Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor β1 (TGF- β 1) as Predictors of Hepatocellular Carcinoma in HCV Related Liver Cirrhosis

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Abstract: Hepatocellular carcinoma (HCC) is one of the most serious complications of liver cirrhosis. Therefore, evaluation of biomarkers that predicts early the occurrence of HCC in patients with hepatitis C virus (HCV) induced liver cirrhosis is of great clinical value from the diagnostic and prognostic points of view. **Aim**: The aim of this work was to study serum levels of TGF-β1 and VEGF in cirrhotic HCV patients with and without HCC. **Subjects and methods**: This research was conducted on 30 patients with chronic HCV and liver cirrhosis (Group I), 30 patients with HCC on top of HCV induced liver cirrhosis (Group II) and 20 healthy controls. .Serum TGF- β1 and VEGF were measured by ELISA. **Results**: Mean VEGF and TGF- β1 levels were significantly higher in patients (Groups I and II) than controls. Furthermore, their values were significantly higher in HCC cases (Group II) than in those with liver cirrhosis (Group I). Significant positive correlations were noticed between each of TGF- β1 and VEGF and Child Pugh score ($p<0.05$). Moreover, statistically significant positive correlations were observed between size of hepatic focal lesions and each of TGF -β1 and VEGF in group II patients ($p<0.05$)
Conclusion: Serum TGF-β1 and VEGF reflect well the degree of hepatic dysfunction. Their serial measurements might be of diagnostic and predictive value for occurrence of HCC in patients with chronic HCV induced liver cirrhosis.

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1. Introduction

HCC is the most common primary malignant liver tumor. It has a fulminant course and a poor prognosis [(1)]. Many etiological factors have been linked to the occurrence of HCC like liver cirrhosis, chronic hepatitis B or C infection and alcohol intake [(2)].

Development of HCC is related to chronic necroinflammatory liver process. The time elapsed between acquiring hepatitis C virus (HCV) infection and HCC development varies between 10-15 years[(3)]. Moreover, 97% of patients with chronic HCV and HCC have liver cirrhosis[(4)]. Factors that predispose to HCC among HCV infected individuals include male gender, old age, HBV and HIV coinfection and heavy alcohol intake as well as HCV genotype and quasi species [(5-7)]

Serum concentration of a variety of cytokines and cytokines antagonists are elevated in patients with liver disease[(8)]. Some have been incriminated in the occurrence of liver cancer. TGF- β1 over expression in transgenic mice is associated with 60% incidence of hepatoma[(9)]. TGF- β1 is an important mediator which plays a role in the development, growth and progression of HCC[(10)].

Tumor angiogenesis is important for growth and spread of cancer and is controlled by angiogenetic factors. HCC is a hypervascular tumor with rich blood supply; therefore, circulating angiogenesis markers have been studied not only as diagnostic but also as predictors and prognostic markers in cancer patients [(11)]. VEGF is the most potent, directly acting mediator of angiogenesis in both physiological and pathological conditions [(12)].

Aim of the work

This study was planned to evaluate serum VEGF and TGF- β1in patients with chronic HCV induced liver cirrhosis with or without hepatocellular carcinoma and their correlation with the size of hepatic focal lesion as determined by triphasic CT.

2. Subjects and Methods

Sixty chronic HCV patients were divided into two groups according to history, examination, ultrasound and biopsy whenever possible;
Group I: 30 chronic HCV patients with liver cirrhosis.

Group II: 30 patients with HCC on top of chronic HCV and hepatic cirrhosis (confirmed by triphasic CT study).

Moreover, 20 healthy Egyptians were included as controls (Group III)

Informed written consent was obtained from all those who were included in this study. The research protocol was approved by the ethics committee of the Faculty of medicine, Alexandria University

Exclusion criteria

Patients with chronic HBV infection or diabetes mellitus as well as those with cardiovascular, chest or renal diseases, alcoholics and those suffering from fever or autoimmune disease were not enrolled in the study.

