CD142, VEGF and Microvascular Density MVD-CD34 Expression in Hepatocellular Carcinoma of Patients with Cirrhosis and Correlation with Tumor Growth and Progression

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Abstract: Background: Angiogenesis is one of the mechanisms most critical to the postoperative recurrence and metastasis of HCC. So, finding the molecular markers associated with angiogenesis may help identify patients at increased risk for recurrence and metastasis of HCC. **Aim:** The aim of this study is to investigate the level of CD142, VEGF, and MVD-CD34 expression in HCC and surrounding cirrhotic liver tissue and their relationship to tumor growth and progression. **Material and Methods:** This study included forty six patients with clinical, radiological and serological diagnosis of HCC arising on top of cirrhosis at Mansoura Gastroenterology Surgical Center during 2010-2011. Tissue samples were obtained from specimens of resected HCC and the surrounding cirrhotic tissue. Immunohistochemical staining for CD142, VEGF and MVD-CD34 antibodies was performed and expression was identified in both HCC tissue, and the surrounding cirrhotic tissue. **Results:** CD142 and VEGF showed significantly increased expression in HCC compared to LC, and showed increased expression from grade I to grade II to grade III, but no significant difference in their expression between grades III and IV. There is highly significant association between CD142 and VEGF expression positivity and tumor size, vascular emboli, intrahepatic metastasis and tumor grade ($P<0.001$). There is highly significant association between CD142 and VEGF expression in both LC and different grades of HCC ($P<0.001$). MVD-CD34 was increased significantly from LC to HCC and increased significantly from grade I to II to III to grade IV HCC ($P<0.001$). The MVD-CD34 was significantly higher in tumors with high immunoreactivity for CD142 than in tumors with low immunoreactivity for CD142 (median, 53.26 *vs* 37.01/HPF, P <0.02). **Conclusion:** Expression of the angiogenic factors CD142, VEGF and MVD-CD34 is increased in HCC relative to LC and correlated with tumor aggressiveness.
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1. Introduction:

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide in terms of cases but because of its very poor prognosis it is the third most common cause of death from cancer (Parkin *et al.,* 2005). The major risk factor is liver cirrhosis associated with chronic hepatitis B and C infection, alcohol and various metabolic disorders (Chen *et al.,* 1996). However, in approximately 10-40% of patients the tumor arises from a non-cirrhotic liver (Nazeako *et al.,* 1996). The tumor from patients with cirrhosis tends to be less well differentiated (high grade), and exhibits local portal invasion and metastasis more often than tumors from patients without cirrhosis (Nazeako *et al.,*1996). Tumor angiogenesis is critical for providing nutrient supply to the tumor and providing the route for tumor survival, growth, invasion and metastasis of various human solid tumors. In fact, solid tumors can't grow 1 to 2 mm in diameter in the absence of angiogenesis (Chebib *et al.,* 2007).

In HCC, the sinusoid endothelial cells lost their structural and phenotypic characteristics and adopted the structure and phenotype of normal capillary endothelial cells, a phenomenon known as capillarization (Nakamura *et al.,* 1997). The association of increased expression of vascular endothelial growth factor (VEGF) with the capillarization of sinusoidal endothelial cells demonstrated in recent studies indicates that the capillarization phenomenon is not merely a change of endothelial cell differentiation but rather represents a process of tumor angiogenesis (Park *et al.,* 2000, Imnra *et al.,* 2004, Huang *et al.,* 2005).

MVD, a quantitative measurement of tumor angiogenesis, has been shown to be of prognostic value in many types of malignancy including HCC, which became more consistent in dysplastic nodules in cirrhotic liver (Poon *et al.,* 2003). Therefore, capillarization of sinusoidal endothelial cells was considered part of the carcinogenesis process in HCC-C (Chebib *et al.,* 2007). MVD-CD34 has been widely

used for the assessment of sinusoidal like neoangiogenesis in HCC (Amarapurkar *et al.,* 2008).

VEGF is a known marker of angiogenesis (Yang *et al.,* 2006). It is thought to be a selective mitogen for endothelial cells. It acts as a link between angiogenesis, immune system and tissue re-modeling (Kim and Hu., 1999). It was found that VEGF secreted by replicating hepatocytes induces sinusoidal endothelial cell proliferation during regeneration after partial hepatectomy in rats (Shimizu *et al.,* 2001). Greene *et al.,* 2003 in their studies on hepatic regenerative process suggested that the regulation of angiogenesis controls the regenerative process.

