Vascular endothelial growth factor and hepatic neoangiogenesis in hepatitis C associated chronic liver disease

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Abstract

Background
Worldwide Egypt had the highest prevalence of hepatitis C virus infection. Angiogenesis is a complex process that regulated by many factors, including vascular endothelial growth factor (VEGF).in the liver HSCs, Kupffer cells, regenerating hepatocytes and existing endothelial cells are responsible for the process of neo angiogenesis and production of vascular endothelial growth factor (VEGF).

Aim of the work: To detect the significance vascular endothelial growth factor and its relation to hepatic neoangiogenesis in hepatitis C associated chronic liver disease.

Methods: A total of 70 adult patients with chronic hepatitis C infection in various stages with no evidence of cirrhosis, were recruited for the study. We studied the expression of VEGF and vascular density in liver specimens from chronic HCV infected patients using a computer-based analysis of immunohistochemical staining and confirmed it by Western Blot.

Results: Relation between stage of fibrosis and laboratory finding was done there were significant relation between the stage of fibrosis and platelet count, also, the level of liver enzyme (AST and ALT) significantly related to the fibrosis stage, Serum albumin significantly related to fibrosis stage. The most important findings that VEGF level were significantly related to fibrosis stage.

Conclusion: Angiogenesis was present in 45.5% cases of chronic liver disease. It was proportional to the increase in stage of fibrosis. Expression of VEGF was commonly found in early stages of fibrosis.

Introduction

One of the most common life-threatening condition affect the Egyptian population is hepatitis c virus infection [HCV], worldwide Egypt is considered as one of the most common country affected by the virus and it had the highest prevalence of hepatitis C virus infection [1].

Angiogenesis, the formation of new blood vessels, occurs in various liver diseases such as hepatocellular carcinoma, it was also described in chronic hepatitis c virus infection [2]. Angiogenesis is a complex process that regulated by many factors, including vascular endothelial growth factor (VEGF) [3]. In the liver HSCs, Kupffer cells, regenerating hepatocytes and existing endothelial cells are responsible for the process of neo angiogenesis and production of vascular endothelial growth factor (VEGF)[4].

There is no clear relation between Chronic Hepatitis C (CHC) and angiogenesis, the aim of this study is to detect the significance of vascular endothelial growth factor and its relation to hepatic neoangiogenesis in hepatitis C associated chronic liver disease.

Material and methods

Patients: A total of 70 adult patients with chronic hepatitis C infection in different stages of hepatic fibrosis, their ages ranged from 20-59 years (40.5 ± 11.4 SD), were recruited for the study. All patients were selected from the outpatient clinic or the inpatient section of the Tropical Medicine and Gastroenterology Department, Sohag University Hospital. The study protocol was approved by the Ethics committee of Sohag Faculty of Medicine. Informed written consent was obtained from all participants. The diagnosis of chronic HCV will be based positive HCV antibody by ELISA test and HCV RNA by PCR for more than 6 months. All the patients had abdominal ultrasonography.

Exclusion criteria were: Co-infection with hepatitis B or HIV infection or Schistosomiasis, Patients with liver cirrhosis, Alcoholic liver disease, Metabolic liver diseases as Wilson's disease and haemochromatosis and nonalcoholic fatty liver disease, Autoimmune hepatitis, Vascular alterations (i.e., portal/arterial thrombosis or stenosis).

Histopathological assessment: Seventy liver biopsies were included in the study and submitted to histopathological examination to assess both the grade and the stage of chronic viral hepatitis, using the METAVIR classification system [5], which evaluates fibrosis stage in a scale from F0 to F4. Necro-inflammatory activity will be scored with a scale from A0 to A3.

Evaluation of immunostaining findings: VEGF protein expression appeared as dark brown granular staining in the cytoplasm of hepatocytes was considered a positive finding (figure 1). The immunoreactive score (IRS) was determined by multiplying an estimate of the percentage of the immunoreactive cells (quantity score; QS) with an estimate of the staining intensity (intensity score; IS).