Beside complete blood picture, ESR and liver function tests

All patients and controls were subjected to the following

1. HBs Ag (13) and Anti-HCV antibodies by ELISA. [14]
2. Serum HCV RNA level was done for cases with positive anti-HCV Ab using quantitative polymerase chain reaction (The Cobas Amplicor HCV Monitor ™ test, Roche molecular systems, Banch burg, NJ, USA) [15] .
3. Estimation of serum alphafetoprotein by Chemiluminescence (Immulite 1000, Siemens, Germany)
4. VEGF was determined in patients and controls sera using VEGF ELISA kit (Peninsula inc. USA) [16] .
5. Patients' and controls' serum TGF- β1 was measured using Human TGF beta ELISA Kit (Abcam Company –USA) [17] .
6. Abdominal ultrasonography
7. Triphasic CT abdomen (Siemens, Germany) after oral water and IV contrast administration and examination in the hepatic arterial phase (HAP), portal venous phase (PVP) and delayed phase was performed for cases of HCC

Statistical analysis

Data were collected, revised and transferred into statistical package for social science (SPSS/ version 10). Results were expressed as means and standard deviation. Statistical tests used in this study were student t test, F test and Pearson correlation. A level of 5% was considered as the cutoff level of significance.

3.Results

Results revealed that group I patients included 5 Child A patients (Mean score 5.6), 12 Child B (Mean score 8.03) and 13 Child C (Mean score 11.76). Besides, in group II 15 patients were Child B (Mean score 8.46) and 15 Child C (Mean score 11.86)

All group I and II patients were HCV positive (HCV Ab +ve and confirmed by PCR for HCV-RNA). None of them had HBsAg.

Tables I, II and III show the blood picture, liver function tests and α-fetoprotein findings in the three studied groups

Table I: Hematological findings in the three studied groups (Mean± SD).

	RBCs(million/µl)	WBCs(x10^3 /µl)	Platelets(10^3 /µl)
Group I (n=30)	4.14±0.80	6.56±1.6	161.2±67.53
Group II (n=30)	3.7±0.82	6.76±2.2	119.4±44.1
Group III (n=20)	4.96±0.34	5.69±1.58	251.60±57.5
F	17.9	2.11	32.45
P value	0.000*(I,II) (I,III) (II,III)	0.000* (II,III)	0.000* (I,II) (II,III), (I,III)

*Significant at p≤0.05

Table II: Liver function tests among the three groups (Mean±SD)

	ALT(U/l) (ULN=40)	AST(U/l) (ULN=40)	S.Bilirubin (mg/dl)	S.albumin gm/dl)	Prothrombin time(sec)
Group I (n=30)	48.33±15.23	71.86±16.97	3.29±.155	2.99±0.32	18.6±2.8
Group II (n=30)	69.70±26.27	113.66±39.20	4.05±2.42	2.83±0.42	19.43±2.34
Group III(n=20)	20.45±5.1	15.35±3.71	0.82±0.14	4.51±0.44	13.14±0.71
F	41.19	84.04	20.88	121.72	50.49
P value	0.001*(I,II), (I,III),(II,III)	0.000*(I,III) (I,II) (II,III)	0.000*(I,III) ,(II,III),	0.000* (I,III) (II,III)	0.000* (I,III) (II,III)

* ULN= upper limit normal *Significant at p≤0.05

Table III: Mean serum α-fetoprotein in the three studied groups (Mean±SD).

1.	α-fetoprotein (ng/ml)
Group I (n=30)	41.13±32.06
Group II (n=30)	467.79±388.45
Group III (n=20)	5.9±1.8
F	31.9
P value	0.000* (I,II) (II,III) (I,III)

*Significant at p≤0.05

Regarding TGF-β1, the mean serum levels were significantly higher in groups I and II than in group III and in group II than in group I. Furthermore, mean serum VEGF was significantly elevated in patients with liver cirrhosis and HCC than in controls and also higher in HCC cases than those with liver cirrhosis (Table IV).

Table IV: Mean serum TGF-β1and VEGF in the three studied groups (Mean±SD).

