



## COMPARISON OF SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) BETWEEN HEPATOCELLULAR CARCINOMA (HCC) PATIENTS WITH CHRONIC HEPATITIS B VIRUS INFECTION

### Medicine

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### ABSTRACT

**INTRODUCTION:** Hepatitis B virus infection is a major cause of acute hepatitis, chronic hepatitis, cirrhosis, and liver cancer in the world. The Hepatitis B virus is estimated to have infected more than 2 billion people until now. Based on the underlying disease, hepatitis B plays a role of up to 80% in all hepatocellular carcinoma events. Hepatocellular Carcinoma (HCC) is one of the important problems in the health sector because it can cause a variety of serious complications and require early treatment to improve prognosis and reduce mortality. Tumor development in HCC conditions will worsen cell hypoxia, which is known to be a Vascular Endothelial Growth Factor (VEGF). VEGF activity inducing condition is a protein that acts as the main regulator of angiogenesis

**METHOD:** The sample size for each study group are 30 people and 20 people. The sample were diagnosed with HCC and Chronic Hepatitis B virus infection and then a serum VEGF are performed. To display epidemiological data the subject of research used tabulation to show the descriptive picture. Data analyzed with SPSS 22nd.

**RESULT:** Based on the t-dependent test, the results showed that VEGF levels were significantly higher in the HCC group than in the chronic hepatitis B group (p-value=0,0001).

**CONCLUSION:** Based on the results and discussion in this study, it can be concluded that serum VEGF levels are significantly higher in HCC patients than in chronic hepatitis B

### KEYWORDS

Hepatocellular Carcinoma, Chronic Hepatitis B virus infection, VEGF, AFP

### 1. INTRODUCTION

Hepatitis B virus infection is a major cause of acute hepatitis, chronic hepatitis, cirrhosis, and liver cancer in the world. The Hepatitis B virus is estimated to have infected more than 2 billion people until now. One third of the world's population is estimated to have been infected with the Hepatitis B virus and around 400 million people are suffering from chronic hepatitis B, while the prevalence in Indonesia is reported to range from 3-17%.<sup>2</sup>

Based on the underlying disease, hepatitis B plays a role of up to 80% in all hepatocellular carcinoma events. Hepatocellular Carcinoma (HCC) is one of the important problems in the health sector because it can cause a variety of serious complications and require early treatment to improve prognosis and reduce mortality. Every year, HCC is diagnosed from half a million people worldwide, of which about three-quarters of cancer cases, HCC is the most common type of liver cancer.<sup>3,4</sup> The incidence is increasing and one of the five malignancies worldwide and the cause third largest death due to cancer after lung cancer and gastric cancer.<sup>5</sup>

Tumor development in HCC conditions will worsen cell hypoxia, which is known to be a Vascular Endothelial Growth Factor (VEGF).<sup>6</sup> VEGF activity inducing condition is a protein that acts as the main regulator of angiogenesis. Other VEGF functions are regulating microvascular permeability and tissue remodeling. VEGF is secreted by various cell types such as muscle cells, glomerulus, choroid plexus, liver, platelets and so on. The main regulators of VEGF expression are hypoxia, inflammatory cytokines, growth factors and hormonal. VEGF in mammals has five isoforms: VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factors (PIGF).<sup>7,8</sup>

Therefore researchers are interested in conducting a study to assess the comparison of serum VEGF levels among HCC patients with chronic hepatitis B virus infection. Comparison of serum VEGF levels may be clinically useful in determining the diagnosis, treatment and prognosis of the disease

### 2. METHOD

#### 2.1 Patient Selection

The research was conducted in a cross sectional design method with

HCC group and chronic hepatitis B virus infection group independently and randomized. Samples to be used in this study were all patients with the diagnosis of HCC at Haji Adam Malik Hospital Medan starting from April 2018 until Juli 2018. The sample size for each study group are 30 people and 20 people.

The sample were diagnosed with HCC and Chronic Hepatitis B virus infection and then a serum VEGF are performed.

