Soluble Vascular Endothelial Growth Factor and its Soluble Receptor 1 in Patients with Post-Hepatitis C Virus Liver Cirrhosis


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Abstract

Background: Vascular endothelial growth factor (VEGF) is a well-known mediator of physiological and pathological angiogenesis and vascular permeability. Vascular endothelial growth factor might be involved in cirrhosis-associated angiogenesis as well as fibrogenesis. There are conflicting results concerning plasma VEGF level in cirrhosis.

Aim: Evaluate the plasma concentration of VEGF and its soluble receptor 1 and their possible association with liver function impairment in post-hepatitis C virus liver cirrhosis patients in attempting to select patients with refractory ascites and who responding to diuretics.

Patients and Methods: A case-control study was conducted on eighty subjects, forty of them were patients with post-hepatitis C virus liver cirrhosis (cases) and another forty were free from hepatitis C virus (controls). Patients were divided into two equal groups according to modified Child Pugh scoring system for cirrhosis. Vascular endothelial growth factor and its soluble receptor 1 were measured in plasma by ELISA.

Results: The mean levels of serum VEGF and its soluble receptor 1 were 145.85 ± 23.78 (pg/ml) and 1.36 ± 0.61 (pg/ml) in Child B, 118.55 ± 39.72 (pg/ml) and 1.84 ± 0.87 (pg/ml) in Child C, 234.98 ± 60.80 (pg/ml) and 0.62 ± 0.26 (pg/ml) in control group. There were statistically significant increase of VEGF level in control group in comparison to patients (p-value <0.05). Moreover, there were statistically significant decrease of its soluble receptor 1 in control group in comparison to patients (p-value <0.05). Plasma VEGF and its soluble receptor 1 showed significant correlation with liver function test and portal hypertension manifestation.

Conclusion: Our result suggest down-regulation of VEGF in chronic hepatitis C patient with manifestation of portal hypertension.

Key Words: Vascular endothelial growth factor – Hepatitis C – Liver cirrhosis – Portal hypertension.

Introduction

EGYPT has the highest prevalence of anti-bodies in the world estimated nationally at 14.7%, an estimated 9.8% are chronically infected [1]. In more than 80% of infected person HCV infection can induce persistent hepatic injury which leads to disease progression from periportal inflammation to chronic hepatitis with bridging fibrosis, to frank cirrhosis [2].

Angiogenesis occurs in the liver of patients infected with hepatitis B or C virus [3]. The pathophysiological significance of chronic viral hepatitis-associated angiogenesis is unclear; it has been proposed to exert a beneficial role by contributing to tissue repair and regeneration after liver damage [4]. It has also been suggested to represent a risk factor for progression to hepatocellular carcinoma in patients with chronic hepatitis C [5].

The process of angiogenesis in chronic liver disease starts with the development of fibrosis, lying down of extracellular matrix leading to hypoxia which acts as a stimulus for neovascularization [6].

Vascular endothelial growth factor (VEGF), a glycosylated peptide with multiple isoforms generated by differential mRNA splicing, potently induces endothelial proliferation on binding with high affinity to one of its two kinase receptors: Kinase insert domain receptor (KDR) or the fms-like tyrosine kinase receptor (Flt-1 or VEGFR1). Also, VEGF contributes to angiogenesis by increasing vascular permeability through disorganization of endothelial junctional proteins [7].

VEGF production is known to be regulated by various substances, including oxygen, steroid hormones, reactive oxygen metabolites and protein kinase C agonists [8].
Vascular endothelial growth factor receptor 1 is a positive regulator of monocyte and macrophage migration, and has been described as a positive and negative regulator of VEGFR2 signalling capacity. Negative regulation is exerted, at least in part, by an alternatively spliced soluble VEGFR 1 variant that binds to VEGF and thereby prevents VEGF from binding to VEGFR2. VEGFR2 is implicated in all aspects of normal and pathological vascular-endothelial-cell [9]. It has been shown that the VEGF/VEGFR system has multiple functions, such as the induction of tumour metastasis, inflammation, neuroprotection, protection of liver and mobilization of marrow-derived stem cells, as well as lymphangiogenesis [10]. An up regulation of VEGF in cirrhotic livers was demonstrated in animal models [11] as well clinical studies and linked with ongoing angiogenesis and portal hypertension [12].

The aim of the present study was to evaluate the plasma concentration of VEGF and its soluble receptor [1] and their possible association with liver function impairment in post-hepatitis C virus cirrhotic patients in attempting to select patients with refractory ascites and who responding to diuretics.

