

Nitric Oxide and von Willebrand Factor Levels as Markers of Endothelial Dysfunction in Liver Cirrhosis

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ABSTRACT

Introduction

A number of investigators have shown that endothelial dysfunction in liver cirrhosis can be indicated by increased levels of nitric oxide (NO) and von Willebrand factor (vWF). The cause of this increase is still unclear. It is believed to be correlated with hyperdynamic circulation and endotoxemia, which are common in liver cirrhosis.

The Aim of This Study

To compare the levels of NO and vWF in liver cirrhosis patients with those in healthy control subjects, and to investigate whether there is a correlation between levels of NO and vWF with the severity of the disease according to the Child Pugh Criteria

Material and Method

This study was conducted from February until June 2001 in 35 liver cirrhosis patients at Dr. Pirngadi and H. Adam Malik Hospital and some private hospitals in Medan. The mean age of patients with liver cirrhosis was 54 ± 12.26 years, the youngest being 31 years and the oldest 75 years, and 20 healthy controls while the mean age of the control subject was 55.20 ± 13.04 years, the youngest being 31 years and the oldest 76 years. Based on Child Pugh criteria, 9 were classified as Child Pugh class A, 13 in class B, 13 in class C. The criteria for liver cirrhosis were based on clinical examination, laboratory findings and liver ultrasound examination. Cirrhotic patients with hypercholesterolemia, hypertension, heart failure, myocardial infarction, renal failure diabetes, COPD were those on drugs, such as antibiotics and bronchodilators were excluded from the study.

Result

The mean level of NO in patients with liver cirrhosis was 6.2600 ± 4.4456 μM , while the mean NO level in control subjects was 3.2325 ± 3.2355 μM , $p < 0.05$. The mean level of NO in Child Pugh class A patients was 6.6889 ± 3.9757 μM , compared to control $p < 0.05$; in Child Pugh class B the mean level was 4.8308 ± 2.4642 μM compared to control $p > 0.05$. There was a significant increase in the level of NO associated with the severity of liver cirrhosis. The mean level of vWF in patients with liver cirrhosis was $399.514 \pm 175.313\%$ while the mean vWF level in control subjects was $139.100 \pm 51.144\%$, $p < 0.05$. The mean level of vWF in Child Pugh class A patients was $231.778 \pm 43.8576\%$, compared to control $p < 0.05$; in Child Pugh class B was $365.846 \pm 110.034\%$, compared to control $p < 0.05$, in Child Pugh class C was $549.308 \pm 164.483\%$, compared to control $p < 0.05$. There was significant increase in the level of vWF correlated with severity of liver cirrhosis.

Conclusion

The level of NO was significant higher in liver cirrhosis patients compared to control subjects, but there was no correlation between the increase in the level of NO with the severity of the disease. The levels of vWF was significantly higher in liver cirrhosis patients compared to control, and there was a correlation between increased levels of vWF and the severity of the disease.

Key Words: Liver cirrhosis, Child Pugh criteria, nitric oxide (NO), von Willebrand Factor (vWF)

INTRODUCTION

Liver cirrhosis is still the most frequent liver disease found in various hospitals in Indonesia.^{1,2} In Southeast Asia, the most common cause of liver cirrhosis is Hepatitis virus B and C.³ Liver cirrhosis is the final stage of a chronic liver disease resulting from an acute process that may not clinically present any significant physical abnormality up to the point of severe clinical condition.

Endothelial dysfunction is defined as imbalance between relaxation factors, and contraction pro-coagulant and anti-coagulant mediators or substances that inhibit or accelerate growth. Biochemical markers that are commonly used to determine endothelial dysfunction as well as improved endothelial function include NO, Endothelin-1, von Willebrand factor (vWF), Thrombomodulin, E-Selectin, sVCAM-1, sICAM-1, PAI-1, Microalbuminuria, and F2 isoprostan.^{4,5}

Several clinical and experimental studies found endothelial dysfunction in liver cirrhosis demonstrated through increased levels of nitric oxide and von Willebrand factor. The cause of such increased levels is still unclear, possibly related to hyperdynamic circulation and endotoxemia commonly encountered in liver cirrhosis.