CD142 (thromboplastin or tissue factor) is a transmembrane glycoprotein that localize the coagulation serine protease factor VII/VIIa (FVII/VIIa) to the cell surface. The primary function of CD142 is to activate the clotting cascade. Recent studies have shown that CD142 is expressed by tumor cells and contributes to a variety of pathologic processes, such as thrombosis, metastasis, tumor growth and tumor angiogenesis (Zhou *et al.,* 2011).

The hypervascularity described in HCC varies according to the progression and differentiation of the tumor, suggesting an angiogenic switch during tumor development. Thus, finding the molecular markers associated with angiogenesis may help identify patients at increased risk for recurrence and metastasis of HCC and thus those who require more aggressive therapy and closer surveillance (Yang *et al.,* 2006). Interfering with angiogenesis may be a potential target to avoid progression of liver disease (Amarapurkar *et al.,* 2007; Zhou *et al.,* 2011).

This study was designed to investigate whether CD142, VEGF and MVD-CD34 expression could serve as a valid prognostic markers in patients with HCC-C and correlation with angiogenesis, progression and differentiation of HCC-C and so the postoperative recurrence and metastasis.

2. Material and methods:

This study was conducted on 46 cases with HCC recruited from the Hepatic Oncology Unit at the Specialized Mansoura Medical Hospital, from 2010 to 2011. All patients were subjected to thorough clinical examination, routine laboratory tests, and abdominal ultrasonography and computed tomography (CT) scan. Patients who are candidate for surgical treatment were referred to the Mansoura Gastroenterology Center for either or liver transplantation or partial hepatectomy. All the cases were HCC on background of cirrhosis and were HCV positive confirmed by real-time polymerase chain reaction. Cases arising in a normal liver, associated with bilharzial fibrosis only with no evident cirrhosis, or received previous adjuvant therapy or chemoembolization were excluded.

Resection specimens, as well as total hepatectomy specimens from the patients who underwent liver transplant surgery were used in the study. No tru-cut biopsy material was used. 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 (4μm) were cut on albuminized glass slides and stained with Hematoxylin & Eosin stain. All sections were subjected to light microscopic examination for evaluating the histopathological and basic classification of cases. The pathologic diagnosis and classification of variables were based on the criteria recommended in the general rules for clinical and pathological study of primary liver cancer (Liver Cancer Study Group Of Japan. 2000) and included age, gender, surrounding nontumerous liver pathology (cirrhosis), tumor size (<5cm vs ≥ 5cm), tumor grade, vascular invasion and multiplicity.

Immunohistochemical Staining of CD142 (tissue factor):

Immunohistochemical staining was performed using the streptavidin-biotin peroxidase complex method. Formalin-fixed, paraffin-embedded sections of 4 μm were deparafinized in xylene and rehydrated in a graded series of ethanol. Endogenase peroxidase was blocked by treating the sections with 3% hydrogen peroxide in methanol for 10 min. The sections were then subjected to antigen retrieval by microwave treatment for 10 min. Afterward, the sections were incubated with normal goat serum for 30 min at 37°C and then with 1:50 diluted TF mouse antihuman monoclonal antibody (American Diagnostica, Inc., product Nos.4509) for 1 h at 37°C. Sections were then incubated with 1:100 diluted biotin-conjugated goat antimouse immunoglobulin secondary antibody (Zymed Laboratory Inc., SanFrancisco, CA) for 1 h at 37°C and developed in 3,3-diamino-benzidine tetrachloride (Dako, Carpinteria, CA). Counterstain the section with Mayer's hematoxylin for 10 minutes then rinsed extensively with distilled water.

Immunohistochemical staining of VEGF:

Formalin-fixed, paraffin embedded sections of tumor tissue and surrounding cirrhotic tissue obtained from the resected liver specimens were cut into 4 microns thick sections and deparafinized in xylene and rehydrated in a graded series of ethanol. Antigen retrieval was performed by using EDTA in case of VEGF for 10 m. The slides were then incubated for 1 hour for mouse polyclonal anti VEGF antibody (1:100 dilution, in vitrogen code No. 726127A). This is followed by biotin-conjugated goat anti-mouse immunoglobulin and horseradish peroxidase-conjugated streptavidine (UltraVision Detection System) DAB was used as chromogenic substrate.

