factors.

Cui et al. [27] have shown moderate to diffuse positivity for CD34 in the majority of well differentiated HCC. Park et al. [24] studied CD34 in HCC and found that 14 out of 21 cases of moderate to poorly differentiated HCC showed strong positivity, while it was seen in three out of 11 cases of well differentiated HCC. The importance of neovascularization in the progress of HCC has been high lightened suggesting that microvessels increase gradually from cirrhotic nodules through low grade and high grade dysplastic nodules with the greatest number recorded in HCC.

According to Yang et al. [13] much attention has been paid to the association between angiogenesis and post-operative recurrence or metastasis. Ma Jee et al. [26] found that diffuse capillarization with overexpression of CD34, collagen IV and laminin are features of HCC. Frequent breaks in and loss and decrease of the basement membrane in poorly differentiated tumors and tumors with portal vein infiltration suggest a potential metastasis of tumor cells and may play a role in metastasis of HCC. They found a significant difference ($p<0.05$) in the expression of CD34 between the well differentiated and moderately differentiated HCC. Amarapukar et al. [10] suggested that agiogenesis as assessed by CD34 expression play an important role in carcinogenesis.

Kim & Hu [14] found that microvascular density in HCC is not directly correlated with VEGF expression, suggested that other angiogenic factors may be involved in neovascularization of HCC.

Piao et al. [32] found that MVD in HCC with metastasis, lower differentiation or without intact capsule was significantly higher than that in HCC with intact capsule, higher differentiation or without metastasis.

Brdosky et al. [30] found that in the early stages of fibrosis, the production of VEGF and the neovascularization increase, whereas in the late stages; cirrhotic nodules in hepatitis C patients are characterized by decreased density of micro-vasclature and that VEGF was higher in HCC and diminished in cirrhotic nodule, thus strongly correlating with the degree of vascularization.

Ivarone et al. [33] concluded that VEGF appears to be involved in the development of HCC and could be a predictor of HCC development in patients with cirrhosis. They found that angiogenic factors were equally expressed in any well differentiated & poorly differentiated tumors.

In conclusion, in the present study, MVD-CD34 increased in LC and in HCC ($p<0.01$) and in HCC from grade I to II to III ($p<0.05$). VEGF positivity increased in CH ($p<0.05$) and, in LC& HCC ($p<0.01$). In HCC higher expression was in grade I and lower in grade II &III. Therefore MVD-CD34 is more indicative of progression of CLD & HCC than VEGF is associated with angiogenesis. Further search for other cells contributing to and factors mediating angiogenesis is needed; which may have implication in providing therapeutic targets to control progress of chronic liver disease and malignancy.

Corresponding author
Olfat Ali Hammam
Departments of Pathology
Theodor Bilharz Research Institute, Ministry of Scientific Research, El-Nil Street, Warrak El-Hadar, P.O. Box 30, Imbaba 12411, Cairo, Egypt.
*totoali1@hotmail.com

References

1. Fattovich G, Stroffolini T, Zagni I, Donato F.Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004 ; 127(5 Suppl 1): S35-50
2. Kung JW, Forbes SJ. Stem cells and liver repair. Curr Opin Biotechnol 2009; 20:568-74
3. Schaffner F, Popper H. Morphologic Studies in Neonatal Cholestasis with Emphasis on Giant Cells. Ann N Y Acad Sci 1963 30; 111:358-374
4. Couvelard A, Scoazec JY, Feldmann G. Expression of cell-cell and cell-matrix adhesion proteins by sinusoidal