Patients and Methods

A case-control study was conducted on eighty subjects, forty of them were patients with chronic hepatitis C virus with cirrhosis (cases) and another forty were free from hepatitis C virus (controls). Cases were selected randomly from the attendants of the Tropical and Internal Medicine Departments of Al Zahraa University Hospital, Cairo, Egypt from January 2013 to June 2013. Patients were divided into two equal groups according to modified Child Pugh scoring system for cirrhosis Table (I) [13].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascites</td>
<td>None</td>
<td>Slight</td>
<td>Moderate/severe</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>None</td>
<td>Slight/moderate</td>
<td>Moderate/severe</td>
</tr>
<tr>
<td>Bilirubine (mg/dl)</td>
<td>&lt;2.0</td>
<td>2.3</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>&gt;3.5</td>
<td>2.8-3.5</td>
<td>&lt;2.8</td>
</tr>
<tr>
<td>Prothrombine time (sec. increased)</td>
<td>1-3</td>
<td>4-6</td>
<td>&gt;6.0</td>
</tr>
<tr>
<td>Total numerical score:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child pugh class:</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
</tbody>
</table>

Inclusion criteria for cases:

Middle age adult (40-65 years old), chronic H.C.V. liver diseases as proved clinically, ultrasonography and laboratory by ELISA technique.

Exclusion criteria for cases:

Chronic hepatitis B patients, Hepatocellular carcinoma patients, alcoholic patients, ischemic heart diseases and renal impairments patients.

Inclusion criteria for controls:

The main inclusion criterion for controls “were being free from HCV” which was proved clinically, ultrasonography and laboratory by ELISA technique. Free from any liver affection (normal liver function). They were age matched to the cases. Matching was carried out by grouping every 5 years.

After explaining the purpose of the study and taking the consents of both cases and controls; data were collected through a personal interview.

The studied subjects were subjected to the following processes:

- Full history taking and thorough clinical examination.
- Investigation were done in the form of complete blood picture by culter, liver function tests by auto-analyzer instrument, Prothrombine time, fasting and post-prandial blood sugar level, kidney function tests.
- Abdominal ultra-sonography examination and E.C.G. examination.
- Laboratory investigations in the form of hepatitis markers for hepatitis C. and B. By ELISA technique by Bio-rav Kits to isolate hepatitis C virus cases and confirm healthy controls.
- Alpha-fetoprotein level by chemilluminace method lot (171 86602) by Roche diagnostics.

Measurement of serum levels of VEGF and its soluble receptor [1]:

The Quantikine Human VEGF Immunoassay (Catalog Number DVE00, SVE00) is a 4.5 hour solid phase ELISA designed to measure VEGF 165 levels in cell culture supernates, serum, and plasma. The assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for VEGF has been pre-coated onto a microplate. VEGF isoforms are differentially expressed during development and in the adult [14].

VEGF dimers bind to two related receptor tyrosine kinases, Soluble VEGF Receptor [1]
(sVEGF-R 1) also called (Flt- 1) and VEGF R2 (Flk-1/KDR), and induce their homodimerization and autophosphorylation. Circulating VEGF levels correlate with disease activity in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus \[15\].

The Quantikine Human VEGF Immunoassay contains Sf 21-expressed recombinant human VEGF 165 and antibodies raised against the recombinant protein. Results obtained for naturally occurring human VEGF and recombinant human VEGF 121 showed linear curves that were parallel to the standard curves obtained using the Quantikine Human VEGF Immunoassay standards. These results indicate that this kit can be used to determine relative mass values for natural human VEGF [16].

Ab119567 VEGF Receptor 1 Human ELISA Kit Abcam Inc. (with color giving dyes) is an enzyme-linked immunosorbent assay for the quantitative detection of human soluble VEGF receptor 1 by Sandwich ELISA. Soluble VEGF-R1 is a naturally occurring endogenous form of the VEGF-R1. It is generated by differential splicing of the flt- 1 gene. In vitro sVEGF-R1 is used to inhibit VEGFA mediated signals in endothelial cells and in vivo it can be used to block physiological angiogenesis in several organs as in the ovary or in bones. Tumor cells transfected with the flt- 1 gene are growth restricted in vivo because of the limitation in developing tumor blood vessels via VEGFA signaling [17].

**Sampling:**

10ml venous blood was taken then put 4ml in vacuum tube with EDTA to measure complete blood picture and prothrombin time. Also, 2ml put in vacuum tube with EDTA which centrifuged for 15 minutes at 1000xg. Remove the plasma and stored at \(\leq -20^\circ\)C for measuring VEGF levels and sVEGF-R1.