Statistical analysis:

Data was analyzed using SPSS computer program version 22.0. Quantitative data was expressed as means ± standard deviation, median and range. One-Way ANOVA test was used for normally distributed data. Spearman's correlation was used for testing of correlation between different quantitative variables. Chi-Square test was used for comparison between qualitative variables. P-values 0.05 were considered statistically significant.
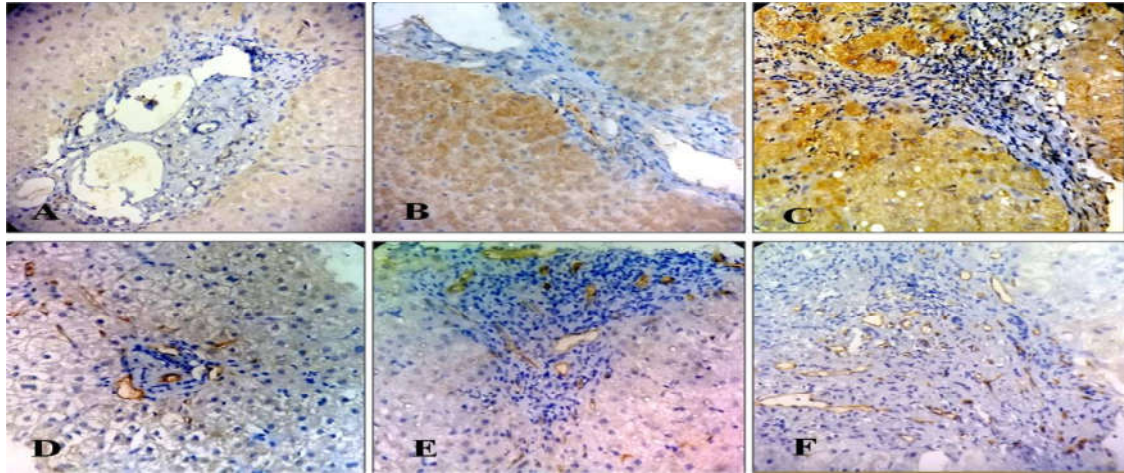
Figure 1: Cytoplasmic expression of VEGF in different grades of inflammation (A) and different stages of fibrosis (F); weak expression in A1F1 chronic viral hepatitis (A), moderate expression in A2F2 (B) and strong expression in A3F3 (C). CD34 expression in cell membrane of endothelial cells in portal tracts; the mean of MVD is 5 microvessel in A1F1 chronic viral hepatitis (D) the mean of MVD is 17 microvessel in A2F2 (E) and the mean of MVD is 57 microvessel in A3F3 (Original magnification is 400X). MVD =

Results

From September 2014 to august 2016, 70 adult patients with chronic hepatitis C infection had fulfilled our inclusion criteria and were included in this study. their ages ranged from 20-59 years (40.5 ± 11.4 SD). 42 males, 28 females. All the patients had complete laboratory investigation.

Table [1]: Comparison of VEGF level according to gender, clinical, radiological data and HCV PCR of the studied patients

Parameter	Mean ± SD	P-value
Gender **Males** **Females**	**8.7 ± 3.1** **8.9 ± 3.1**	**0.843**
History **RT hypochondrial pain (2)** **Accidently discovered (68)**	**7.5 ± 2.1** **8.8± 3.1**	**0.516**
Examination **Free (60)** **Diabetes (5)** **Hypertension (2)** **Hypertension &Diabetes (3)**	**∧.5 ± 3.1** **9.6 ± 3.3** **10.5 ± 0** **11.3 ± 1.2**	**0.291**
Liver ultrasonography **Bright liver (9)** **Bright coarse liver (10)** **Bright enlarged liver (6)** **Coarse liver (15)** **Enlarged liver (2)** **Normal (28)**	**9.7 ±2.9** **١٠.6 ±2.1** **∧.2 ±3.7** **∧.1 ±3.6** **5.5 ±3.5** **8.6 ±11.4**	**0.183**
Spleen ultrasonography **Normal (63)** **Mild splenomegaly (7)**	**8.9 ± 2.9** **8.1 ± 14.7**	**0.875**
HCV PCR		
Low viremia (4) **Moderate viremia (15)** **High viremia (51)**	**9.7 ± 4.5** **9.3 ± 2.6** **8.6 ± 3.1**	**0.532**

There was no significant relation between VEGF and laboratory parameter of the study population.