endothelial cells in the normal and cirrhotic human liver. Am J Pathol 1993; 143:738-52
5. Tosh D, Strain A. Liver stem cells prospects for clinical use. J Hepatol 2005; 42 Suppl :S75-84
6. Fausto N. Liver regeneration and repair: Hepatocytes, progenitor cells, and stem cells. Hepatology 2004; 39: 1477-1487
7. Herrera MB, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, Bussolati B, Camussi G. Isolation and characterization of a stem cell population from adult human liver. Stem Cells 2006 ;24 :2840-50
8. 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;29:955-65
9. Poon RT, Lau CP, Ho JW, Yu WC, Fan ST, Wong J. Tissue factor expression correlates with tumor angiogenesis and invasiveness in human hepatocellular carcinoma.Clin Cancer Res 2003 ;9:5339-4
10. Amarapurkar AD, Vibhav, Kim V. Angiogenesis in liver cirrhosis and hepatocellular carcinoma. Indian J Pathol Microbiol 2008; 5:323-8
11. Mazzanti R, Messerini L, Monsacchi L, Buzzelli G, Zignego AL, Foschi M, Monti M, Laffi G, Morbidelli L, Fantappié O, Bartoloni Saint Omer F, Ziche M. Chronic viral hepatitis induced by hepatitis C but not hepatitis B virus infection correlates with increased liver angiogenesis. Hepatology 1997;25 :229-234
12. Ohmori S, Shiraki K, Sugimoto K, Sakai T, Fujikawa K, Wagayama H, Takase K, Nakano T. High expression of CD34-positive sinusoidal endothelial cells is a risk factor for hepatocellular carcinoma in patients with HCV-associated chronic liver diseases. Hum Pathol 2001;32 :1363-70
13. Yang LY, Lu WQ, Huang GW, Wang W. Correlation between CD105 expression and postoperative recurrence and metastasis of hepatocellular carcinoma. BMC Cancer 2006 2;6:110
14. Kim YO, Hu R. Immunohistochemical expression of CD 34 and vascular endothelial growth factor in hepatocellular carcinoma. J Korean Cancer Ass 1999; 31(40):802-810
15. Shimizu H, Miyazaki M, Wakabayashi Y, Mitsuhashi N, Kato A, Ito H, Nakagawa K, Yoshidome H, Kataoka M, Nakajima N.Vascular endothelial growth factor secreted by replicating hepatocytes induces sinusoidal endothelial cell proliferation during regeneration after partial hepatectomy in rats. J Hepatol 2001; 34:683-689
16. Greene AK, Wiener S, Puder M, Yoshida A, Shi B, Perez-Atayde AR, Efstathiou JA, Holmgren L, Adamis AP, Rupnick M, Folkman J, O'Reilly MS. Endothelial directed hepatic regeneration after partial hepatectomy. Ann Surg 2003; 237:530-535
17. Amarapurkar AD, Amarapurkar DN, Vibhav S, Patel ND. Angiogenesis in chronic liver disease. Ann Hepatol 2007; 6:170-3
18. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ.Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994; 19 :1513-1520
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, Raine L. Protein A, avidin and biotin in immunohistochemistry. J Histochem Cytochem 1981; 29: 1349-1353
22. Gasparini G, Harris AL: Clinical importance of the determination of tumor angiogenesis in breast carcinoma: Much more than a new prognostic tool. J Clin Oncol 1995 13: 765-782
23. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995 15; 55:3964-8
24. Park YN, Yang CP, Fernandez GJ, Cubukcu O, Thung SN, Theise ND. Neoangiogenesis and sinusoidal "capillarization" in dysplastic nodules of the liver. Am J Surg Pathol 1998; 22:656-62
25. Di Carlo I, Fraggetta F, Lombardo R, Azzarello G, Vasquez E, Puleo S. CD 34 expression in chronic and neoplastic liver diseases. Panminerva Med 2002; 44:365-7
26. Ma Jee M, Xiaojun z, Taihe Z, guigin S, Kui M. Pathological observation of sinusoidal lining endothelial cells and the basement membrane in human hepatocellular carcinoma. Chinesa J of Digestive Diseases 2006; 2 : 83-87
27. Cui S, Hano H, Sakata A, Harada T, Liu T, Takai S, Ushigome S. Enhanced CD34 expression of sinusoid-like vascular endothelial cells in hepatocellular carcinoma. Pathol Int 1996; 46:751-6
28. Saaristo A, Karpanen T, Alitalo K. Mechanisms of angiogenesis and their use in the inhibition of tumor growth and metastasis. Oncogene 2000 11;19: 6122-9
29. Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Hicklin DJ, Wu Y, Yanase K, Namisaki T, Yamazaki M, Tsujinoue H, Imazu H, Masaki T, Fukui H.Vascular endothelial growth factor and receptor interaction is a prerequisite for murine hepatic fibrogenesis. Gut 2003; 52:1347-54
30. Brdoskey SV, Mendolov N, Melamed M, Ramaswamy G. Vascular density and VEGF expression in hepatic lesions. J Gaterointestin Liver Dis 2007,16 ;4:343-377
31. Namashima A, Nakayama T, Sumida Y, Abo T, Takeshita H, Shibata k, Hidaka SH, Sawai T, Yasutake T, Nagayasu T. Relationship between microvessel count & posthepatectomy survival in patients with hepatocellular carcinoma. World J Gateroenterol 2008; 14:4815-4922
32. Piao YF, He M, Shi Y, Tang TY. Relationship between microvessel density& telomerase activity in hepatocellular carcinoma. World J Gateroenterol 2004; 10:2147-2149
33. Iavarone M, Lampertico P, Iannuzzi F, Manenti E, Danato MF, Arosio F, Bertotini F, Primignani M, Sangiovanni A, Colombo M. Increased expression of vascular endothelial growth factor in small hepatocellular carcinoma. J Virology Hepat 2007; 14: 133-139.

5/22/2011