	TGFβ1(pg/ml)	**VEGF(pg/ml)**
Group I (n=30)	345±182.01	260.2±120.4
Group II (n=30)	868±164.6	981.06±34.1
Group III (n=20)	30.50±8.82	127.3±35.15
F	199	113.5
P value	0.000* (I,II) (II,III) (I,III)	0.000* (I,II) (I,III) (II,III)

- Significant at p≤ 0.05

In patients with liver cirrhosis (Group I), the mean serum TGF-β1 was significantly higher in Child C patients than in Child A and B ones and also in Child B cases than in Child A ones. As regards VEGF, its mean was significantly higher in Child C cases than in Child A and B ones, while no significant difference was found between Child B and Child A patients (Table V).

Table V: VEGF and TGF-β1in group I patients (Mean±SD).

	TGF-β1 (pg/ml)	VEGF(pg/ml)
Child A	73.7±30.17	166.6±10.47
Child B	304.16±52.91	203.66±23.48
Child C	487.65±151.04	348.38±138.81
F	28.58	10.40
P	0.001* A&B, A&C, B&C	0.001* A&C, B&C

- Significant at p≤ 0.05

In patients with HCC (Group II) the mean serum TGF-β1 and VEGF levels were significantly higher in Child C patients than in Child B ones (Table VI).

Table VI: VEGF and TGF-β1in group II patients (Mean±SD).

	TGF-β1 (pg/ml)	VEGF(pg/ml)
Child B	773.33±153.09	726±204.6
Child C	960.8±119.6	1236.13±253.16
T	3.96	6.06
P	0.001*	0.001*

- Significant at p≤ 0.05

As regard correlation studies, a significant positive correlation was detected between TGF-β1 and VEGF on one hand and Child Pugh score on the other hand in groups I and II (p<0.05) (Figs.1 and 2). Furthermore, a significant positive correlation was observed between VEGF and TGF-β1 in the same groups (r= 0.74 and 0.47, respectively) (p= 0.000 and 0.00, respectively)

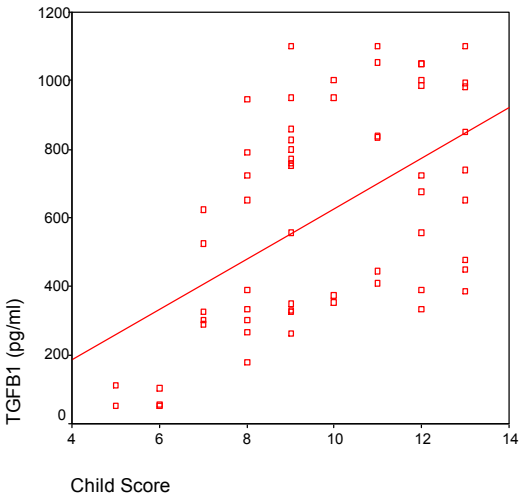

Figure 1: correlation between TGF-β1 and Child Pugh score

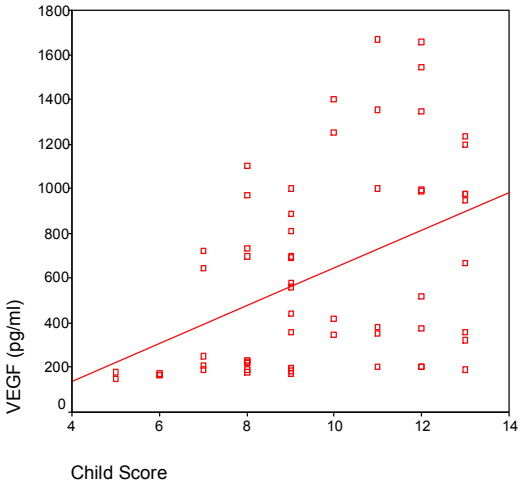

Figure 2: Correlation between VEGF and Child Pugh score.

In group II patients (HCC cases) triphasic CT revealed solitary hepatic focal lesion in 28 patients and multiple hepatic lesions in 2 patients. The lesions showed blush enhancement in the arterial phase with washout at the venous and delayed phases (Figs. 3 and 4). Furthermore, a significant positive correlation was observed between lesion size and each of VEGF and TGF-β1 in group II patients (r= 0.62 and 0.40, respectively) (p= 0.000 and 0.02, respectively).