Counterstain the section with Mayer's hematoxylin for 10 minutes then rinsed extensively with distilled water. The brown precipitate was identified as positive staining.

Immunohistochemical staining of CD34:

Formalin-fixed, paraffin embedded sections of tumor tissue and surrounding cirrhotic tissue obtained from the resected liver specimens were cut into 4 microns thick sections and deparafinized in xylene and rehydrated in a graded series of ethanol. Sections were immersed in citrate buffer (pH 7.0) for antigen retrieval and incubated in a water bath for 40 min 98˚c. After endogenous peroxidase was blocked, the sections were incubated with monoclonal mouse antihuman CD34 antibody (Clone QBEnd/10, Thermo Fisher Scientific, Fremont, CA, USA) for 60 min at room temperature. Tissues were treated with the (EnVision+system, Dako, Glostrup, Denmark) for 30 min at room temperature, and the reaction was visualized by DAB until color developed. The cell nuclei were counterstained with Mayer's hematoxylin.

The expression of the three markers was correlated with positive controls (cancer colon for VEGF, cancer breast for CD142, and internal control, vessels in the portal tracts and fibrous septa for CD34) and negative controls. The expression of three markers was correlated with pathological data collected. For this purpose patients were divided into two age groups (<60 years & ≥ 60 years), size (<5cm & ≥5cm), and tumors were graded according to Edmonson and Steiner four tiered grading system (Edmondson & Steiner, 1956, Goodman *et al.,* 2012).

Immunohistochemical scoring of VEGF and CD142:

For VEGF, cytoplasmic staining of more than 10% of the tumor cells was defined as positive. The intensity of immunoreactivity was graded as weak (+), moderate (++) and marked (+++) (Moon *et al.,* 2003). The immunoreactivity of CD142 was classified as high if >50% of the tumor cells were stained positively and low if <50% of the tumor cells were stained positively (Poon *et al.,* 2000).

Determination of microvascular density (MVD):

MVD was evaluated according to the method described by Weinder *et al.*(1993). Brown-stained endothelial cell or endothelial cell cluster, which was clearly separate from adjacent microvessels and other connective tissue elements, was considered a single, countable blood vessel. Screening of the cores was first performed at a low power (40 X) to identify areas of the highest MVD. Counting was performed in the three highest MVD areas at high power (400 X). The mean value of the counted three fields was considered as the MVD of an individual case.

Statistical analysis:

The Statistical Package for Social Sciences Immunohistochemical expression of CD142

Immunohistochemical staining of 46 cases for CD142 showed expression of the biomarker in both tumor tissue and the surrounding cirrhotic tissue. There were 2 cases (4.3%) negative for CD142 and 44 (95.7%) of cases were positive for CD142.

As regard CD142 expression in cirrhotic tissue, there were 30 cases (65.2%) with low expression (Fig.3), and14 cases (30.4%) showed high expression (Fig. 4). However, there were 17(37.0%) cases with low CD142 expression and 27(58.7%) cases showed high CD142 expression in HCC (Fig. 5). There was significantly higher CD142 expression in HCC cases relative to LC($P<0.05$).

In our study, there was no significant association between CD142 expression positivity both in cirrhosis and tumor tissue and age or sex ($P>0.05$). However, there is highly significant association between CD142 expression positivity and tumor size, vascular emboli and tumor grade ($P<0.001$).

CD 142 expression increased significantly from grade I to grade II to grade III. But there was no significant difference in CD142 expression between grade III and grade IV (Table 2). (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 was used with Post Hoc test for internal comparisons. Comparison between positive cases was calculated by Chi-square test. *P* value <0.05 was considered statistically significant.

3. Results:

Patients were 40 males (87%) and 6 females (13.0%), with age range (41-69 years) with a mean of 52.91±6.98 years. Frequency of vascular invasion was insignificantly higher ($P>0.05$) in patients with tumor size >5cm (87.5%) compared to tumor size <5cm (76.7%). On the other hand, there was significant association between vascular emboli and high tumor grade (P =0.03).

