Serum Vascular Endothelial Growth Factor (VEGF) Levels in Liver Diseases

Karaciğer Hastalıklarında Serum Vasküler Endotelyal Büyüme Faktörü (VEGF) Düzeyleri

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Geliş Tarihi/*Received:* 12.04.2010
Kabul Tarihi/*Accepted:* 20.12.2010

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ABSTRACT Objective: Vascular endothelial growth factor (VEGF) is a protein with 45 kDA molecular weight, the encoding gene is located at the short arm of 6th chromozome (6p12). VEGF levels and the role of VEGF in the pathophysiology of several liver diseases such as liver cirrhosis, acute hepatitis, chronic hepatitis, hepatocellular carcinoma, and other benign liver diseases have been investigated. We investigated the clinic importance of VEGF levels in the evaluation of liver diseases, together with other markers of liver function. **Material and Methods:** We included 126 patients with diagnosed liver disease due to nonalcoholic fatty liver disease, acute hepatitis, chronic hepatitis, liver cirrhosis. The control group included 28 healthy adults. All patients were evaluated with their disease history, biochemical blood tests, viral markers, autoantibodies and abdominal ultrasound. Blood samples were collected from the patients when the liver disease was detected. The VEGF level was determined by Biosource Human VEGF Immunassay Kit with ELISA. **Results:** A total of 126 patients (68 men/58 women) were evaluated. Median age of patient group was 47. Thirtytwo patients had liver cirrhosis (25.4%), 10 acute hepatitis (7.93%), 72 chronic hepatitis (57.1%) and 12 nonalcoholic fatty liver disease (9.5%). Twentyeight (7 men/ 21 women) healthy controls were included in control group. The median age was 46.6. We found significantly higher serum VEGF levels in acute hepatitis and nonalcoholic fatty liver disease patients. Contrary to the consensus achieved in acute hepatitis, there are inconsistencies regarding serum VEGF levels reported in chronic hepatitis and liver cirrhosis. We also found no significant difference in serum VEGF levels between patients with liver cirrhosis and chronic hepatitis. **Conclusion:** Our results suggest that VEGF levels may be affected by acute hepatitis and nonalcoholic fatty liver disease, but not liver cirrhosis and chronic hepatitis.

Key Words: Fatty liver; hepatitis; liver; liver cirrhosis; vascular endothelial growth factor

ÖZET Amaç: Vasküler endotelyal büyüme faktörü (VEGF) 45 kDA ağırlığında 6. kromozomun kısa kolu (6p12) tarafından kodlanan protein yapıda büyüme faktörüdür. VEGF düzeyi ve karaciğer sirozu, akut hepatit, kronik hepatit, karaciğer kanserleri ve benign karaciğer hastalıkları gibi karaciğer hastalıklarının patofizyoljisindeki rolü değerlendirilmiştir. Bu çalışmada VEGF düzeylerinin karaciğer hastalıklarındaki klinik önemini diğer karaciğer fonksiyon testleri ile birlikte değerlendirdik. **Gereç ve Yöntemler:** Akut hepatit, kronik hepatit, nonalkolik yağlı karaciğer hastalığı, ve karaciğer sirozu tanılı 126 hasta ve kontrol grubu olarak 28 sağlıklı erişkin çalışmaya alındı. Hastalar anamnezleri, biyokimyasal testler, viral belirteçler, otoantikorlar ve abdominal ultrasonografi ile değerlendirildi. Tanı anında hastalardan serum örnekleri toplandı. VEGF düzeyleri Biosource Human VEGF Immunassay Kiti ile ölçüldü. **Bulgular:** Toplam 126 (68 erkek/58 kadın) hasta değerlendirildi. Hasta grubunun ortanca yaşı 47 idi. Otuz iki (%25.4) hastanın karaciğer sirozu, 10 (%7.93) hastanın akut hepatit, 72 (%57.1) hastanın kronik hepatit ve 12 (%9.5) hastanın nonalkolik yağlı karaciğer hastalığı mevcuttu. Yirmi sekiz (7 erkek/21 kadın) sağlıklı gönüllüden kontrol grubu oluşturuldu. Ortanca yaş 46.6 idi. Akut hepatit ve nonalkolik yağlı karaciğer hasta gruplarında istatistiksel olarak anlamlı yüksek VEGF düzeyleri saptandı. Bunun yanı sıra, kronik hepatit ve karaciğer sirozlu hastalarda serum VEGF düzeyleri arasında anlamlı fark saptanmadı. **Sonuç:** Bizim sonuçlarımız serum VEGF düzeylerinin karaciğer sirozu ve kronik hepatitten farklı olarak akut hepatit ve nonalkolik yağlı karaciğer hastalıklarından etkilenebileceğini göstermiştir.