#### 2.2. Inclusion criteria and exclusion criteria

Inclusion criteria are subjects with age above 18 years and were diagnosed with HCC with 3 Phase Liver CT Scan. Subjects receive informed consent for physical and laboratory examination and desired by the Medical Research Ethics Committee of USU Medical Faculty. Exclusion criteria are patients with systemic diseases and other malignancies, pregnant women and the patient is not cooperative

#### 2.3. Statistical Methods

To display epidemiological data the subject of research used tabulation to show the descriptive picture. Data was processed and analyzed using the SPSS 22nd program with a significance limit of  $p < 0.05$ . Furthermore, the data will be analyzed by t-dependent test if the data is normally distributed or Wilcoxon test if the data is not normally distributed.

### 3. RESULT

This study was attended by 50 patients who met the inclusion criteria, divided into 2 groups in the form of HCC group (n = 30) and chronic hepatitis B group (n = 20). In the HCC group, 21 people (70.0%) were male and 9 people (9.0%) were female. The average age is 55.9 + 8.5 years, with the majority of jobs being employees and entrepreneurs as many as 10 people (33.3%) then housewife as many as 6 people (20.0%), and civil servant as many as 4 people (13.3%). The HCC group suffered the most among people with Batakese as many as 19 people (63.3%), followed by Javanese people 6 people (20%), Acehese 5 people (16.7%). The education level of the HCC group patients came mostly from high school graduates as many as 16 people (53.3%), followed by junior high school graduates as many as 6 people (20.0%), and finally from elementary and bachelor as many as 4 people (13.3%)

In the chronic hepatitis B group, 11 people (55.0%) were male and 9 people (45.0%) were female. The average age is 59.8 + 7.5 years, with the majority of jobs being 7 employees (35.0%) then housewife as many as 6 people (30.0%), entrepreneurs as many as 5 people (25.0%) and civil servant 2 people (10.0%). The group with chronic hepatitis B suffered the most with people with Bataknese as many as 15 people (75.0%), followed by Javanese with 4 people (20%), and Acehnese with 1 person (5%). The education level of patients with chronic hepatitis B group mostly came from high school graduates as many as 14 people (70.0%), followed by graduates of bachelor as many as 5 people (25.0%), and the last from junior high school graduates as much as 1 person (5.0%).

In the HCC group the mean VEGF level found was 1307.59+ 578.17 ng / ml, while the AFP content found was 670.98 + 847.04 ng / ml. In the chronic hepatitis B group, the mean VEGF level found was 71.83 + 21.81 ng/ml

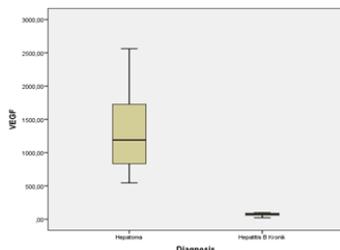
Based on table 1, characteristics of the research subjects above, it can be explained that there was no difference in sex and age between the HCC group with chronic hepatitis B (p = 0.279; p = 0.159). In this study, we compared VEGF levels in patients who experienced HCC with patients with chronic hepatitis B. From the results of bivariate analysis using an independent t-test, the results showed that VEGF levels were significantly higher in the HCC group than in the chronic hepatitis B group (p-value = 0,0001).

In the normality test of VEGF levels between hepatoma groups with chronic hepatitis B, using the Shapiro Wilk test the results were p = 0.109 so that the data was normally distributed.