Immunohistochemical expression of VEGF

Immunohistochemical staining of 46 cases for VEGF shows expression of the biomarker in both tumor tissue and the surrounding cirrhotic tissue, and the endothelial cells lining the blood vessels in the tumor area mainly and in the nearby periphery of the tumor. Two cases (4.3%) were negative for VEGF and 44(95.7%) cases were positive for VEGF.

In the cirrhotic liver, 12(26.1%) cases showed weak staining (+), 18(39.1%) cases were of moderate staining (++) and 14(30.4%) cases showed marked staining (+++). In patients with HCC, 4 cases (8.7%) showed weak staining, 13 cases (28.3%) showed moderate staining, and 27 cases (58.7 %) showed marked staining (Fig. 1). In 15(39.1%) cases, the stain

was more intense at the periphery of the tumor than in the center (Fig. 2).

In our study, there was no significant association between VEGF expression positivity both in cirrhosis and tumor tissue and age or sex ($P > 0.05$). However, there is highly significant association between VEGF expression positivity and tumor size, vascular emboli, intrahepatic metastasis and tumor grade ($P < 0.001$).

There is significant increase in VEGF expression in HCC cases relative to LC ($P < 0.05$). VEGF expression increased significantly from grade I to grade II to grade III ($P < 0.05$). But there was no significant difference in VEGF expression between grade III and grade IV (Table 1).

Immunohistochemical staining of MVD-CD34

Immunohistochemical staining of 46 cases for CD34 showed expression of the biomarker in both tumor tissue and the surrounding cirrhotic tissue. In liver cirrhosis, there was one case (2.17%) negative for CD34 and 45 (97.83%) cases were positive for CD34, while all cases of HCC were positive for CD34.

There was significant increase ($P < 0.05$) in CD34 expression from cirrhotic nodules to dysplastic nodules to HCC (Fig. 6).

In liver cirrhosis, CD34 staining was confined to large vessels in the portal tracts. In cases of HCC-C the density of microvessels was higher in the peripheral tumor tissue close to the margin than in the central areas

The mean MVD-CD34 in liver cirrhosis was significantly lower than the MVD-CD34 in HCC (8.28 ± 6.62 vs 46.24 ± 24.04, $P < 0.001$) (Table 3).

Specific staining of capillary-like vessels by anti-CD34 antibody was observed in tumor specimens and between cancer cells. CD34 expression was found to be significantly increased with the tumor grade ($P < 0.05$) (Figs.7,8).

Correlation between CD142 immunoreactivity and MVD-CD34:

Immunohistochemical staining of the tumor sections showed that CD142 was expressed to a variable extent by HCC tumor cells in 95.7% of the tumors studied, with high immunoreactivity in 27 patients and low immunoreactivity in 17 patients. Specific staining of capillary-like vessels by anti-CD34 was observed in 97.82%of tumor specimens. The mean tumor MVD was

52.1/HPF (range, 0–78.9).The MVD was significantly higher in tumors with high immunoreactivity for CD142 than in tumors with low immunoreactivity for CD142 (median, 53.26 vs 37.01/HPF, $P < 0.02$).

Correlation between CD142 immunoreactivity and VEGF:

Highly statistically significant ($P < 0.001$) association was found between VEGF and CD142 expression in both LC and different grades of HCC. All cases of LC and HCC with high CD142 immunoreactivity also showed marked VEGF expression. On the other hand, all cases of LC and HCC with low CD142 immunoreactivity also showed mild to moderate VEGF expression. Furthermore, cases with negative CD142 immunoreactivity showed negative expression for VEGF in both LC and HCC.

Table 1: VEGF expression levels in the paracarcinomatous cirrhotic liver and in different grades of HCC-C.

| Groups | No. of positive cases for VEGF | VEGF | | |
| | | Percentage of positive area /3 microscopic fields | | |
		Mild	Moderate	Marked
LC (n=46)	44/46	12 (26.1)	18(39.1)	14(30.4)[a]
HCC(n=46)	44/46	4(8.7)	13(28.3)	27(58.7)
GI (n=3)	2/3	0(0.0)	1(33.33)	1(33.33)[b]
GII (n=23)	22/23	2(8.7)	11(47.8)	9(39.1)[c]
GIII (n=14)	14/14	2(14.3)	0(0.0)	12(85.7)[c,d]
GIV(n=6)	6/6	0(0.0)	1(16.7)	5(83.3)[c,d]