Anahtar Kelimeler: Yağlı karaciğer, hepatit; karaciğer; karaciğer sirozu; vasküler endotelyal büyüme faktör A

Turkiye Klinikleri J Gastroenterohepatol 2010;17(2):76-80

Vascular endothelial growth factor (VEGF) is a protein with 45 kDA molecular weight, the encoding gene is localized at the short arm of 6th chromozome (6p12). Megakaryocytes are important sources for VEGF is stored in the alfa granules of platelets[1]. It is the most important factor in physiologic and pathologic angiogenesis.[2,3] VEGF is a critical survival factor for quiescent endothelial cells. Besides the stimulation of vascular development via selective mitogenic effect on endothelial cells, VEGF plays an important role in morphogenesis and chemotaxis. It also increases vascular permeability and this effect is 50.000 times stronger than that of histamine. This effect leads to protein extravasation, the development of fibrin matrix and invasion of stromal cells.[4-7]

Sinosoidal endothelial cells and hepatocytes synthesize VEGF in the liver. Variable amounts of expression have been reported in Kuppfer cells. An important role has been shown in liver regeneration.[8,9] VEGF levels and its role in the pathophysiology of several liver diseases such as liver tumours, liver cirrhosis (LC), acute hepatitis (AH), and chronic hepatitis (CH) have been evaluated.[10-13] When angiogenesis decreases, reparation also decreases substantially. Acute hepatocellular damage results in secretion of VEGF into the blood whereas, in chronic liver diseases (CLD) such as CH and LC, serum VEGF concentrations decrease, indicating that there might be a correlation between VEGF levels and the severity of CLD.[11] Vascular growth factor levels, such as VEGF and FGF-beta (b) were found higher[12], on the contrary lower[11] in different studies. In the present study, we aimed to evaluate the clinical importance of VEGF levels and other liver function tests in patients with various liver diseases.

MATERIAL AND METHODS

A total of 126 patients, 68 men and 58 women, followed for a liver disease categorized into 4 groups as LC, AH, CH and nonalcoholic fatty liver disease (NAFLD). The control group included 28 healthy adults. All patients were evaluated with their disease history, biochemical blood tests, viral markers, autoantibodies and abdominal ultrasound.

Patients with acute hepatitis were diagnosed with anti-HAV IgM, anti-HBc IgM, HBsAg; chronic hepatitis and liver cirrhosis were evaluated with history (ethanole), physical examination, biochemistry, viral markers (anti-HCV, HBsAg, anti-HDV IgM), otoantibodies [anti-nuclear antibody (ANA)], smooth muscle antibody (SMA), liver kidney microsomal antibody (LKM-1), soluble liver antigen antibody (SLA), antimicrosomal antibody (AMA), copper excretion in 24 hours urine, seruloplazmin and upper abdomen ultrasonography. NAFLD was diagnosed with history (ethanole), physical examination, biochemical parameters (lipid profile) and ve upper abdomen ultrasonography.

Blood examples were collected from the patients when the liver disease was detected. The VEGF levels were determined by Biosource Human VEGF Immunassay Kit with ELISA. The linear range of the method was shown as 5-1500 pg/mL.