Vallance and Moncada stated that increased synthesis and release of nitric oxide is caused by endotoxin and/or cytokine may be responsible for arteriolar vasodilatation and related to ascites in cirrhosis.⁶ Guarner et al also found a correlation between endotoxin in cirrhosis and nitric and nitrate levels, which are the final products of nitric oxide metabolism.⁷ Even though the role of endotoxin and/or cytokine is still under debate, further study support the role of NO as the chief vasodilator responsible for the development of a hyperdynamic circulation in cirrhosis.⁸

Von Willebrand factor is a multimer glycoprotein synthesized by vascular endothelial cells and megakaryocytes, secreted and stored in intraendothelial Weibel Pallade bodies and platelet alpha-granules.⁹ Plasma von Willebrand factors are increased in various conditions during endothelial dysfunction. The cause of such increase in von Willebrand factor is unknown, possibly due to

endothelial activation by cytokines and/or endothelial damage.^{8,10}

Ferro et al found increased von Willebrand factor due to endotoxemia in liver cirrhosis and such increase is correlated with the degree of liver failure based on Child-Pugh criteria.¹¹ Albornoz et al also found a correlation between increased levels of von Willebrand factor and NO levels in patients with cirrhosis, where an increase in these two endothelial factors are also correlated with the severity of the disease.

In the last few years, there has been additional proof that mechanical stimulation can directly influence endothelial structure and function. It has been shown that sheer stress can increase endothelial NOS and NO production. Thus, high levels of endothelial stress in liver cirrhosis caused by hemodynamic changes may be involved in causing excessive production of NO. On the other hand, local endothelial damage may cause a disturbance in Weibel Pallade bodies and endothelial membranes, thus releasing or causing leakage of von Willebrand factor (vWf).^{8,10,11}

From the account above, a study was conducted to determine whether there is a difference in the levels of NO and von Willebrand factor in patients with liver cirrhosis and normal subjects, and if the levels of the two endothelial factors were in accordance with the severity of disease according to the Child Pugh criteria.

AIM OF STUDY

- To determine whether there is an increase in the levels of NO and von Willebrand factor in patients with liver cirrhosis compared to healthy subjects
- To determine whether the levels of NO and von Willebrand factor in patients with liver cirrhosis is in line with the severity of illness according to the Child Pugh criteria
- To determine whether there is a correlation between the levels of NO and von Willebrand factor in patients with liver cirrhosis

MATERIALS AND METHOD

This descriptive analytic study was conducted from February 2001 to July 2001 at Dr. Pringadi General Hospital and Adam Malik General Public Hospital, Medan.

Subjects consisted of ambulatory and in-patients with liver cirrhosis. Subjects underwent routine laboratory evaluation, liver function test, SPE, prothrombine time, and liver ultrasound.

Control subjects consisted of healthy subjects (with normal findings on physical and laboratory evaluation). Case and control subjects consented to participate in the studies. Case and control subjects underwent evaluation for nitric/nitrate acid with enzymatic method using Griess diazotasion reaction, while von Willebrand factor levels were determined using Elisa method. Nitric/nitrate levels were stated in umol/L, while von Willebrand factor antigen stated in %.

Case subjects were chosen according to the following criteria for inclusion: patients with liver cirrhosis based on clinical, laboratory, and ultrasound evaluation.

Criteria for exclusion: patients with liver cirrhosis and control subjects suffering from hypercholesterolemia, hypertension, heart failure, myocardial infarction, renal failure, diabetes, chronic pulmonary obstruction disease, malignancy, pregnancy, less than 2 weeks following surgery, following heavy and moderate exercise, use of

antibiotics that are not absorbed in the bowel or current use of vasodilators.

DATA ANALYSIS

- Independent t-test to compare the mean value of NO and von Willebrand factor between case and control groups.
- Spearman or Pearson correlation to see the correlation between NO and von Willebrand factor in patients with liver cirrhosis.

RESULTS

Out of 35 patients with liver cirrhosis included in this study, 27 were male and 8 were female, with an average age of 54 ± 12.26 years, with the youngest being 31 years and the oldest 75 years. While in control subjects, 20 are healthy subjects with an average age of 55.20 ± 13.04 years, the youngest being 31 years of age, and the oldest being 76 years. There is no significant difference in the age of case and control subjects.

Out of the 35 patients with cirrhosis, 22 of them scored less than 9 in the Child Pugh classification, and 13 patients had a score of over 9; 23 patients suffered from ascites and 12 did not.

The results of nitric oxide and von Willebrand factor evaluation in patients with cirrhosis and control subjects can be found in the following tables.