[a]P value <0.05 LC relative to HCC --[b]P<0.05 between different HCC grades
[c]P<0.05 GI relative to GII, GIII, GI -- [d]P<0.05 GII relative to GIII, GIV

Table 2: CD142 expression levels in paracarcinomatous cirrhotic liver and indifferent grades of HCC-C.

| Groups | CD142 | | |
| | No. of positive cases for CD142 | Percentage of positive cases | |
		Low	High
LC (n=46)	44/46	30(65.2)	14(30.4)[a]
HCC(n=46)	44/46	17(37.0)	27(58.7)
GI (n=3)	2/3	1(33.33)	1(33.33)[b]
GII (n=23)	22/23	13(56.5)	9(39.1)[c]
GIII (n=14)	14/14	2(14.3)	12(85.7)[c,d]
GIV(n=6)	6/6	1(16.7)	5(83.3)[c,d]
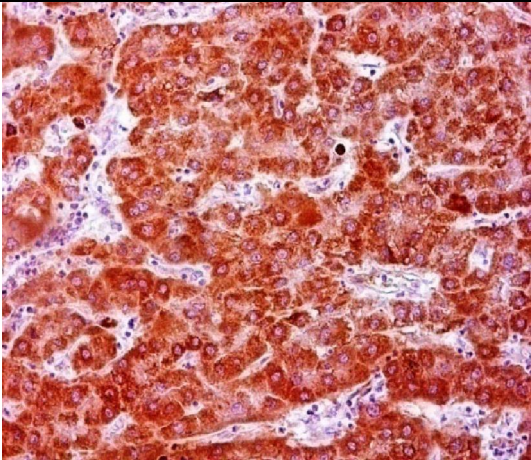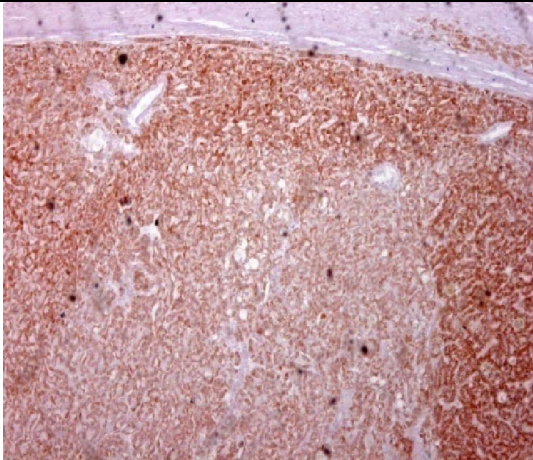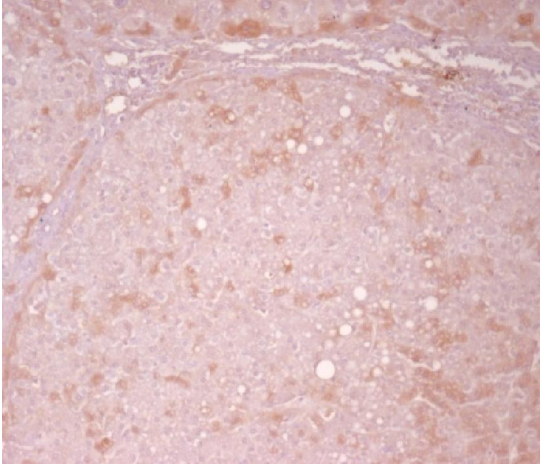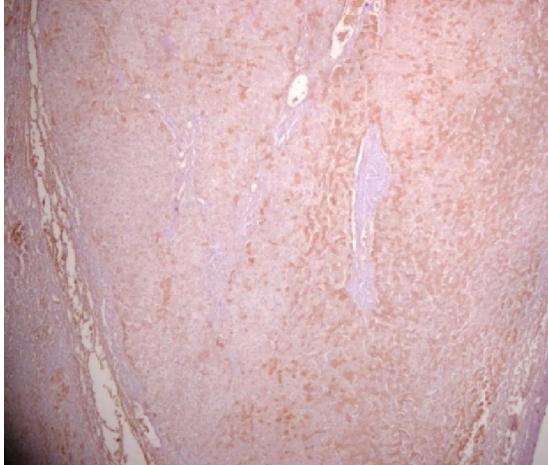
[a]P value <0.05 LC relative to HCC –[b]P<0.05 between different HCC grades
[c]P<0.05 GI relative to GII, GIII, GIV –[d]P<0.05 GII relative to GIII, GIV.
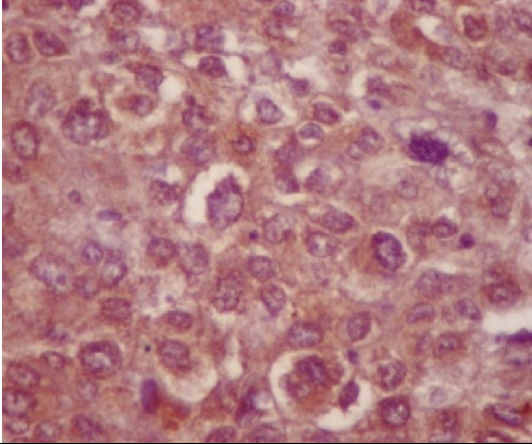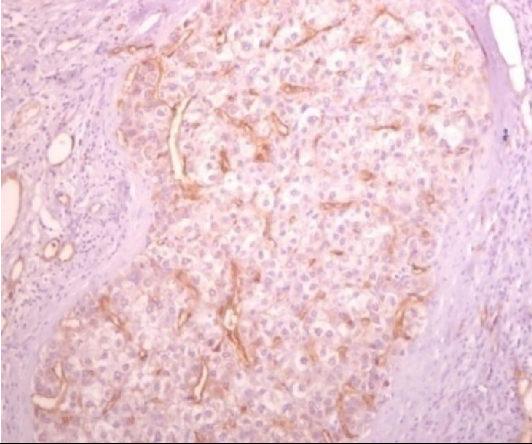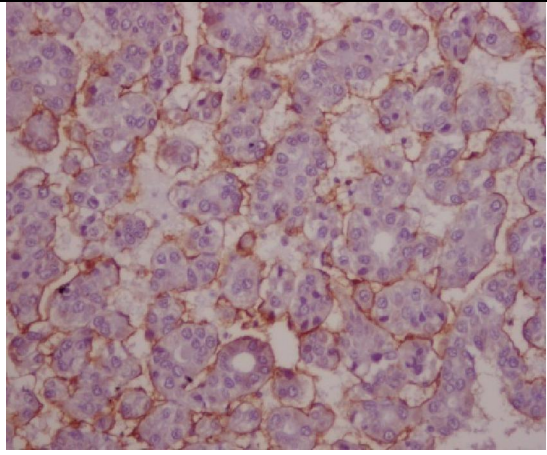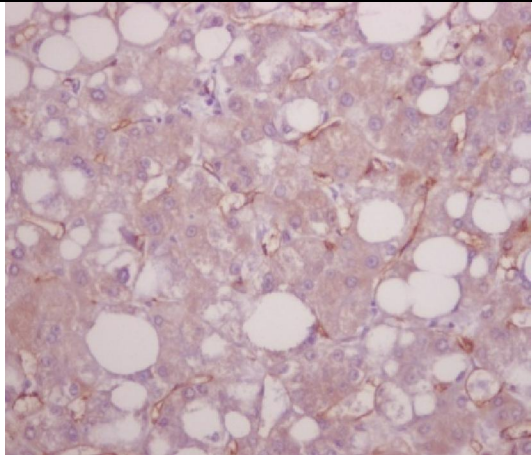
Table 3: MVD-CD34 expression levels in paracarcinomatous cirrhotic liver and in different grades of HCC-C.