Because VEGF, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were not distributed normally, non-parametric tests were applied. The level of significance was set at $p< 0.05$ levels. This study protocol was conformed to the ethical guidelines of Declaration of Helsinki by Haseki Education and Research Hospital Ethical Council Regulations. Written informed consent was required for enrollment.

RESULTS

A total of 126 (68 men/58 women) patients were included. Median age of patient group was 47 (R: 16-79). 32 (25.4%) patients had LC, 10 (7.93%) AH, 72 (57.1%) CH and 12 (9.5%) NAFLD. In the control group, 28 (7 men/21 women) healthy controls were included. The median age was 46.6 (R: 27-73) (Table 1).

Median VEGF in the study group was 130.09 pg/mL (R: 5-674), mean VEGF in the healthy group was 67.93 pg/mL (R: 5-172). Kruskall Wallis Test was used since data did not distribute normally, and statistically significance was found according to disease type ($p< 0.004$). To show the

TABLE 1: Group characteristics.

	Characteristics of patients	
Number of patients	126	%
Age	47.5 (range 16-79)	
Sex		
Male	67	53
Female	59	47
Patients		
Chronic hepatitis		57.1
HBV	39	
HCV	30	
HDV	1	
Ethanol	2	
Acute hepatitis		7.9
HBV	8	
HAV	2	
Liver cirrhosis		25.3
HBV	12	
HCV	5	
Ethanol	7	
Wilson	4	
Primary bilier cirrhosis	1	
Autoimmune hepatitis	1	
Cryptogenic	2	
Nonalcoholic fatty liver disease	12	9.5

significance between groups, Mann Whitney U Test was used. The reason for this difference was related with AH and NAFLD group (Table 2).

Thirty two patients were included in to the LC group whom 15 women and 17 men. Median age was 53.5 (R: 28-79), median VEGF value was 121 pg/mL (R: 5-401). A total of 10 patients with 6 women, 4 men were in AH group. Median age was 31.5 (R: 16-56), mean VEGF value was 243.04 pg/mL (R: 5-674). A total of 72 patients with 30 women, 42 men were in CH group. Median age was 45.4 (R: 18-77), median VEGF value was 110.44 pg/mL (R: 5-471). A total of 12 patients with 9 women and 3 men were in NAFLD group. Median age was 51,25 (R: 26-77), median VEGF value was 221.10 pg/mL (R: 32-524). In the healthy group median VEGF was 67.93 pg/mL (R: 5-172).

TABLE 2: VEGF levels of groups (Kruskall Wallis Test).

Groups	n	Mean rank	Sd	χ^2	P
Liver cirrhosis	32	121	4	15.473	0.004
Chronic hepatitis	72	110.44			
Nonalcoholic fatty liver disease	12	221.10			
Acute hepatitis	10	243.04			
Control	28	67.93			

Disturbance of ethiologic reason; liver cirrhosis group included total of 32 patients associated with HBV (n= 12), HCV (n= 5), Wilson's Disease (n= 4), Ethanole (n= 7), primary biliary cirrhosis (n= 1), otoimmune hepatitis (n= 1), cryptogenic cirrhosis (n= 2). Chronic hepatitis group included total of 72 patients associated with HBV (n= 41), HCV (n= 30), HDV (n= 1), Ethanole (n=1). Acute hepatitis group included total of 10 patients associated with HBV (n= 8), HAV (n= 2). Sera were collected in icteric period from patients with acute hepatitis. Liver transplantation was not performed to any patients with acute hepatitis.

Spearman Brown Correlation Coefficients was used to show the positive correlation between serum VEGF levels of AH group and platelet counts ($p= 0.001$), ALT ($p< 0.0001$), AST ($p< 0.0001$), bilirubin ($p= 0.04$), LDH ($p= 0.041$) that were all statistically significant. There were no correlation between serum VEGF and ALP, GGT levels ($p> 0.05$).

