ORIGINAL ARTICLE

Endothelin-1 level as a predictor of hepatopulmonary syndrome in liver cirrhosis

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ABSTRACT

Aim To determine the role of endothelin (ET)-1 in predicting hepatopulmonary syndrome (HPS) in patients with liver cirrhosis.

Methods A cross sectional study involving 80 liver cirrhosis patients aged 18 years or older was conducted in Adam Malik General Hospital Medan, Indonesia between July 2017 and June 2018. HPS diagnosis was confirmed from the presence of liver cirrhosis, abnormal oxygenation, and intrapulmonary vascular dilatations (IPVD). ET-1 level was measured from serum sample using ELISA method. Patients with coexisting primary pulmonary pathology and intrinsic heart disease were active smokers, and those who declined to participate were excluded. Statistical analysis was conducted at 95% confidence interval. $p<0.05$ was considered significant.

Results Majority the patients were male (56.3%) and had higher educational background (62.5%). Mean age of the patients was 51.3 (SD=12.6) years. The prevalence of HPS was 21.2%. The patients with HPS had higher ET-1 level compared to those without HPS ($p<0.001$). The patients with hepatic encephalopathy had 2.917 times higher risk for suffering from HPS, while the patients with Child Pugh score A had lower risk (0.738 times) for having HPS compared to subjects with Child Pugh score B and C. ET-1 level >187.5 mg/L had sensitivity and specificity for predicting HPS in subjects with liver cirrhosis of 82.35% and 81.25%, respectively.

Conclusion ET-1 could be used as a promising marker for HPS in patients with liver cirrhosis. ET-1 level of >187.5 mg/l had a good accuracy in predicting HPS in liver cirrhosis patient.

Key words: endothelin, hepatopulmonary syndrome, liver cirrhosis

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INTRODUCTION

Liver cirrhosis is the end stage of liver disease and carries significant morbidity and mortality. Liver cirrhosis is the 13th most common cause of death globally (1,2). In the United Kingdom, mortality rate from liver cirrhosis as end stage liver disease between 1968 and 2011 was 5.4 per 100 00 person-years (3). In Adam Malik General Hospital, the 4-year prevalence of liver cirrhosis was 4% (4).

The most common etiologies for liver cirrhosis are chronic hepatitis viral infection, alcoholic liver disease, and nonalcoholic fatty liver disease (1,5). Patients with liver cirrhosis are at risk of several complications such as hepatocellular carcinoma, hepatic encephalopathy, hepatorenal syndrome, and hepatopulmonary syndrome (HPS) (1,2,4).

Hepatopulmonary syndrome (HPS) is marked by end stage liver failure, arterial hypoxemia (PaO2 <70 mmHg or alveolar-arterial oxygen gradient >20 mmHg), and intrapulmonary vascular dilatation without underlying cardiopulmonary disease (4). The prevalence of HPS ranges from 4 to 47% among liver cirrhosis patients with mortality rate of 16% (4,6). Liver failure causes imbalance between vasodilators production and clearance, which is the underlying etiology of HPS (4). Endothelin (ET)-1 is one of vasodilators produced in HPS. It is consisted of 21 amino acids with a free amino terminus and C-terminal carboxyl acid. ET-1 is produced in endothelial cells and work in autocrine fashion to induce vasodilatation (7).

The HPS as a complication of liver cirrhosis impairs survival rate of the patients, thus prompt diagnosis and management are mandatory. Classically, HPS is diagnosed based on clinical manifestations supported by echocardiography findings, and blood gas analysis (6,8). Appropriate marker for HPS is needed to assist an accurate diagnosis.

Several studies (9,10) reported that ET-1 was associated with severity of liver disease and HPS. To our knowledge, there is no study regarding ET-1 as a predictor of HPS in patients with liver cirrhosis. We hypothesized that ET-1 plays a significant role in predicting HPS in patients with liver cirrhosis in Indonesian population, particularly in Medan. Due to high morbidity and mortality rates due to HPS in liver cirrhosis, ET-1 is expected to become a promising marker for HPS in this population and further to suggest a therapeutic target based on pathogenesis.