RESULT OF NO EVALUATION

Table 1. Comparison of NO Levels Between Cirrhotic and Control Patients

Variable	Subject Group	N	X	SD	p
NO level	Liver cirrhosis	35	6.2600	4.4456	0.010
	Control	20	3.2325	3.2355	

NO: Nitric oxide

The mean NO level in patients with liver cirrhosis was significantly higher than in control subjects ($p < 0.05$)

Table 2. Comparison of NO Levels Between Patients with Child Pugh <9 and Control

Variable	Subject Group	N	X	SD	p
NO level	Child Pugh <9	22	5.5909	3.2196	0.023
	Control	20	3.2325	3.2355	

NO: Nitric oxide

The mean NO level in patients with liver cirrhosis with a Child Pugh score of less than 9 was significantly higher than in control subjects ($p < 0.05$)

Table 3. Comparison of NO Levels Between Patients with Child Pugh >9 and Control

Variable	Subject Group	N	X	SD	p
NO level	Child Pugh >9	13	7.3923	5.9705	0.014
	Control	20	3.2325	3.2355	

NO: Nitric oxide

The mean NO level in patients with liver cirrhosis with a Child Pugh score of more than 9 was significantly higher than in control subjects ($p < 0.05$)

Table 4. Comparison of NO Levels Between Patients with Child Pugh <9 and >9

Variable	Subject Group	N	X	SD	p
NO level	Child Pugh <9	22	5.5909	3.2196	0.253
	Child Pugh >9	13	7.3923	5.9705	

NO: Nitric oxide

The mean NO level in patients with liver cirrhosis with a Child Pugh score of less than 9 was not significantly higher than in case subjects with a Child Pugh score of more than 9 ($p > 0.05$)

Table 5. Comparison of NO Levels Between Cirrhotic Patients with and without Ascites

Variable	Subject Group	N	X	SD	p
NO level	Ascites	23	6.4652	5.0983	0.711
	No ascites	12	5.8667	2.9742	

NO: Nitric oxide

The mean NO level in liver cirrhosis patients with ascites was higher than in liver cirrhosis patients without ascites, but the difference was not statistically significant ($p > 0.05$)

Table 6. Comparison of vWf Levels Between Cirrhotic and Control Patients

Variable	Subject Group	N	X	SD	p
vWf level	Liver cirrhosis	35	399.514	175.313	0.0000
	Control	20	139.100	51.144	

The mean vWf level in patients with liver cirrhosis was significantly higher than in control subjects ($p < 0.05$)

Table 7. Comparison of vWf Levels Between Patients with Child Pugh <9 and Control

Variable	Subject Group	N	X	SD	p
vWf level	Child Pugh <9	22	311.000	110.468	0.0000
	Control	20	139.100	51.144	

The mean vWf level in patients with liver cirrhosis with a Child Pugh score of less than 9 was significantly higher than in control subjects ($p < 0.05$)

Table 8. Comparison of vWf Levels Between Patients with Child Pugh >9 and Control

Variable	Subject Group	N	X	SD	p
vWf level	Child Pugh >9	13	549.308	164.483	0.0000
	Control	20	139.100	51.144	

The mean vWf level in patients with liver cirrhosis with a Child Pugh score of more than 9 was significantly higher than in control subjects ($p < 0.05$)

Table 9. Comparison of vWf Levels Between Patients with Child Pugh <9 and >9

Variable	Subject Group	N	X	SD	p
vWf level	Child Pugh <9	22	311.000	110.468	0.0000
	Child Pugh >9	13	549.308	164.483	

The mean vWf level in patients with liver cirrhosis with a Child Pugh score of less than 9 was significantly higher than in case subjects with a Child Pugh score of more than 9 ($p < 0.05$)

Table 10. Comparison of vWf Levels Between Cirrhotic Patients with and without Ascites

Variable	Subject Group	N	X	SD	p
vWf level	Ascites	23	455.087	176.076	0.007
	No ascites	12	293.000	118.991	

The mean vWf level in liver cirrhosis patients with ascites was significantly higher than in liver cirrhosis patients without ascites ($p < 0.05$)

CORRELATION BETWEEN NO AND VWF LEVELS IN LIVER CIRRHOSIS

No linear correlation was found between NO and fvW levels of liver cirrhosis, with a Pearson correlation test of $r = -0.053$ and $p > 0.05$.

DISCUSSION

NO levels in Liver Cirrhosis

Several clinical studies have found increased NO levels in liver cirrhosis. Vallance and Moncada stated that endotoxemia and/or cytokine related with cirrhosis can induce excessive NO production through the synthesis of iNOS, and is responsible for increased NO in cirrhosis.⁶ Guarner et al found increased NO levels in patients with liver cirrhosis, and the increase is higher in patients with ascites. Increased NO levels is also correlated with the level of endotoxemia.⁷ Barak et al found a higher Nitric/Nitrate level in patients with cirrhosis compared to in control subjects.¹² Campillo found higher NO levels in patients with severe ascites compared to healthy subjects, while patients with mild ascites or no ascites demonstrate normal NO levels. Albomoz et al found higher levels of NO in patients with cirrhosis compared to control subjects, and the increase in NO is in line with the severity of disease, with a higher level of NO in cirrhotic patients with a Child Pugh score of over 9, compared to cirrhotic patients with a Child Pugh score of less than 9.⁸

In this study, we found a mean NO level of 6.2600 ± 4.4456 mmol/l, while the mean NO level in control subjects was 3.2325 ± 3.2325 mmol/l, demonstrating a statistically significant difference with a p of less than 0.05 (Table 1). Such result is the same as that found by several previous researchers.

