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• CLINICAL RESEARCH •

Spider angiomas in patients with liver cirrhosis: Role of vascular endothelial growth factor and basic fibroblast growth factor

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Abstract

AIM: To investigate whether vascular endothelial growth factor (VEGF) and basic fibroblastic growth factor (bFGF) are associated with spider angiomas in patients with liver cirrhosis.

METHODS: Eighty-six patients with liver cirrhosis were enrolled and the number and size of the spider angiomas were recorded. Fifty-three healthy subjects were selected as controls. Plasma levels of VEGF and bFGF were measured in both the cirrhotics and the controls.

RESULTS: Plasma VEGF and bFGF were increased in cirrhotics compared with controls (122±13 *vs*. 71±11 pg/mL, *P*=0.003 for VEGF; 5.1±0.5 *vs*. 3.4±0.5 pg/mL, *P*=0.022 for bFGF). In cirrhotics, plasma VEGF and bFGF were also higher in patients with spider angiomas compared with patients without spider angiomas (185±28 *vs*. 90±10 pg/mL, *P*=0.003 for VEGF; 6.8±1.0 *vs*. 4.1±0.5 pg/mL, *P*=0.017 for bFGF). Multivariate logistic regression showed that young age and increased plasma levels of VEGF and bFGF were the most significant predictors for the presence of spider angiomas in cirrhotic patients (odds ratio [OR]=6.64, 95 % confidence interval [CI]=2.02-21.79, *P*=0.002; OR=4.35, 95 % CI=1.35-14.01, *P*=0.014; OR=5.66, 95 % CI=1.72-18.63, *P*=0.004, respectively).

CONCLUSION: Plasma VEGF and bFGF are elevated in patients with liver cirrhosis. Age as well as plasma levels of VEGF and bFGF are significant predictors for spider angiomas in cirrhotic patients.

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INTRODUCTION

Liver cirrhosis is a major disease in Asian countries and causes marked morbidity and mortality. Spider angioma is a common presentation of liver cirrhosis[1,2]. It appears frequently in alcoholic cirrhotics or when liver function deteriorates[2-4] and may be associated with esophageal variceal bleeding[5]. However, the exact pathogenesis has been unclear.

Angiogenesis is a possible mechanism in the pathogenesis of spider angiomas and has not been well investigated. Serum vascular growth factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), have been found to be elevated in cirrhotic patients[6-9]. These vascular growth factors may play a role in the neovascularization and formation of spider angiomas in patients with liver cirrhosis.

The aim of this study was to evaluate the predictive value of plasma VEGF and bFGF for the presence of spider angiomas in patients with liver cirrhosis.

MATERIALS AND METHODS

Study patients

Eighty-six consecutive liver cirrhotic patients from Taipei Veterans General Hospital were enrolled into this study. Fifty-three age- and sex-matched subjects from apparently healthy adults who were admitted to our hospital for routine physical checkups were randomly selected as healthy controls. The etiologies of liver cirrhosis included hepatitis B in 37 patients (43 %), hepatitis C in 18 patients (21 %), alcoholism in 12 patients (14 %), primary biliary cirrhosis in 2 patients (2 %), hepatitis B and alcoholism in 7 patients (8 %), hepatitis C and alcoholism in 6 patients (7 %), and being cryptogenic in 4 patients (5 %). The diagnosis of cirrhosis was confirmed by liver biopsy or peritoneoscopy in 8 patients, and based on typical clinical findings (splenomegaly, ascites, and/or esophageal varices), imaging studies (abdominal sonography[10], computerized tomography, and/or angiography), and characteristic laboratory findings in the remaining 78 patients. The severity of cirrhosis was categorized according to the Child-Pugh classification[11]. Patients with hypertension, diabetes mellitus, atherosclerosis, uremia, and peripheral vascular occlusive diseases were excluded. None of these patients had received antibiotics or vasoactive drugs in the previous week before blood sampling. All the subjects gave informed consent to participate in this study, which was approved by the Hospital Ethics Committee. This study also conformed to the provisions of the World Medical Association Declarations of Helsinki.