The aim of this study was to determine the role of ET-1 in predicting HPS in patients with liver cirrhosis.

PATIENTS AND METHODS

Patients and study design

This cross sectional study was conducted at Haji Adam Malik General Hospital Medan, North Sumatera, Indonesia. A total of 80 patients were enrolled in this study from July 2017 to June 2018. An inclusion criterion was liver cirrhosis patients aged 18 years or older. The HPS was confirmed from the presence of liver cirrhosis, abnormal oxygenation, and intrapulmonary vascular dilatations (IPVD).

Abnormal oxygenation was defined by elevated alveolar-arterial oxygen gradient >15 mmHg or >20 mmHg in patients older than 64 years while breathing room air at rest in the sitting position. Exclusion criteria were patients with coexisting pulmonary pathology, coexisting intrinsic heart disease, active smokers, and those who declined to participate in the study. The selection of the patients was made using consecutive sampling method.

A written consent was obtained from each patient before enrolling to the study.

The Ethics Committee of the School of Medicine of Universitas Sumatera Utara was obtained prior investigation.

Methods

The IPVD was diagnosed by contrast-enhanced transthoracic echocardiography.

Every patients was interviewed using questionnaire as a guideline to obtain demographic data. Routine blood count, hepatitis viral marker, bilirubin level, liver enzymes, renal function test, CRP, and ET-1 measurements were done for each patient.

ET-1 was measured from serum sample using Quantikine ELISA kit DET100 (R&D Systems, Inc., Minneapolis, USA).

The Child-Pugh scoring system was used to determine the severity of liver cirrhosis. It consists of several parameters. Each parameter has a range of score from 1 to 3 (Table 1). Based on the total score, a patient is categorized into grade A

Table 1. The Child-Pugh score system*

Variable	Description/value according to the score		
	1	2	3
Encephalopathy	None	Minimal	Advanced (coma)
Ascites	Absent	Controlled	Refractory
Bilirubin (μmol/L)	<34	34-51	>51
Albumin (g/L)	>35	28-35	<28
Prothrombin (second)	<4	4-6	>6

*grade A (total score of 5-6), grade B (total score of 7-9), grade C (total score of 10-15)

(total score of 5-6), B (total score of 7-9), and C (total score of 10-15). The higher the score, the more severe the disease becomes (11).

Statistical analysis

Normality of continuous data distribution was determined using Kolmogorov-Smirnov test. $\chi 2$ test was used to analyse the association between gender, hepatic encephalopathy, and Child Pugh score and HPS. To assess the difference of laboratory parameters in patients with and without HPS, we used independent T test with Mann-Whitney test as an alternative. Receiver operating curve (ROC) analysis was conducted to determine the accuracy of ET-1 to predict HPS in patients with liver cirrhosis. Statistical analysis was conducted at 95% confidence interval, using $p<0.05$.

RESULTS

A total of 80 patients were enrolled in this study, of whom majority were male, 45 (56.3%) and had higher educational background, 50 (62.5%). Mean age of patients was 51.3 (SD=12.6) years. More than a half of patients, 42 (52.5 %) had positive hepatitis viral marker with hepatitis B as the most frequent. The prevalence of HPS was 21.2%. Sixty-percent patients had Child Pugh score C (Table 2).