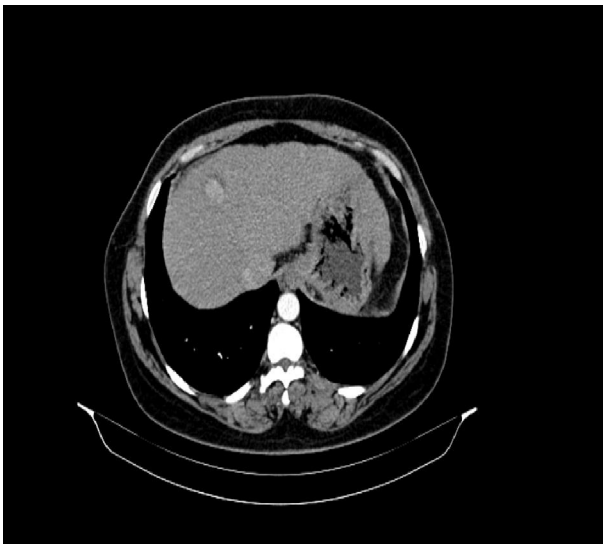

Figure 3: Shrunken liver showing established cirrhotic changes with 3cm hepatic focal lesion noted at right lobe that expressed moderate homogenous blush enhancement at HAP phase

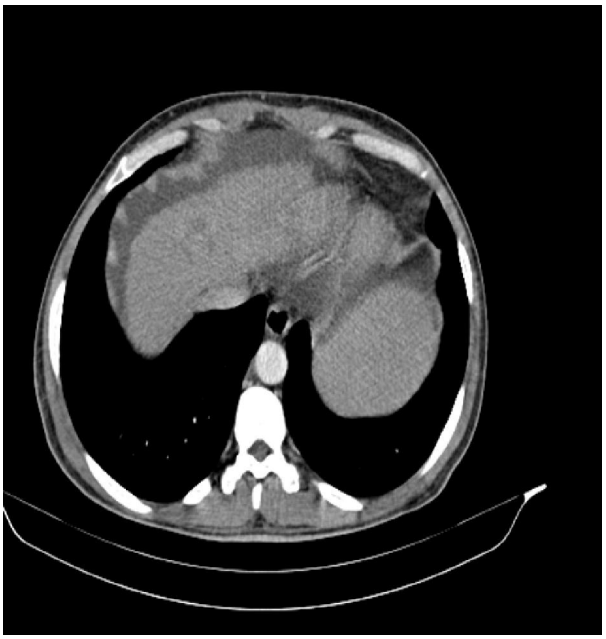

Figure 4: Shrunken liver with established cirrhotic changes and medium sized 4x 4cm expanding hepatic focal lesion affecting area VIII of right hepatic lobe that expressed mild heterogeneous capillary blush enhancement at the late HAP

4. Discussion

Association between chronic HCV infection and HCC has been established. HCV is likely to predispose to HCC via viral proteins as well as enhanced hepatocyte turnover that happens in an attempt to replace infected hepatocytes which have attacked by immune cells [2].

Alphafetoprotein (α-fetoprotein) has been utilized as a marker for HCC, despite its low sensitivity and positive predictive value. Moreover, it has been estimated that up to 30% of HCC patients have normal α-fetoprotein levels [18]. Therefore, novel biomarkers are needed to be used for early detection of cases with HCC. With the advance of cellular and biological techniques, many molecular markers have been studied such as angiogenic factors [19].

VEGF is a well known angiogenic 46k glycoprotein which accelerates vascular permeability and has a role in proliferation of endothelial cells [20]. Various types of human cancer secrete VEGF, and its expression by tumor is closely linked to tumor progression, prognosis and even metastases [21,22]. Production of VEGF is regulated by oxygen, steroid hormones and protein C agonists [23].

In the present study, serum VEGF was significantly higher among patients than controls. Furthermore, it was more significantly elevated in HCC cases than in those with liver cirrhosis. Similar results were reported by **Abdel Haleem *et al*.** [24] and **Abdelmoaty *et al*.** [25]. Moreover, **Jerzy *et al*.,** recorded elevated circulating levels of VEGF and its receptors in patients with liver cirrhosis [26].