All the patients received a complete physical examination to reveal the number and size of the spider angiomas. Serum albumin (reference range 3.7-5.3 g/dL), bilirubin (0.2-1.6 mg/dL), aspartate transaminase (AST, 5-45 U/L), alanine transaminase (ALT, 0-40 U/L), creatinine (0.7-1.5 mg/dl), and blood urea nitrogen (7-20 mg/dL) concentrations were measured in each patient using standard laboratory methods (Hitachi Model 736 automatic analyzer, Tokyo, Japan) on the same day the blood was sampled for assays of plasma vascular growth factors. Each cirrhotic patient underwent an upper GI endoscopy (Olympus GIF-XQ240; Olympus Corp., Taipei, Taiwan) to

document the presence of esophageal varices. The severity of varices was graded F1: small straight varices, F2: enlarged tortuous varices, and F3: largest-sized coil-shaped varices, as suggested by Beppu *et al*[12].

Determination of plasma levels of VEGF and bFGF

All the plasma samples were centrifuged at 3 000 rpm for 10 minutes at 4 ℃ and stored at -80 ℃ until tested. Samples from all the patients and controls were coded so that the technicians running the assays were blind to the sources of the samples. Plasma levels of VEGF and bFGF were measured by using commercially available enzyme-linked immunoabsorbent assay kits (R&D Systems Inc., Minneapolis, MN) according to the manufacturer's instructions. Standard curves were constructed using serial dilutions of recombinant VEGF and bFGF. Optical densities were determined using a micro-titer plate reader (Bio-Kinetics Reader, Bio-Tek Instruments, VT). Tests were performed in duplicate. The intra- and inter-assay variations of these assays were less than 10 %.

Statistical analysis

Results were expressed as mean ± SD. Unpaired Student *t*-test was used to analyze continuous variables between groups. Chi-square test or Fisher's exact test was used for comparison of categorical variables. Pearson correlation coefficient was used to determine the relationship between numerical variables, such as plasma levels of VEGF and the size of spider angiomas. Cut-off values were determined for each serum angiogenic factor according to the best discrimination between patients with or without spider angiomas regarding optimal values of sensitivity and specificity using the receiver operating characteristics (ROC) curve analysis. Logistic regression was used to assess the relationship of independent variables with the presence of spider angiomas in cirrhotic patients. Statistical analyses were performed using the SPSS software (SPSS 10. 0, SPSS Inc., Chicago, IL, USA). Results were considered statistically significant at $P<0.05$.

RESULTS

Plasma angiogenic factors in patients with liver cirrhosis

Plasma VEGF and bFGF levels in patients with liver cirrhosis are listed in Table 1. Plasma levels of VEGF and bFGF were increased in patients with liver cirrhosis compared with healthy controls ($P<0.05$). There was no difference in age, sex, and serum levels of creatinine between the two groups (data not shown).

Table 1 Plasma levels of VEGF and bFGF

Parameters	Healthy controls (n=53)	Cirrhotics (n=86)	P Value
VEGF (pg/mL)	71±11	122±13	0.003
bFGF (pg/mL)	3.4±0.5	5.1±0.5	0.022

VEGF=vascular endothelial growth factor, bFGF=basic fibroblast growth factor.

Patient characteristics

Characteristics of the 86 patients with liver cirrhosis are listed in Table 2. Plasma VEGF and bFGF were increased in the 31 cirrhotic patients with spider angiomas compared with the 55 patients without spider angiomas (185±28 pg/mL *vs*. 90±10 pg/mL, $P=0.003$ for VEGF; 6.8±1.0 pg/mL *vs*. 4.1±0.5 pg/mL, $P=0.017$ for bFGF). Plasma VEGF was also higher in the 26 patients with alcohol-related liver cirrhosis compared with the 60 patients with non-alcoholic liver cirrhosis (167±29 pg/mL *vs*. 103±13 pg/mL, $P=0.04$).

Table 2 Characteristics of patients'

	No. of patients	VEGF (pg/ml)	bFGF (pg/ml)
Sex			
Male	67	121±14	4.8±0.5
Female	19	123±27	6.1±1.5
Age			
≤60 years	36	149±24	5.2±0.8
>60 years	50	103±13	5.1±0.6
Etiology			
Alcohol-related	26	167±29*	4.6±0.7
Non-alcoholic	60	103±13	5.4±0.6
Albumin (g/dL)			
≤3.6	48	132±15	5.1±0.7
>3.6	38	109±21	5.2±0.7
Creatinine (mg/dL)			
≤1.5	83	122±13	5.2±0.5
>1.5	3	125±73	4.5±2.6
ALT (U/L)			
≤40	37	136±22	4.1±0.6
>40	49	111±15	5.9±0.7
Total bilirubin (mg/dL)			
≤1.6	54	115±16	5.2±0.6
> 1.6	32	133±20	5.0±0.9
Child-Pugh score			
A	47	98±19	5.5±0.7
B, C	39	141±16	4.8±0.7
Prothrombin time prolongation >4 s			
Yes	27	145±23	5.0±1.0
No	59	112±15	5.3±0.6
Platelet count (/μL)			
≤150 000	71	109±12†	6.8±1.5
> 150 000	15	204±41	4.7±0.5
Esophageal varices			
Yes	59	122±14	5.0±0.6
No	27	119±28	5.8±1.0
Spider angioma			
Yes	31	185±28[b]	6.8±1.0[a]
No	55	90±10	4.1±0.5