Table 2. Demographic, social and clinical characteristics of the patients

Characteristic	No (%) of patients
Gender	
Males	45 (56.3)
Female	35 (43.8)
Mean age (SD) (years)	51.3 (12.60)
Education status	
Primary	8 (10.0)
Secondary	(22 (27.5)
Higher secondary and above	50 (62.5)
Ethnicity	
Bataknese	45 (56.3)
Javanese	28 (35.0)
Other	7 (8.8)
Viral marker	
HBsAg (+)	32 (40.0)
Anti HCV (+)	10 (12.5)
Hepatopulmonary syndrome (HPS)	
Absence	63 (78.8)
Presence	17 (21.2)
Child Pugh score	
A	15 (18.8)
B	17 (21.3)
C	48 (60.0)

SD, standard deviation; HBsAG, hepatitis B surface antigen; HCV, hepatitis C virus;

From laboratory parameters, there was no significant difference between patients with and without HPS except for ET-1 level. The patients with HPS had higher ET-1 level compared to those without HPS ($p<0.001$) (Table 3).

There were significant relationships between hepatic encephalopathy, Child Pugh score, and HPS. Patients with hepatic encephalopathy had

Table 3. Difference in laboratory parameters between the patients with and without hepatopulmonary syndrome (HPS)

Variables	Reference values	Absence of HPS (n = 63)	HPS (n = 17)	p
Mean haemoglobin (SD) (g/dL)	12-17	10.2 (2.62)	9.7 (2.22)	0.517
Mean haematocrit (SD) (%)	36-51	30.6 (7.63)	28.9 (6.32)	0.414
Median WBC (min-max) (cells/μL)	4 500-11 000	7 170 (1 830-29 000)	7 470 (1 866-35 190)	0.711
Median platelet (min-max) (cells/μL)	150 000-350 000	142 000 (15 000-509 000)	164 000 (12 000 – 654 000)	0.353
Mean albumin (SD) (g/dL)	3.5-5.4	2.5 (0.6)	2.3 (0.4)	0.060
Median INR (min-max)	2.0-3.0	1.3 (0.8 – 3.7)	1.1 (0.9 – 1.6)	0.193
Median total bilirubin (min-max) (mg/dL)	0.3-1.2	2.1 (0.2-13.6)	3.3 (0.6 – 21.9)	0.104
Median direct bilirubin (min-max) (mg/dL)	0.0-0.3	0.9 (0.1 – 9.6)	1.5 (0.2 – 15.2)	0.189
Median AST (min-max) (U/L)	0-35	57 (15 – 216)	49 (15 – 377)	0.837
Median ALT (min-max) (U/L)	0-35	43 (8 – 246)	40 (11 – 146)	0.356
Median ALP (min-max) (U/L)	36-150	117 (39 – 331)	122 (43 – 178)	0.791
Median GGT (min-max) (U/L)	8-78	78 (13 – 346)	83 (13 -628)	0.742
Median RBG (min-max) (mg/dL)	<140	105 (41 – 295)	100 (65 – 163)	0.298
Median ureum (min-max) (mg/dL)	8-20	30 (11 – 263)	40 (17 – 146)	0.270
Median creatinine (min-max) (mg/dL)	0.7-1.3	0.8 (0,4 – 7)	1.1 (0.5 – 3.6)	0.107
Median CRP (min-max) (mg/dL)	<0.5	2.2 (0.1 – 8.4)	2.8 (0.8 – 14)	0.214
Median ET-1 level (min-max) (mg/L)	N/A	86 (60 – 120)	320 (150 – 400)	<0.001

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, c-reactive protein; ET-1, endothelin-1; GGT, gamma-glutamyl transpeptidase (GGT); INR, international normalized ratio; RBG, random blood glucose; WBC, white blood cell;

2.917 times higher risk for suffering from HPS, while patients with Child Pugh score A had the lower risk (0.738 times) for having HPS compared to patients with Child Pugh score B and C. There was no association between gender and HPS (Table 4).