Groups	MVD-CD34	
	No. of positive cases for CD34	MVD μ^2/ 3 microscopic field Mean ± SD
LC (n=46)	45/46	8.28±6.62[a]
HCC(n=46)	46/46	46.24± 24.04
GI (n=3)	3/3	13.53±18.80[b]
GII (n=23)	23/23	44.77±22.94[c]
GIII (n=14)	14/14	50.31±19.99[c]
GIV(n=6)	6/6	57.32±18.64[c]

[a]$P<0.001$ LC relative to HCC–[b]$P<0.05$ between different HCC grades
[c]$P<0.05$ GI relative to GII, GIII, GIV.

Figure 1: Marked intensity of VEGF immunostaining in case of HCC (original magnification X 200).

Figure 2: Increased intensity of the VEGF staining at the periphery of the tumor. (Original magnification X 100).

Figure 3: CD142 immunostaining (low expression) in liver cirrhosis. (original magnification X 100)

Figure 4: CD142 immunostaining (high expression) in liver cirrhosis. (original magnification X 100)

Figure 5: CD142 immunostaining (high expression) in grade III HCC. (original magnification X 400)

Figure 6: A case of dysplastic cirrhotic nodule showing high MVD as assessed by anti-CD34 immunostaining. (original magnification X 200)

Figure 7: A case of grade I HCC stained by anti CD34 immunostaining showing high MVD. (original magnification X 200)

Figure 8: A case of grade III HCC stained by anti CD34, showing high MVD. (original magnification X 200)

4.Discussion

HCC is a highly malignant tumor with a propensity for vascular invasion and metastasis. Hepatic resection is the treatment of choice for HCC, but the prognosis after resection remains unsatisfactory because of a high incidence of recurrence related to tumor metastasis (Poon *et al.,* 2000). Tumor angiogenesis is an important determinant of invasiveness and progression of HCC (Poon *et al.,*2001; Sugimachi *et al.,*2002; Poon *et al.,*2002). However, little is known about the regulatory mediators of angiogenesis in HCC.

This study showed a positive correlation between VEGF degree of staining and vascular emboli ($P<0.001$). There was 27 cases (58.7%) associated with vascular invasion and showed marked degree of VEGF staining. Deli *et al.* (2005) found that VEGF expression in HCC has a significant correlation with vascular invasion, intrahepatic metastasis and shortened survival rates. Also, this study has demonstrated a positive correlation between tumor size and intrahepatic metastasis and expression of VEGF and this is consistent with that reported by the Moon *et al.,*study (Moon *et al.,*2003).

According to Saaristo *et al.,* 2000 VEGF is a cell specific mitogen and is a major inducer of angiogenesis in human cancers. There is some evidence that VEGF is an important angiogenic mediator of HCC (Weidner *et al.,*1993; Yoshiji *et al.,* 1998; Li *et al.,*1998; Park *et al.,*2000). Yoshiji *et al.,* 2003 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. Ivarone *et al.,* 2008 concluded that VEGF appears to be involved in the development of HCC and could be a predictor of HCC development in patients with cirrhosis.

In our study, there was positive correlation between grades of the tumor and degree of VEGF expression ($P<0.05$). In agreement with our results, Yoo *et al.,*2003 reported increased expression of VEGF in poorly differentiated tumors and decreased in well differentiated ones. However, Hammam *et al.,* 2011 found that VEGF expression showed insignificant increase in HCC compared to LC. Moreover, they found that with differentiation of HCC, VEGF showed high expression in grade I and decreased in grade II and grade III.

Increased expression of VEGF receptors in HCC was demonstrated on different levels, including mRNA and protein (Shimamura *et al.,*2000; Yamaguchi *et al.,*2000). The patterns of VEGF expression (mRNA or protein expression) in HCC and surrounding liver tissue are still controversial. Most of the studies report that the mRNA VEGF expression level is higher in HCC than in the surrounding hepatic tissue (Miura *et al.,*1997, Yao *et al.,* 2005). The expression of VEGF protein is inconsistent, demonstrating a higher level either in HCC or in the surrounding liver tissue (El-Assal *et al.,*1998; Yamaguchi *et al.,*1998).

In our study, VEGF expression is increased significantly in HCC relative to LC. Shimamura *et al.,* 2000 and Hassan *et al.,* 2009 studies concluded that the increased level of VEGF in HCV-related HCC is the result of VEGF gene amplification. In the study of Tseng *et al.,* 2008 the presence of viremia (either HBV or HCV) was associated with VEGF overexpression in patients with HCC, being a poor prognosis factor in these patients. As all our cases were HCV positive, this may explain the high expression levels of VEGF (95.7% of cases) in this study.

In our study, 39.1% of cases showed that VEGF expression was more intense at the marginal area of the tumor than in the center. This can be explained by the fact that rapid cell proliferation in the center of a tumor can lead to increased interstitial fluid pressure, which may result in compression closure of capillaries and consecutive tissue necrosis. Central necrosis areas cause a suppression of VEGF protein synthesis (Deli *et al.,* 2005).