DISCUSSION

Neovascularization increases substantially during liver regeneration.[14-16] VEGF acts an important role as an angiogenic factor in the growth of hepatic parenchymal cells. However its role to reflect the severity of liver disease has not been thoroughly evaluated yet.

VEGF induces capillary permeability, hepatocyte regeneration and new vessel formation, in correlation with the inflammatory changes seen in hepatitis. In Akiyoshi et al.'s study,[11] patients with AH had significantly higher VEGF levels compared to control group. Serum VEGF levels in patients with AH changes synchronous with ALT and

AST levels. Patients with LC had significantly lower VEGF levels than control group, suggesting that serum VEGF levels could be related to degree of hepatocyte regeneration.[11] In our study, we also found significantly higher synchronous VEGF, ALT and AST levels in patients with AH. In a study, Andus et al. create the acute phase response after local damage in AH, including the secretion of cytokines such as IL-1, IL-6, IL-11 and TNFα.[17,18] We believe that this increase can be explained with acute phase proteins produced by liver and/or from leakage from hepatocytes.

Shimoda et al.[19] found no clear difference of VEGF in patients with NAFLD, chronic C hepatitis and hepatocellular carcinoma (HCC). In contrast, we found a significant difference in VEGF levels between non-alcoholic fatty liver disease (NAFLD) and CH, LC, control groups. We concluded that this difference could be explained with coexisting NAFLD diseases. Moreover, as the diagnosis of NAFLD was performed by imaging method, we thought that the histology would also have been important.

Makhlouf et al.[20] investigated the patients with CH, LC and HCC. Higher VEGF levels were established in patients with liver diseases than control group. Similarly in a recent study, the association between development of hepatic angiogenesis in patients with HCV and expression of TGF-β and VEGF[21] was showed. In the present study, there was no significant difference in VEGF levels between CH, LC and the control group.

Vascularization factors, such as VEGF, play a key role in cirrhosis process. There is a tendency to shrink in the cirrhotic liver but it is still not clear whether neovascularization is good or bad as a part. El-Assal et al.[12] found that VEGF production in cirrhotic liver tissue without tumor was much more than in the liver without cirrhosis. All the same, the VEGF levels were clearly low in AH, CH, otoimmune hepatitis (OH) and primary biliary cirrhosis (PBC) patients. In chronic liver disease cells, the ongoing decrease in oxygen pressure due to decreased blood flow, stimulates the production of VEGF and other factors such as, FGF-b and TGF-b.[22] Desideri et al.[23] showed a relationship between serum VEGF concentration and liver dysfunction in patients with LC and CH. VEGF levels were lower in patients with cirrhosis than hepatitis, and hepatitis were lower than the control group[23]. In our study, Child-Pugh classification was not performed in patients with LC, therefore whether a correlation does or does not exist with liver dysfunction could not be evaluated. In the present study, VEGF levels are not related to blood biochemical markers of hepatocyte function and/or damage. Recently, VEGF levels in platelets have been investigated, and determined that they are secreted throughout coagulation during their activity.[24,25] Low platelet count in cirrhotic patients with portal hypertension (PH) is a frequent finding and probably the reason for low serum VEGF levels in this group.[25] In Genesca et al.'s study,[26] they found a strong relationship between VEGF levels and platelet counts similar to our study. The lack of investigation of viral replication, histopathological evaluation in study group and Child-Pugh classification in cirrhosis were limitations of present study.

Contrary to a general agreement achieved in AH, there are controversial regarding serum VEGF levels reported in CH and LC. In our study, we found no significant difference in serum VEGF levels between patients with LC and CH. As conclusion, we think that VEGF levels may be affected from acute hepatitis and nonalcoholic fatty liver disease, however not liver cirrhosis and chronic hepatitis. Further studies are needed to clarify the regulatory mechanisms of VEGF secretion and the role of VEGF in liver diseases.

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