Table 4. Associations between gender, hepatic encephalopathy, and Child Pugh score and hepatopulmonary syndrome (HPS)

Variable	No (%) of patients			p	PR (95%CI)
	With HPS	Without HPS	Total		
Gender					
Male	10 (22.2)	35 (77.8)	42 (100.0)	0.810	1.111 (0.47-2.62)
Female	7 (20.0)	28 (80.0)	28 (100.0)		
Hepatic encephalopathy					
Yes	5 (50.0)	5 (50.0)	10 (100.0)	0.031	2.917 (1.30-6.53)
No	12 (17.1)	58 (82.9)	70 (100.0)		
Child Pugh Score					
Class A	0 (0.0)	15 (100.0)	15 (100.0)	0.032	0.738 (0.64-0.85)
Class B+C	17 (26.2)	48 (73.8)	65 (100.0)		

CI, confidence interval; HPS, hepatopulmonary syndrome; PR, prevalence ratio

ROC analysis resulted in area under the curve of 0.908 for ET-1 level >187.5 mg/L in predicting HPS (Figure 1). Further analysis showed that ET-1 level >187.5 mg/L had sensitivity and sensitivity for predicting HPS in patients with liver cirrhosis of 82.35% and 81.25%, respectively (Table 5).

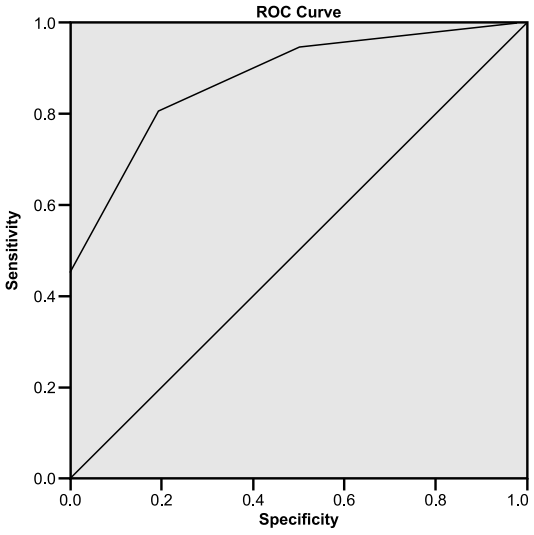

Figure 1. Receiver operating curve (ROC) for endothelin-1 (ET)-1 in predicting hepatopulmonary syndrome (HPS) in liver cirrhosis

DISCUSSION

The prevalence of liver cirrhosis increases significantly probably due to alcohol consumption, obesity, and viral infection (3). Males were affected more commonly compared to females with a ratio of 2.1:1 (2,5). Most patients were older than 50 years and had obesity and/or type 2 diabetes mellitus as comorbidities (1,5). In patients with liver cirrhosis, the liver function deteriorates significantly. The process involves changes in metabolic enzymes and transport proteins (12). The enzymes are downregulated and reduced functionally (12). Transport proteins suffer molecular changes, and as a result, energy expenditure and wasting are disturbed (12). In our study involving patients with liver cirrhosis, male gender was dominant (56.3%). Hepatitis viral infection was the etiology of liver cirrhosis in 52.5% patients. Mean age of patients was 51.3 (SD 12.60) years. These findings are in accordance with the data from the previous reports (1-3, 5).

The association between chronic liver disease, cyanosis, and clubbing finger was introduced firstly by Fluckinger in 1884 (13). In 1966, Berthelot et al. reported pulmonary arterial dilatation in patients with liver cirrhosis (4). The HPS terminology was firstly introduced by Kennedy and Knudson in 1977 after reporting a patient with liver cirrhosis and hypoxemia (4,13). The prevalence of HPS ranges from 4 to 47% among liver cirrhosis patients (4,6). The pathogenesis of HPS remains uncertain. Liver damage causes an increase in synthesis and decrease in metabolism of vasodilator substances (13,14). On the other hand, vasoconstrictor substances production is decreased and lung's sensitivity toward vasoconstrictors is decreased, which ends with vasodilatation in pulmonary capillaries and precapillaries (6,15,16). One of vasoactive substances is ET-1. ET-1 induces vasodilatation by increasing nitric oxide production (4,6,15,16). Vasodilatation leads to ventilation-perfusion mismatch and decreased oxygen saturation from longer distance for oxygen molecules to reach red blood cells in the centre of the pulmonary capillaries (4,6,15,16). In our study, the prevalence of HPS was 21.2%