Data were presented as mean ±SD. [a]$P<0.05$, [b]$P<0.005$. VEGF=vascular endothelial growth factor, bFGF=basic fibroblast growth factor.

Plasma angiogenic factors and clinical features

Table 3 summarizes the clinical features of cirrhotic patients with spider angiomas and those without. Cirrhotic patients with spider angiomas were younger (54±2 years *vs*. 66±1 years, $P<0.001$) and had higher serum bilirubin (3.1±0.5 mg/dL *vs*. 1.7±0.2 mg/dL, $P=0.03$), longer prothrombin time (16.6±0.7 sec *vs*. 14.8±0.4 sec, $P=0.015$), and higher proportion of alcoholism (45 % *vs*. 20 %, $P=0.014$) than those without. Sex, serum albumin, creatinine, AST, ALT, platelet count, size of esophageal varices, and Child-Pugh score did not differ between the two groups.

In the cirrhotics, plasma VEGF was significantly correlated with the size of spider angiomas ($r=0.38$, $P<0.001$). Plasma VEGF level also showed correlation with serum bilirubin level ($r=0.3$, $P=0.006$) and platelet count ($r=0.5$, $P<0.001$).

Univariate analysis of predictive factors for spider angiomas

Univariate analysis of factors predicting the presence of spider angiomas in patients with liver cirrhosis by using logistic regression is shown in Table 4. The cut-off values of 134 pg/mL

for VEGF and 4.8 pg/mL for bFGF obtained by the ROC analysis were used in the univariate analysis. Young age, elevated serum AST and bilirubin, prolonged prothrombin time, elevated plasma VEGF and bFGF, and alcoholism were associated with the presence of spider angiomas in cirrhotic patients.

Table 3 Clinical features of cirrhotic patients with and without spider angiomas

Parameters	Spider (+) (n=31)	Spider (-) (n=55)	P Value
Age (years)	54±2	66±1	<0.001
Sex (male / female)	27 / 4	41 / 14	0.17
Albumin (g / dL)	3.4±0.1	3.5±0.1	0.69
Creatinine (mg / dL)	1.0±0.1	1.1±0.1	0.24
AST (U / L)	91±9	89±13	0.93
ALT (U / L)	59±9	72±11	0.36
Platelet count (/ uL)	108 097±12 379	94 762±7 541	0.33
Esophageal varices (nil / F1 / F2 / F3)	7 / 6 / 12 / 6	18 / 12 / 14 / 11	0.88
Child-Pugh score	8.2±0.5	7.3±0.3	0.99
Bilirubin (mg / dL)	3.1±0.5	1.7±0.2	0.03
Prothrombin time (s)	16.6±0.7	14.8±0.4	0.015
Alcoholism	14 (45 %)	11 (20 %)	0.014

AST=aspartate transaminase, ALT=alanine transaminase.

Table 4 Univariate analysis of predictive factors for spider angiomas

Parameters	Odds ratio	95 % Confidence interval	P Value
Age (≤60 years)	5.12	1.97-13.28	0.001
Albumin (≥3.7 g / dL)	0.66	0.27-1.64	0.369
Creatinine (>1.5 mg / dL)	0.58	0.06-5.81	0.642
AST (>45 U / L)	5.76	1.56-21.3	0.009
ALT (>40 U / L)	1.24	0.51-3.02	0.634
Bilirubin (>1.6 mg / dL)	2.71	1.09-6.74	0.031
Platelet count (>150 000 / uL)	1.41	0.44-4.52	0.563
Prothrombin time prolongation (>4 s)	2.96	1.15-7.58	0.024
Child-Pugh score C	1.64	0.59-4.53	0.344
VEGF (≥134 pg / mL)	5.33	1.93-14.74	0.001
bFGF (≥4.8 pg / mL)	4.38	1.67-11.5	0.003
Alcoholism	3.29	1.25-8.67	0.016

AST=aspartate transaminase, ALT=alanine transaminase, VEGF=vascular endothelial growth factor, bFGF=basic fibroblast growth factor.