This study showed higher expression of VEGF in the surrounding cirrhotic liver tissue compared to HCC in some cases. This is explained by Deli *et al.,* 2005 who suggest that the sustained mechanically-reduced blood flow affects the hepatocytes at the cirrhotic area. This leads to decreased oxygen pressure and strongly up-regulates VEGF transcription and protein synthesis in the cirrhotic area.

By immunohistochemical staining, we showed that CD142 was expressed by HCC tumor cells in 44(95.7%) of the 46 specimens. However, there was a wide variation in CD142 expression among different grades of tumor. In our analysis, we observed significantly high ($P<0.05\%$) expression of CD142 in HCC compared to LC. There are no previous studies for CD142 expression in cirrhotic liver tissue adjacent to HCC. Also, there was positive correlation between grades of the HCC and degree of CD142 expression ($P<0.05$). This result is consistent with the Poon *et al.,* 2003 study who concluded that TF expression was up-regulated in moderately or poorly differentiated HCC compared with well differentiated HCC.

Our study showed a positive correlation between CD142 and VEGF expression in both cirrhosis and the tumor. This may explain the possible pathway through which CD142 is involved in the angiogenesis of HCC. Rickles *et al.,* 2001 has reported that CD142 may regulate tumor angiogenesis in HCC via up-regulation of VEGF.

According to our results, CD142 expression has a significant correlation with vascular invasion, intrahepatic metastasis and tumor size. On the other hand, Poon *et al.,* 2003 found no significant correlation between tumor CD142 expression and tumor size and reported that tumor CD142 expression may influence tumor invasiveness independent of tumor size.

In our study, there was significant increase ($P<0.05$) in MVD-CD34 expression from cirrhotic nodules to dysplastic nodules to HCC. This result is similar to that obtained by Park *et al.,* 1998, Kim & Hu, 1999 and Ma Jee *et al.,* 2006 study. CD34 was closely associated with neo-vascular process in cirrhosis and hepatocellular carcinoma. Di Carlo *et al.,* 2002 studied by immunohistochemistry, the expression and distribution of CD34 in liver cirrhosis and 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.,*2006 concluded that CD34 is a useful marker for distinguishing HCC from non-cancerous liver tissue.

The study by Namashima *et al.,* 2008 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. Also, Poon *et al.,*2003 found that high MVD-CD34 is predictive of early post-resection recurrence in patients with HCCs ≤ 5 cm.

According to the results of our study, MVD-CD34 expression was significantly increased ($P<0.05$) from grade I to grade II to grade III to grade IV. Park *et al.,*1998 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. Ma Jee *et al.,* 2006 found significant difference (P<0.05) in the expression of CD34 between well differentiated and moderately differentiated HCC. Amarapukar *et al.,* 2008 suggested that angiogenesis as assessed by CD34 expression play an important role in carcinogenesis.

According to our analysis, MVD-CD34 expression has a significant correlation with vascular invasion and tumor size. This result is similar to that obtained by Messerini *et al.,*2004.

This study has demonstrated that MVD-CD34 was significantly higher in tumors with high immunoreactivity for CD142 than in tumors with low immunoreactivity for CD142. Also, we found highly significant association between VEGF and CD142 expression in both LC and different grades of HCC. All cases of LC and HCC with high CD142 immunoreactivity also showed marked VEGF expression. On the other hand, all cases of LC and HCC with low CD142 immunoreactivity also showed mild to moderate VEGF expression. Furthermore, cases with negative CD142 immunoreactivity showed negative expression for VEGF in both LC and HCC. To our knowledge; this is the first study that evaluated the CD142 expression in LC and its relationship with angiogenic factors VEGF and MVD-CD34 expression in LC and different grades of HCC.

Conclusion|

Overexpression of CD142, VEGF and CD34 is correlated with the factors of poor prognosis, like tumor size, vascular invasion and intrahepatic metastases. Expression of CD142 in HCC is related to tumor angiogenesis and invasiveness through up-regulation of VEGF and MVD-CD34. This novel finding may provide insight into a new therapeutic strategy for HCC by inhibiting CD142 expression as it plays an important role in the development and progression of HCC. Also, there is a link between HCV infection, angiogenesis, and hepatocarcinogenesis.

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