The rest of 4ml blood sample put in a serum separator tube. Allow the blood samples to clot for 30 minutes before centrifugation for 15 minutes at 1000xg. Remove serum into two tubes. One of them assays immediately for bilirubin, albumin, and liver function tests, fasting blood sugar level and kidney function tests.

The other tube stored at \(\leq -20^\circ\)C for measuring Alpha-fetoprotein level by chemilluminescent method by Roche diagnostics (171 866-02) by using cobas c411 instrument.

**Statistical methods:** Coding and statistical analysis of collected data were done using SPSS program (statistical package of social science) version 15.

**A- Descriptive statistics:** Mean and standard deviation (SD) were calculated to measure central tendency and dispersion of quantitative data.

**B- Analytic statistics:** Comparing groups was done using: Kruskal wallis test: For comparison of non parametric data between more than two groups. Analysis of variance (ANOVA) test for comparison of quantitative parametric data between more than two groups. Correlation Coefficient was done to determine the association between two variables. The level of significance was taken at \(p\)-value of <0.05.

**Results**

The studied sample included eighty subjects. Forty of them were chronic hepatitis C virus patients with cirrhosis who were compared to equal numbers of normal (controls). Cases were divided into two equal groups as Group 1 was (ChildB) and Group 2 was (Child C) according to modified Child Pugh scoring system for cirrhosis.

Table (1) demonstrates the mean levels of bilirubine, Prothrombine time, and albumin among the three groups. Control subjects has the lowest levels of both bilirubine and Prothrombine time (Mean±SD=0.53±0.15 and 12.86±0.38 respectively), while Albumin among them was measured to be of the highest level (4.28±0.43) followed by group 1 (2.54±0.44), then group 2 (2.10±0.36) with statistically significant differences (\(p\)-value <0.05).

Table (2) shows the mean levels of serum VEGF and s VEGF receptor 1 among the studied groups. There were statistically significant increase of VEGF level in control group in comparison to group 1 and group 2 (cases), also, there were statistically significant increase of VEGF level in group 1 in comparison to group 2 (\(p\)-value <0.05). Moreover, there were statistically significant decrease of s VEGF receptor 1 in control group in comparison to group 1 and group 2 (cases), also, there were statistically significant decrease of s VEGF receptor 1 in group 1 in comparison to group 2 (\(p\)-value <0.05).

Table (3) illustrates the correlation between VEGF and both ascites and hepatic encephalopathy. There were significant negative correlations between VEGF and both ascites and hepatic encephalopathy (\(p\)-value <0.05). Also, it portrays the correlation between sVEGFR1 and both ascites
and hepatic encephalopathy. There were significant positive correlations between sVEGFR1 and both ascites and hepatic encephalopathy (*p-value <0.05).

Table (4) shows the correlation between VEGF and albumin, bilirubine, and prothrombine time. There were significant negative correlations between VEGF and both bilirubine and prothrombine time.

Table (1): Mean levels of bilirubine, prothrombine time, and albumin among the studied groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 Child B</th>
<th>Group 2 Child C</th>
<th>Control Group</th>
<th>Statistical test (one-way ANOVA)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubine mg/dl</td>
<td>1.80±0.65</td>
<td>3.63±1.62</td>
<td>0.53±0.15</td>
<td>F=83.88</td>
<td>0.000*</td>
</tr>
<tr>
<td>Prothrombine time</td>
<td>16.76±1.68</td>
<td>20.40±4.14</td>
<td>12.86±0.37</td>
<td>F=220.87</td>
<td>0.000*</td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>2.54±0.44</td>
<td>2.10±0.36</td>
<td>4.28±0.38</td>
<td>F=78.39</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

* Significant difference (*p-value <0.05).

Table (2): Mean levels of serum VEGF and sVEGFR1 among the studied groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 Child B</th>
<th>Group 2 Child C</th>
<th>Control Group</th>
<th>Statistical test (Kruskal Wallis test)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pg/ml)</td>
<td>145.85±23.78</td>
<td>118.55±39.72</td>
<td>234.98±60.80</td>
<td>50.75</td>
<td>0.000*</td>
</tr>
<tr>
<td>VEGFR1 (pg/ml)</td>
<td>1.36±0.61</td>
<td>1.84±0.87</td>
<td>0.62±0.26</td>
<td>46.10</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Table (3): Correlation between VEGF and VEGF1 with both ascites and hepatic encephalopathy.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation coefficient (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF and Ascites</td>
<td>-0.82</td>
<td>0.000*</td>
</tr>
<tr>
<td>VEGF and HE</td>
<td>-0.40</td>
<td>0.011*</td>
</tr>
<tr>
<td>VEGF1 and Ascites</td>
<td>0.58</td>
<td>0.000*</td>
</tr>
<tr>
<td>VEGF1 and HE</td>
<td>0.40</td>
<td>0.010*</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (2-tailed).

