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Original Research Paper

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COMPARISON OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN CHRONI HEPATITIS B, CIRRHOSIS AND HEPATOCELULLAR CARCINOMA

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ABSTRACT

BACKGROUND: Hepatitis B is one of the causes of liver cirrhosis and hepatocellular carcinoma. Cirrhosis that has turned into hepatocellular carcinoma requires more nutrients and oxygen so the process of angiogenesis through the release of VEGF (Vascular Endothelial Growth Factors) increases. Therefore, studies on the comparison of VEGF levels in chronic hepatitis B, liver cirrhosis and hepatocellular carcinoma were conducted. **METHODS:** 97 patients who went to Haji Adam Malik Hospital and Universitas Sumatera Utara Hospital who passed the inclusion criteria from March to June 2019 were tested for blood and serum VEGF, ultrasonography, 3-phase liver CT scan. **RESULTS:** Patients consisted of 62 men (63.9%) and 35 women (36.1%) with mean age of 55.5 years. VEGF levels were obtained in patients with chronic hepatitis B 467 pg / mL, Cirrhosis of the liver 468 pg / mL, hepatocellular carcinoma 872 pg / mL. In comparison of VEGF levels with BCLC degrees, BCLC A 588.2 ± 182.09 pg / mL, BCLC B 707.57 ± 161.69 pg / mL, BCLC C 1249.34 ± 412.96 pg / mL, BCLC D 1562.33 ± 134.02 pg / mL. In comparison of VEGF levels with Child Pugh, Child Pugh obtained a 312.55 ± 298.4 pg / mL, Child Pugh B 613.76 ± 158.17 pg / mL, Child Pugh C 963.95 ± 364.81 pg / mL. **CONCLUSION:** There is a significant relationship between the increase in VEGF levels in Chronic hepatitis B, liver cirrhosis and hepatocellular carcinoma

KEYWORDS : VEGF, Chronic Hepatitis B, Cirrhosis, Hepatocellular Carcinoma

1. INTRODUCTION

Hepatitis B virus infection is a major cause of acute hepatitis, chronic hepatitis, cirrhosis, and liver cancer in the world. More than 2 billion of the world's population are infected with the hepatitis B virus and more than 360 million become chronic sufferers who have a ratio of cirrhosis and liver cancer. Indonesia is classified as a country with hepatitis B number 3 in the Asia Pacific region after China and India. Around 13 million people in Indonesia have been infected with hepatitis B and 4 million are infected with hepatitis C. An increase in hepatitis infection has doubled compared to 2007 based on health research in 2013. The prevalence of hepatitis B by 21.8% and hepatitis C by 2.5% (Kumar et al, 2012)(Kementrian Kesehatan, 2014). HCC follows diverse causes of liver damage (including chronic alcohol use, chronic hepatitis B and C infection, and nonalcoholic fatty liver disease)(European Association for the Study of the Liver (2018), common associated findings are hypervascularity and marked vascular abnormalities (Zhu et al, 2011), such as arterialization and sinusoidal capillarization (Yang & Poon, 2008). Increased tumor vascularity may result from sprouting angiogenesis or by recruiting existing vessels into the expanding tumor mass

Angiogenesis plays a very important role in various physiological and pathological processes. Pathological angiogenesis is an integral part of tumor development and is associated with inflammatory diseases including hepatic cirrhosis. This has led to various attempts to manipulate the process of angiogenesis in the type of therapy (Lee et al, 2011). One regulator of the angiogenesis process that is stimulating and specific to endothelial target cells is Vascular Endothelial Growth Factor (VEGF). VEGF will play a role in the process of angiogenesis that affects the development of hepatitis, cirrhosis and hepatocellular carcinoma (Abdelmoaty et al, 2009).

Increased expression of VEGF in liver tissue in chronic liver disease (cirrhosis and chronic hepatitis B) occurs due to the stimulation of fibroblast in the formation of fibrous tissue. In hepatocellular carcinoma increased VEGF expression occurs due to tumor invasion and metastasis in the intrahepatal. VEGF expression and VEGF receptor (VEGFR) in liver tissue correlates with levels in the blood (Poon & Yang, 2008). But there is no research on the comparison of VEGF in subjects with hepatitis, HCC and chronic liver disease.