Table [2]: Correlation between VEGF and laboratory parameters of the studied patients (n= 70).

Group	r	P-value
HB (g/dl)	**0.085**	**0.485**
RBCS (x1,000,000/mm3)	**0.154**	**0.202**
WBCS (x1000/mm3)	**-0.154**	**0.202**
Platelets (x1,000/mm3)	**-0.131**	**0.279**
ALT (IU/l)	**0.110**	**0.366**
AST (IU/l)	**0.083**	**0.495**
Bilirubin (mg/dl)	**-0.030**	**0.807**
Albumin (mg/dl)	**-0.051**	**0.676**
Prothrombin time (sec)	**0.057**	**0.641**
Prothrombin concentration	**-0.166**	**0.169**
Alpha fetoprotein	**0.194**	**0.108**
HCV PCR	**-0.171**	**0.156**

Relation between stage of fibrosis and laboratory finding was done there were significant relation between the stage of fibrosis and platelet count, also, the level of liver enzyme (AST and ALT) significantly related to the fibrosis stage, Serum albumin significantly

related to fibrosis stage. The most important findings that VEGF level were significantly related to fibrosis stage.

Table [3]: Relation between fibrosis stage and laboratory data of the studied patients.

Parameter	Fibrosis stage			P-value
	F1 (N= 28)	F2 (N= 22)	F3 (N= 20)	
HB (g/dl) Mean± S.D.	14.4 ± 1.5	14.1 ± 2.1	13.9 ± 1.8	0.640
RBCS (x1,000,000/mm³) Mean± S.D.	5.5 ± 0.9	5.1 ± 0.7	5.2 ± 0.8	0.305
WBCS (x1000/mm³) Mean± S.D.	6.9 ± 2.2	7.4 ± 3.5	5.7 ± 1.5	0.142
Platelets (x1,000/mm³) Mean± S.D.	244.3 ± 62.1	257.9 ± 65.6	201.6 ± 55.9	0.011
ALT (IU/l) Mean± S.D.	46.2 ± 81.3	41.8 ± 26.4	73.2 ± 45.9	0.001
AST (IU/l) Mean± S.D.	33.6 ± 28.8	41.7 ± 19.9	61.9 ± 32.9	< 0.001
Bilirubin (mg) Mean± S.D.	0.7 ± 0.3	1.6 ± 3.7	0.7 ± 0.3	0.582
Serum albumin (mg/dl) Mean± S.D.	4.2 ± 0.7	4.1 ± 0.4	4.01 ± 0.3	0.605
Prothrombin time (sec) Mean± S.D.	11.9 ± 2.04	11.6 ± 0.9	12.6 ± 0.8	0.001
Prothrombin concentration Mean± S.D.	93.3 ± 12.6	96.6 ± 9.3	86.4 ± 7.6	< 0.001
Alpha fetoprotein Mean± S.D.	3.05 ± 1.3	5.01 ± 5.1	25.6 ± 38.4	0.001
HCV PCR Low viremia (%) Moderate viremia(%) High viremia (%)	0 (0.0%) 8 (28.6%) 20(71.4%)	1 (4.5%) 4 (18.2%) 17 (77.3%)	3 (15%) 3 (15%) 14 (70%)	0.205
VEGF level Mean± S.D.	9.1 ± 2.6	7.5 ± 3.3	9.9 ± 2.7	0.045

Discussion

Angiogenesis or the process of new vessel formation that accompanies chronic hepatitis C virus infection is integral part of tissue remodeling and provide a portal of entry for the continuing recruitment of inflammatory cells [6].

Angiogenesis had a beneficial role in the process of tissue repair and regeneration after liver necrosis [7]. Although the mechanism and pathophysiological changes that accompanies chronic hepatitis C virus infection are still unclear [8]. The present study investigated the expression of VEGF in liver tissue from patients with CHC to evaluate their role in disease progression. In this study, there is meaningful relationship between VEGF expression in liver biopsy and fibrosis stage.