Table 5 Multivariate analysis of predictive values of VEGF, bFGF, and age for spider angiomas

Parameters	Odds ratio	95% confidence interval	P Value
Age (≤60 years)	6.64	2.02-21.79	0.002
VEGF (≥134 pg / mL)	4.35	1.35-14.01	0.014
bFGF (≥4.8 pg / mL)	5.66	1.72-18.63	0.004

VEGF=vascular endothelial growth factor, bFGF=basic fibroblast growth factor.

Multivariate analysis of plasma VEGF and bFGF, age and their predictive values for spider angiomas

Multivariate analysis with logistic regression showed that young age and elevated plasma levels of VEGF and bFGF were the most independent predictive factors for spider angiomas in cirrhotic patients, as shown in Table 5. The predictive values of plasma VEGF for spider angiomas were the following: sensitivity, 53 %; specificity, 82 %; positive predictive value, 64 %; and negative predictive value, 75 %. The predictive values of plasma bFGF for spider angiomas were: sensitivity, 60 %; specificity, 75 %; positive predictive value, 58 %; and negative predictive value, 76 %.

DISCUSSION

Spider angioma has been commonly seen in patients with liver cirrhosis[1,2]. The pathogenesis is still unknown. Neovascularization is a likely mechanism, but has not been proved. In the current study, we found significantly elevated plasma levels of VEGF and bFGF in cirrhotic patients compared with healthy controls. Elevated plasma VEGF was correlated with the size of spider angiomas. To our knowledge, this is the first study to demonstrate that age and plasma VEGF and bFGF are the most significant independent predictors of spider angiomas in cirrhotic patients.

VEGF has been found to be a glycoprotein that selectively induces endothelial proliferation, angiogenesis, and capillary hyperpermeability[13-15]. VEGF gene was expressed in a wide variety of normal human tissues, including the liver[16,17]. Blood levels of VEGF in patients with cirrhosis remain controversial[6,18]. Our results showed that plasma VEGF was elevated in patients with liver cirrhosis. In addition, plasma VEGF levels were negatively correlated with liver function reserve in patients with liver cirrhosis. The elevated VEGF might be due to ischemic or damaged liver cells, which released cellular VEGF to facilitate damage repair by stimulating angiogenesis[19]. Increased production of VEGF by the cirrhotic liver has also been reported[20], and may subsequently lead to the formation of spider angiomas.

bFGF has also been found to be a potent stimulator of endothelial cell proliferation, migration, and angiogenesis[21]. Our results showed that plasma bFGF was elevated in patients with liver cirrhosis which was consistent with previous reports[7-9]. The elevated bFGF might be due to a release from damaged liver cells[22] or increased production by the cirrhotic liver[9]. bFGF could also stimulate the production of VEGF and enhance angiogenesis[23].

The presence of spider angiomas has been reported to be associated with esophageal variceal bleeding[5]. The pathophysiological mechanism has been unclarified. Spider angiomas originate from arterioles, while esophageal varices are one kind of veins. They are different in nature. In this study, there was no relationship between the presence of spider angiomas and the degree of esophageal varices. In addition, there was no association between esophageal varices and these angiogenic factors.

A positive correlation was also found between serum VEGF level and the platelet count. This result was in accordance with the findings reported by others[24]. Platelets release a variety of vasoactive substances, including VEGF, and promote angiogenesis, endothelial permeability, and endothelial growth[25]. Although there was a positive correlation between the serum VEGF level and platelet count, there was no significant association between platelet count and spider angioma in the present study. Serum VEGF level was an independent predictor of spider angioma.

Plasma VEGF was increased in alcoholic cirrhotics compared with non-alcoholic cirrhotics in our study. Ethanol could induce expression of vascular endothelial growth factor and stimulate angiogenesis[26]. This may lead to the high prevalence of spider angiomas in alcoholic cirrhotic patients.

Young age was a significant predictor for the presence of spider angiomas in cirrhotics in our study. This was in

conformity with previous reports[1]. The underlying mechanism is unknown. A decline in angiogenic capacity in the aged[27,28] may cause impaired neovascularization and formation of spider angiomas in cirrhotic patients.

In summary, plasma VEGF and bFGF are elevated in patients with liver cirrhosis, especially in those with spider angiomas. Age-related angiogenic capacity as well as VEGF and bFGF may play important roles in the formation of spider angiomas in cirrhotic patients.

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