2. METHODS

2.1 Patients

97 patients who had hepatitis B, Cirrhosis and hepatocellular carcinoma were treated in Haji Adam Malik hospital and Universitas Sumatera Utara hospital from March to June 2019.Patients were selected by consecutive sampling. Patients who pass the inclusion criteria were filling the informed consent after getting explanation.

Patients' blood samples were draw for routine blood tests, PT, APTT, INR, HBsAg, Anti HCV, SGOT, SGPT, alkaline phosphatase, gamma GT, total bilirubin, urea, creatinine, albumin, blood glucose and serum VEGF. Serum concentrations of VEGF were measured in duplicate with an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN, USA), by an investigator who was blinded to the clinical information of the patients. The diagnosis of HCC was performed using clinical criteria and the findings obtained by B-mode ultrasonography (US), 3 phase Liver computed tomography (CT).

2.2 Statistical analysis

A Cross-sectional study which was analyzed by using the Statistical Package for the Social Sciences (SPSS version 11.0 ;SPSS, Chicago, IL, USA).

3. RESULTS

Most subjects were male (63.9%) with an average age of 55.5 years. Most ethnic groups are Batak (64.9%), followed by Java (25.8%) and Acehnese (9.3%). Most jobs are private employees (45.4%). Generally subjects with high school education (60%). Median subject VEGF levels were 586 pg / mL. Of 97 study subjects, 32 patients (33%) had HCC, 40 patients (41.2%) had liver cirrhosis, and 25 patients (25.8%) had chronic hepatitis B. Of the 32 HCC patients, most subjects were classified as BCLC C (43.75%), followed by BCLC B (31.25%), BCLC A (15.6%), and BCLC D (9.4%). Of the 40 liver cirrhosis patients, 25% were Child Pugh A, 37.5% for Child Pugh B and C. respectively

Table 1. Baseline and clinical characteristic of subjects

Characteristic	n = 97
Gender a Male	62 (63.9)
Female	35 (36.1)
Age (years) b	55.5 + 8.15
Ethnic a	
Batak	63 (64.9%)
Java	25 (25.8%)
Acehnese	9 (9.3%)
Occupation a	
Private employee	44 (45.4%)
Housewive	24 (24.7%)
Entrepreneur	18 (18.6%)
Civil servants	11 (11.3%)
Education a	
Elementary school	8 (8.2%)
Junior High School	12 (12.4%)
High School	60 (61.9%)
University	17 (17.5%)
VEGF (pg/mL) c	586 (32.6 – 2,561.4)
Diagnosis a	
Hepatocellular carcinoma	32 (33)
BCLC A	5 (15.6)
BCLC B	10 (31.25)
BCLC C	14 (43.75)
BCLC D	3 (9.4)
Liver cirrhosis	40 (41.2)
Child Pugh A	10 (25)
Child Pugh B	15 (37.5)
Child Pugh C	15 (37.5)
Chronic hepatitis B	25 (25.8)

a Categoric data: n(%)
b Numeric data, normal distribution: mean ± SD
c Numeric data, not normal distribution: median (min-max)

There were differences in VEGF levels among patients with HCC, liver cirrhosis, and chronic hepatitis B ($p < 0.001$). VEGF levels were significantly higher in HCC patients compared to liver cirrhosis or chronic hepatitis B.

Table 2. The difference of VEGF levels among patients with HCC, liver cirrhosis, and chronic hepatitis B

Diagnosis	VEGF levels (pg/mL)	p
HCC	872 (396 – 1,921) # !	<0.001*
Liver cirrhosis	468 (119.6 – 2,561.4)	
Chronic hepatitis B	457 (32.6 – 955.5)	

Kruskal-Wallis H test
*$p < 0.05$, significant compared to liver cirrhosis (#) and chronic hepatitis B (!)

There were differences in VEGF levels among BCLC degrees in patients with HCC ($p < 0.001$). VEGF levels were significantly higher in HCC patients with BCLC C and D compared to BCLC A and B.