These results are supported by the results of the study that done by Hassan et al. who reported increased VEGF immunostaining of liver biopsy specimens from HCV infected populations. They suggested that VEGF production is stimulated by HCV infection through a mechanism including stabilization of hypoxia inducible factor-1α (HIF-1α) [9].

Also, the study done by Abe et al., [10] demonstrated that HCV core protein has the distinct potential to up regulate and sustain HIF-1α expression under hypoxia, thereby contributing to increased VEGF expression, through the HCV core/NF-kB axis). Abe et al., [10] reported that the distinct angiogenic potential of HCV core protein under hypoxia might help transformed cells survive in the hypoxic environment of the cirrhotic liver by supplying them with oxygen and nutrients. Moreover, HCV-infected hepatocytes secrete VEGF, which induces a localized depolarization of hepatocytes that promotes viral transmission between adjacent hepatocytes [11].

On the contrary side, the results of this study disagree with the study done by Mukozu, et al., [12] who found that there was no significant difference between the control group and the CHC group as regards VEGF.

Also, the results of this study similar to the studies have done by of Helaly and AbouShama [13] and Janczewska-Kazek et al [14]. And Sieghart et al. [15]. Moreover, Brdoskey et al found that in the initial stages of fibrosis, the production of VEGF and the neovascularization increases; whereas in the late stages, cirrhotic nodules in hepatitis C patients are characterized by decreased density of micro vasculature and decreased VEGF production. [16].

In this study, no significant correlation between liver enzymes (AST and ALT) and VEGF P value=0.279 and 0.366 respectively. This matches with the result of the study done by Mohsen M. Maher, MD et al. who found no significant correlation between ALT and serum VEGF [17]. Mukozu et al., [12] found no significant correlation between VEGF levels and the degree of hepatic dysfunction. This could be explained by the fluctuations that occur in liver enzymes during the disease period [18]. Also, it is well known that in liver cirrhosis due to viral hepatitis serum levels of AST is usually higher than ALT with higher AST/ALT ratio [19].

Absence of any elevation does not rule out significant injury or hepatic fibrosis. Liver enzyme tests do not reveal the true status of hepatic function [20]. On the other hands, our results disagree with the study of Talaat who found clear

correlation between liver enzymes, which are surrogate markers of liver disease, and serum VEGF, supporting the claim that hepatocellular damage leads to marked VEGF release into the blood stream [21].

In our study, no sincere relationship between tissue VEGF and serum albumin level and This was in accordance with the study of Assy et al. who found no significant correlation between serum VEGF and serum albumin and they suggested that serum VEGF didn't reflect hepatic synthetic function [22]. Although the study of Mohsen, et al. and Yao et al. who found a highly significant negative correlation between serum VEGF and serum albumin level [17,23].

Principally, serum VEGF is a combination of both VEGF released from platelets and the circulating plasma VEGF. Platelets may normally contain a certain amount of VEGF in their granules. Therefore, serum VEGF level can be influenced by platelet count [24] In the present study, no significant correlation between VEGF and platelet counts and this was similary to the study of Assy et al the study of Mohsen, et al[17,22] suggesting that VEGF is not stored in platelets only.

On the contrary Kim et al. [25] found a significant correlation between serum VEGF and platelet count in HCC patients, they didn't find a similar correlation in liver cirrhosis patients. They suggested that serum VEGF/platelet count might be used as an indicator of the development of HCC in patients with liver cirrhosis during their follow up [25]. Also, Gunsilus and Gastl, found a significant correlation between peripheral platelet count and absolute value of serum levels of VEGF in their work [26]

Conclusion: Angiogenesis have a role in development of liver fibrosis and carcinogenesis in chronic HCV infection. Neovascularization was shown to be correlated with VEGF and it increased significantly with progression of liver fibrogenesis and carcinogenesis. Hence understanding the process of angiogenesis is of great help in developing new therapeutic approaches for the chronic liver disease patients.

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