Table (4): Correlation between VEGF and VEGF1 with albumin, bilirubine, and prothrombine time.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation coefficient (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF and bilirubine mg/dl</td>
<td>-0.53</td>
<td>0.000*</td>
</tr>
<tr>
<td>VEGF and prothrombine time sec</td>
<td>-0.47</td>
<td>0.000*</td>
</tr>
<tr>
<td>VEGF and albumin g/dl</td>
<td>0.69</td>
<td>0.000*</td>
</tr>
<tr>
<td>VEGF1 and bilirubine mg/dl</td>
<td>0.56</td>
<td>0.000*</td>
</tr>
<tr>
<td>VEGF1 and prothrombine time sec</td>
<td>0.38</td>
<td>0.003*</td>
</tr>
<tr>
<td>VEGF1 and albumin g/dl</td>
<td>-0.63</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Table (4): Correlation between VEGF and VEGF1 with albumin, bilirubine, and prothrombine time.

Discussion

Vascular endothelial growth factor is a specific mitogen for endothelial cells. In contrast to other cytokines, VEGF stimulates endothelial cell proliferation, acting as a circulating hormone rather than a paracrine factor [18]. At the liver level, VEGF is significantly expressed by sinusoidal endothelial cells and hepatocytes [19,20].

Our results show down regulation of VEGF in cirrhotic livers patient, these results may be due to VEGFR-1 is a negative regulator of angiogenesis during early development, but plays an important role in angiogenesis under pathological conditions.

Also due to VEGFR-1 -blocking antibodies prevent the migration but not proliferation of HUVECs (human vascular endothelial cells) indicating the involvement of VEGFR-1 in endothelial cell migration [21]. VEGF production is known to be down-regulated by several substances including angiostatin, and endostatin [22].

Thus, the low serum VEGF in these patients may be related to the increased activity of these inhibitors [23].

Our results agree with Assy, et al., [24] who studied fifty-three patients with HCV, HBV, and cryptogenic liver cirrhosis their patients were classified according to Child-Pugh-s class, they found that expression of VEGF in the serum is down-regulated in the presence of moderate to severe portal hypertension. This finding supports the notion that decreased VEGF serum levels are related to not only increased portal hypertension but also reduced regenerative capacity. This has
an important clinical implication since the clinical outcome of these patients may be related to liver regenerative capacity.

Serum VEGF concentrations are decreased in chronic liver diseases, such as chronic hepatitis and liver cirrhosis, which suggests that VEGF levels might correlate with disease severity [25].

Also, Saphr et al., [26] showed that prolonged administration of octreotide in liver cirrhosis decreased hepatic vein pressure gradient (HVPG) which was associated with the decrease in circulating VEGF, these suggests an improvement in splanchnic hyperemia.

Our results are in contrary with Jaroszewicz et al., [27] who measured VEGF in 78 patient with liver cirrhosis (alcohol related liver cirrhosis, primary biliary cirrhosis, HCV, HBV and autoimmune hepatitis) showed up regulation of VEGF in cirrhotic livers, these results indicate possible association between VEGF signalling pathway and enhanced angiogenesis during liver cirrhosis and portal hypertension results from a combination of increased intrahepatic vascular resistance and increased blood flow through the portal venous system, different mechanisms have been proposed to be involved in its pathogenesis. However,our results show increase of VEGF-R1. These results are in consistent with Jaroszewicz et al., [27] who found increase concentration of VEGF-R1 with advanced stages of liver cirrhosis, which was reflected by a positive correlation with Child-Pugh score. Also, they observed highest concentration of VEGF and VEGF-1 in patients with refractory ascites and correlated with the degree of liver insufficiency.

Recommendations: Further prospective studies on a large number of patients to evaluate levels and correlations of VEGF and VEGF-R1 to manifestations of portal hypertension, for possible Hepatocellular carcinoma development and their possible role in treatment of complications of post-hepatitis C virus cirrhosis.

References