**Table 1. Baseline Characteristic in HCC and Chronic Hepatitis B Virus Infection Group**

Characteristics	HCC group (n = 30)	Chronic HBV group (n = 20)	p-value
Gender, n (%)			0,279
Male	21 (70,0)	11 (55,0)	
Female	9 (30,0)	9 (45,0)	
Age (years), (mean ± SD)	55,9 + 8,5	59,8 + 7,5	0,159
Tribe, n (%)			N/A
Bataknese	19 (63,3)	15 (75,0)	
Javanese	6 (20,0)	4 (20,0)	
Acehnese	5 (16,7)	1 (5,0)	
Education level, n (%)			
Elementary	4 (13,3)	0 (0)	
Junior High School	6 (20,0)	1 (5,0)	
Senior High School	16 (53,3)	14, (70,0)	
Bachelor	4 (13,3)	5 (25,0)	
Work, n(%)			
Employee	10 (33,3)	7 (35,0)	N/A
Entrepreneur	10 (33,3)	5 (25,0)	
Housewife	6 (20,0)	6 (30,0)	
Civil Servant	4 (13,3)	2 (10,0)	
AFP, (mean ± SD)	670,98+ 847,04	N/A	N/A
VEGF, (mean ± SD)	1307,59 ± 578,17	71,83 ± 21,81	0,0001

In Figure 1 showed that VEGF levels were higher in the HCC group compared to the chronic hepatitis B virus infection group



**Figure 1. Boxplot diagram, comparison of serum VEGF level between HCC group with Chronic Hepatitis B virus infection group**

In table 2 shows the VEGF mean value in female HCC patients of 1288.1 ng / mL, higher than the average VEGF in male patients of 1188.4 ng / mL. The mean VEGF in HCC patients aged ≤ 60 years is 1422.0 ng / mL, higher than the average VEGF in patients > 60 years old with an average of 1160.9 ng / mL. The results of the analysis using the Mann Whitney test showed no significant difference for VEGF levels based on gender and age in HCC patients (p = 0.309; p = 0.769).

**Table 2 VEGF Levels based on gender and age in HCC patients**

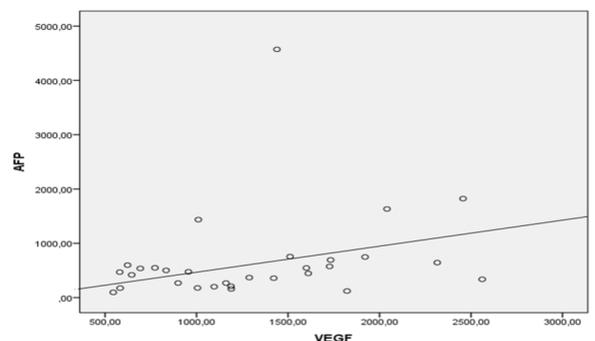
Characteristic	N	VEGF, median (min-max)	P
Gender			
Male	21	1188,4 (544,6 - 2315,6)	0,309
Female	9	1288,1 (645,5 - 2561,4)	
Age			
>60 years	21	1160,9 (544,6 - 2315,6)	0,769
≤ 60 years	9	1422,0 (582,4 - 2561,4)	
*Mann Whitney			

In Table 3 shows the average VEGF value in chronic hepatitis B patients of 74.5 ng / mL is higher than the mean VEGF in female patients with an average of 72.9 ng / mL. The mean VEGF in hepatitis B patients aged > 60 years is 87 ng / mL, higher than the average VEGF in patients aged ≤ 60 years with a mean of 62.3 ng / mL. The results of the analysis using the Mann Whitney test showed no significant difference for VEGF levels based on gender and age in chronic hepatitis B patients (p = 0.879; p = 0.342)

**Table 3 VEGF Levels based on gender and age in chronic Hepatitis B virus infection patients**

Characteristic	N	VEGF, median (min-max)	p
Gender			
Male	11	74,5 (22,1 - 97,0)	0,879
Female	9	72,9 (54,3 - 99,7)	
Age			
>60 years	11	87,0 (22,1 - 99,7)	0,342
≤ 60 years	9	62,3 (32,6 - 97,0)	
*Mann Whitney			

In this study a correlation test between serum VEGF levels and AFP levels was carried out using the Spearman correlation test. In Figure 2 and Table 3, there is a positive correlation between serum VEGF levels and AFP levels in HCC patients with a weak correlation strength. (p = 0.043; r = 0.371).