Table 3. The difference of VEGF levels among BCLC degrees in patients with HCC

BCLC	VEGF levels (pg/mL)	p
A	588.2 + 182.09	<0.001*
B	707.57 + 161.69	
C	1,249.34 + 412.96 # !	
D	1,562.33 + 134.02 # !	

One-Way ANOVA test
*$p < 0.05$, significant compared to BCLC A (#) and BCLC B (!)

There were differences in VEGF levels among child pugh in patients with liver cirrhosis ($p = 0.001$). VEGF levels were significantly higher in liver cirrhosis patients with child pugh C compared to child pugh A.

Table 4. The difference of VEGF levels among child pugh in patients with liver cirrhosis

Child Pugh	VEGF levels (pg/mL)	p
A	312.55 + 298.42	0.001*
B	613.76 + 158.17	
C	963.95 + 364.81#	

One-Way ANOVA test

*$p < 0.05$, # significant compared to Child Pugh A

4. DISCUSSION

The process of angiogenesis in cirrhosis can be triggered by inflammation and hypoxia. An ongoing inflammatory condition causes activation of endothelial cells. Activated endothelial cells cause increased vascular permeability and inflammatory factors. This causes the production of cytokines and growth factors that induce proliferation and migration of endothelial cells which are important for the process of angiogenesis (Iwakiri & Groszmann, 2007). The condition of liver fibrosis that occurs in cirrhosis will worsen cell hypoxia. Hypoxia inducible factor-1 (HIF-1) is a transcriptional regulator induced by hypoxia and activates the transcription of a number of pro-angiogenic molecules, including VEGF(Schiff et al, 2003). Cirrhosis will at last develop to hepatocellular carcinoma. Hepatocellular carcinoma need huge supply of nutrition and oxygen, this will induce angiogenesis by releasing VEGF. When angiogenesis is not able to reach the need of the cancer, hypoxia occurs and this will induce more VEGF production. In our study, VEGF was increased in cirrhosis and more over, VEGF in HCC is the higher than others. This study is consistent with research conducted by Yvamoto et al. (2015), which states that higher serum VEGF levels were found in patients with KHS (G1 = 588.0 ± 501.0 pg / mL), compared with patients with cirrhosis (G2 = 173.0 ± 113.0 pg / mL), patients with HCV (G3 = 273.0 ± 189.0 pg / mL) and groups control (G4 = 264.0 ± 194.0 pg / mL) ($P < 0.02$ for all groups). Expression VEGF represents a significant increase along with disease progression ($p < 0.01$) (Hammam, et al, 2013).

Arles et al (2015) study found that stage A BCLC had average VEGF level of 288.26 ± 156.6 pg/mL, stage B BCLC group had average VEGF level of 434.96 ± 164.8 pg/mL, stage C BCLC had average VEGF level of 785.57 ± 194.25 pg/mL, and stage D BCLC group had average VEGF level of 1537.97 ± 660.62 pg/mL VEGF was increased in Hepatocellular carcinoma base on BCLC staging($p < 0.001$). It was found that the higher the BCLC staging, the higher the VEGF increase (12). Our study also found the same result with this study ($p < 0.001$).

In a study conducted by Abdelmoaty et al(2009) where the study compared the VEGF levels of liver cirrhosis patients with Child Pugh degrees. Reported that the VEGF level in Child Pugh A is 52.28 ± 9.5, Child Pugh B 72.95 ± 15.3 and in Child Pugh C is 193.12 ± 24.2.There is a significant difference between Child Pugh A and Child Pugh B; Child Pugh A with Child Pugh C, Child Pugh B and Child Pugh C ($p < 0.005$)(7). The highest concentration of VEGF is observed in patients with advanced stages of cirrhosis liver especially Child Pugh C., VEGF was found significantly increased in Child Pugh A, B and C ($p < 0.004$). This study is consistent with our study($p = 0.001$).

5. CONCLUSION

VEGF was significantly increased in chronic hepatitis B , cirrhosis and hepatocellular carcinoma and probably can be used as marker of severity of chronic hepatitis B, cirrhosis and hepatocellular carcinoma

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