In the current work, Child class C patients had significantly higher serum VEGF than Child class A and B ones. Moreover, a significant positive correlation was noticed between VEGF and Child Pugh score. These results were in agreement with those of **Jerzy *et al*.** [26] and **Abdelmoaty *et al*.** [25].

Poon et al, reported that serum VEGF level is a predictor of microscopic venous invasion in HCC, suggesting that it may be useful as a biologic marker of tumor invasiveness [27]. Similar results were obtained by Chao et al who concluded that preoperative serum VEGF is a significant independent predictor of tumor recurrence [28]. Furthermore, **Poon *et al*.**, reported that high serum VEGF is predictor of poor outcome after resection of HCC [27].

In agreement with the previous studies, the present research revealed a significant positive correlation between serum VEGF and tumor size. This positive correlation could be attributed to the fact that angiogenesis is essential for tumor growth and invasion. This was supported by Folkman ***et al*** who clarified that neovasculature facilitates shedding of tumor cells into surrounding blood vessels [29]. Later on, this finding was supported by Jinno ***et al*** who recorded elevated serum VEGF in HCC patients with distant metastases [30].

Salgado *et al*., showed that platelets are able to store circulating VEGF [31]. It has been postulated that platelet adhering to circulating tumor cells may be activated to release VEGF. They also suggested that fast growing tumors may release thrombopoietic cytokines in addition to VEGF. **Hino *et al*.**, reported that HCC could express thrombopoeitin which could be a mediator in inducing thrombocytosis [32].

However, such correlation between VEGF and platelets could not be detected in this study. This can be attributed to the fact that most patients had thrombocytopenia as a result of liver cirrhosis and splenomegaly. So, high serum VEGF in included patients could be attributed to its production by tumor cells.

VEGF has been linked to hepatic dysfunction and this was proved in the current work by the presence of a significant positive correlation between VEGF & Child Pugh score. It could be suggested that elevated VEGF may contribute to enhanced hepatic fibrosis through induction of proliferation of hepatic stellate and sinusoidal cells [26].

Geert's *et al*., found that angiogenesis is increased in the mesenteric microvasculature in animal models with portal hypertension and cirrhosis. They also reported high VEGF in the mesentery of animal models suggesting its contribution to portal hypertension [33].

This observation was previously reported by **Fernandez *et al*.**, who found decreased intestinal neovasculature, splanchnic blood flow and porto-systemic collaterals in portal hypertensive rats following administration of anti- VEGF receptor 2 monoclonal antibodies [34].

In the present study, patients with HCC had significantly higher TGF-β1 than cirrhotic patients. Furthermore, a significant positive correlation was noticed between serum TGF-β1 and Child Pugh score.

Neuman *et al*., recorded that serum TGF-β1 could reflect the degree of fibrosis in HCV patients [35]. Moreover, **Flisiak *et al*.**, suggested the possible use of plasma TGF-β1 as a good marker of liver function impairment [36]. **Sacco *et al*.,** reported elevated serum TGF-β1 in HCC patients in 23% of cases with normal α-fetoprotein [37]. In the present work, α-fetoprotein was normal in 2 patients proved to have HCC by triphasic CT. These patients had elevated TGF-β1.

This elevation in HCC cases could be attributed to its production by the tumor. This was in accordance with the present results by the positive correlation between tumor size and TGF-β1.

Moreover, Okumoto et al, showed overexpression of TGF-β1 in HCC tissues which correlated well with carcinogenesis and tumor progression [38].

In clinical cases where high TGF-β1 could be correlated with tumor, attempts to decrease or inhibit TGF-β1 action by blocking its receptors may be used to treat advanced or metastatic disease [39].

In the current work, a significant positive correlation was found between TGF-β1 and VEGF. This finding was in accordance with **Chun *et al.***, who clarified that TGF-β1 can activate macrophages to express angiogenic mediators such as VEGF [40].

Conclusion

Serum TGF-β1 and VEGF reflect well the degree of hepatic dysfunction. Their serial measurements might be of diagnostic and predictive value for occurrence of HCC in patients with chronic HCV induced liver cirrhosis.

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