Table 5. Accuracy of endothelin-1 level in predicting hepatopulmonary syndrome (HPS) in liver cirrhosis

Cut off	Sensitivity	Specificity	PPV	NPV	PLR	NLR	Accuracy
ET-1 (>187.5 mg/l)	82.35%	81.25%	53.84%	94.44%	4.32	0.22	90.8%

ET-1, endothelin-1; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value

which is within the range of prevalence reported in the literature (4,6).

Reportedly, the mortality rate for HPS is 41% (6). The presence of HPS significantly worsens the outcome of patients with liver cirrhosis and hampers their quality of life (6,13). Liver transplantation is the only therapeutic option to overcome HPS (6,13,15). Elevated ET-1 level also induces proliferation, hypertrophy, and synthesis of extracellular matrix (13). Grebes et al. reported that ET-1 level is higher in patients with liver cirrhosis compared to normal one; their study involving 18 patients with liver cirrhosis and eight healthy patients shows a significant difference in arterial ET-1 level (17.8 pg/mL versus 9.2 pg/mL) (14). Another study by Alam et al. (9) confirmed this result: hepatic ET-1 level was measured and the result was higher in patients with liver cirrhosis compared to controls. This condition was still observed even if patients had undergone transjugular intrahepatic portosystemic shunt (9). The result of Kamath et al. study was in concert with this result; ET-1 levels in hepatic tissue, peripheral vein, hepatic vein, and portal vein were higher in patients with liver cirrhosis (17).

A study by Kaffarnik et al. reported that plasma ET-1 level is positively correlated with the severity of liver failure; the more severe the liver damage, the higher the serum ET-1 level (10). This is in line with the result from Alam et al., who also found that ET-1 level has a positive correlation with liver disease severity based on Child Pugh score (9). Our study is in accordance with both studies, all patients were suffering from liver cirrhosis and we found that ET-1 level was significantly higher in patients with HPS compared to those without HPS. But there are two studies that contradict this result. Koch et al. and Kamath et al. reported that ET-1 level does not correlate with the severity of liver dysfunction (15,10).

Abdel-Razik et al. conducted a study in Egypt enrolling 42 liver cirrhosis patients with hepatorenal syndrome. They found that ET-1/NO ratio can predict therapeutic response toward terlipressin and albumin; early reduction of ET-1/NO ratio gave better response toward therapy in their study (18). A prospective study by Koch et al. in the USA tried to determine the association between ET-1 level and intrapulmonary vasodilatation in patients with liver cirrhosis, and found that ET-1 level in patients with intrapulmonary vasodilatation is higher than those without vasodilatation (9.1 pg/mL versus 2.1 pg/mL) (15).

To date, there is no study determining the role of ET-1 as a predictor of HPS in patients with liver cirrhosis. We tried to answer the question and found that ET-1 is a promising predictor for HPS in that population. Our results showed that ET-1 level had sensitivity and specificity of 82.35% and 81.25%, respectively in predicting HPS with a cut off value of >187.5 mg/L.

This study has a few limitations. The low prevalence of liver cirrhosis in our Centre makes it difficult to gather more patients for the study. In addition, the proportion of patients with and without HPS in this study is not balanced. A larger study involving more patients and several centres is mandatory to confirm the result of this study.

In conclusion, the patients with liver cirrhosis and HPS had a higher ET-1 level compared to those without HPS. ET-1 could be used as a promising marker for HPS in patients with liver cirrhosis. ET-1 level of >187.5 mg/l had a good accuracy in predicting HPS in liver cirrhosis patients.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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