**Figure 2 Scatter dot between VEGF levels with AFP**

**4. DISCUSSION**

In this study it was found that VEGF levels were found to be higher in the HCC group when compared with the chronic hepatitis B group. In the HCC group the mean VEGF level found was 1307.59 + 578.17 ng / ml, whereas in the chronic hepatitis B group, the average VEGF level found was only 71.83 + 21.81 ng / ml. There was a significant difference in VEGF levels between the HCC group and the chronic hepatitis B group (p-value = 0,0001). This is in line with the research conducted by Yvamoto et al., Which states that higher serum VEGF levels were found in patients with HCC (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 the control group (G4 = 264.0 ± 194.0 pg / mL) (P <0, 02 for all groups). VEGF expression illustrates a significant increase along with disease progression (p <0.01).<sup>9</sup>

The study conducted by El-Sadek et al. Also found that serum VEGF levels were significantly higher in the HCC group ( $488.46 \pm 139.07$  pg / mL) than in the non-HCC group ( $197.93 \pm 50.37$  pg / mL), and both of these groups had significantly higher VEGF levels than the control group ( $134.13 \pm 51.94$  pg / mL) ( $P = 0.001$ ). VEGF from liver tissue increased significantly higher in the HCC group ( $1.92 \pm 0.6$ ) compared to the non-HCC group ( $0.62 \pm 0.28$ ).<sup>10</sup>

The results of this study indicate that there is no difference in serum VEGF levels between male and female sex or between age > 60 years and  $\leq$  60 years in hepatocellular carcinoma patients ( $p = 0.309$ ;  $p = 0.769$ ). In this study also showed that there was no difference in serum VEGF levels between male and female or between age > 60 years and  $\leq$  60 years in chronic hepatitis B patients ( $p = 0.879$ ;  $p = 0.342$ ). This is similar to the results of a study reported by Malamitsi et al where serum VEGF levels by gender were not significantly different.<sup>11</sup> In addition, the same study conducted by Yamamoto et al analyzed 184 people from the age of 21-59 years and reported no correlation between VEGF levels and age.<sup>9</sup> Another study by Kumar et al analyzed 136 individuals from ages 20 to 80 years and found no correlation between VEGF levels and age.<sup>1</sup> These results are supported by Ventriglia et al. And Kaya et al. Found that there was no significant difference in VEGF levels based on age.<sup>12,13</sup>

Plasma VEGF levels are significantly correlated with the number of HCC focal lesions, portal venous thrombosis, and aspartate aminotransferase and AFP levels. Increased liver angiogenesis in chronic HCV and HBV can provide a molecular basis for liver carcinogenesis and contribute to an increased risk of HCC in patients with cirrhosis due to HCV and / or HBV.<sup>14</sup> Increased regulation of VEGF and its receptors shows that VEGF can have an important role in tumor angiogenesis in HCC.<sup>15</sup>

Various cut-off values for AFP have been used for further evaluation in HCC patients. Previous studies in the surveillance population using the most common cut-off values of 20 ng / mL showed specificities of 89.8% and 90.0%.<sup>16</sup> In this study found high levels of AFP in patients with HCC. In this study found AFP level average was  $670.98 + 847.04$  ng / mL. The same was found by Bird et al, who found that about 48% of patients with HCC had high AFP levels ( $> 6$  kU / L) at diagnosis.<sup>17</sup> Hernandez et al. Also found that AFP levels in patients also had higher HCC than control patients ( $110.78$  UI / mL vs.  $7.40$  UI / mL) ( $p < 0.001$ ).<sup>18</sup>

In this study also conducted a correlation test between serum VEGF levels and AFP levels which showed that there was a positive correlation between serum VEGF levels and AFP levels in HCC patients with a weak correlation strength. ( $p = 0.043$ ;  $r = 0.371$ ). This is in line with the research conducted by Yvamoto et al, which states that serum VEGF levels have a weak positive correlation with AFP levels in HCC patients.<sup>9</sup>

## 5. CONCLUSION

Based on the results and discussion in this study, it can be concluded that serum VEGF levels are significantly higher in HCC patients than in chronic hepatitis B

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