The cause for increased NO levels in cirrhosis is still unclear. It is suspected to be related to a hyperdynamic condition and the presence of chronic endotoxemia in liver cirrhosis. Such hyperdynamic circulation causes high shear stress, which can directly affect endothelial structure and function. Shear stress increases eNOS enzyme activity, which then further increases NO production. In addition, general endotoxemia found in cirrhosis can induce excessive production of NO through the synthesis of iNOS enzyme. Aside from shear stress and endotoxemia, catecholamine, estrogen, and P substance, which are all increased in cirrhosis, increase iNOS enzyme synthesis.^{14,15,16,17}

From this study, patients with cirrhosis were classified into two groups compared to liver function reserve (Child Pugh classification, with a score of less

than 9 and over 9). There is a significant difference in the mean NO level in subjects with a Child Pugh score of less than 9 is 5.5909 ± 3.2196 mmol/l, compared to that of control subjects (3.2325 ± 3.2325 mmol/l), with a p of less than 0.05 (Table 2). However, there was no significant difference in the mean NO level in patients with a Child Pugh score of less than 9 compared with those with a Child Pugh score of over 9 (7.3923 ± 5.9705 mmol/l), $p > 0.05$ (Table 4).

From the data above, we can see that patients with cirrhosis are found with higher NO levels compared to those in control subjects. However, the increase is not in line with the severity of disease according to the Child Pugh criteria. Such results were different from that found by a previous researcher, which found increased NO levels in line with the severity of liver cirrhosis. Various factors might have caused such difference, such as long-standing cirrhosis, which could cause a chronically hyperdynamic condition, or the patient might have received antibiotics or nitrate drugs that were not discovered during history-taking. Antibiotics, particularly those that are not absorbed in the bowel, reduces the level of endotoxin, which thus reduces the synthesis of iNOS enzyme.^{7,8} While nitrate drugs act by releasing NO from its molecule, thus increasing the level of NO in the blood.¹⁸ To avoid such problem, administration of all drugs should be terminated several days to weeks prior to taking blood samples, to ensure that the patient is completely free from the influence of drugs. In this study, drug administration was not terminated. Albomoz et al, in a study on 27 patients with liver cirrhosis, found a higher NO level in patients with ascites compared to those without.

In this study, out of 35 patients with liver cirrhosis, 23 patients suffered from ascites, while 12 patients did not. The mean NO level of patients with ascites was 6.4652 ± 5.0983 mmol/l, while the mean NO level in patients without ascites was 5.8667 ± 2.9742 mmol/l.

The mean NO level in patients with ascites was still relatively higher than in those without ascites, but the difference was not statistically significant (Table 5). From the data above, we can see that ascites does not influence the level of NO in liver cirrhosis. This differs from what was found by the two previous researchers mentioned above. The difference might be caused by the difference in the severity and duration of ascites.

Von Willebrand Factor Levels in Liver Cirrhosis

Several clinical studies found a higher level of von Willebrand factor in patients with liver cirrhosis. The cause of such increase is still unclear. It may be due to

cytokine activation or endothelial damage, or perhaps high levels of von Willebrand factors may be associated with a compensation mechanism for hemostatic disturbance in chronic liver disease. Local endothelial damage may cause a disturbance in Weibel-Pallade Bodies, thus releasing or causing leakage of von Willebrand factors.^{8,11} Ferro et al found a progressive increase of von Willebrand factor and endotoxemia in accordance with the severity of disease according to Child-Pugh criteria.¹¹ Beer et al found a von Willebrand factor of over 400% normal levels. Albornoz et al found a higher level of von Willebrand factor in cirrhotic patients compared with normal subjects, and the increase was in line with the severity of disease according to the Child-Pugh criteria. In this study, we found an average von Willebrand factor level of 399 ± 175.313 % in patients with liver cirrhosis and a von Willebrand factor level of 139 ± 51.144 % in control subjects. This difference was statistically significant ($p < 0.05$) (Table 6). The average level of von Willebrand factor in liver cirrhosis scoring less than 9 in the Child-Pugh classification was 311.000 ± 110.468 %, which was significantly higher than control according to statistics ($p < 0.05$) (Table 7). The average level of von Willebrand factor in cirrhotic patients with a Child-Pugh classification of over 9 is 549.308 ± 164.483 %, which was significantly higher than control according to statistics ($p < 0.05$) (Table 8). The average von Willebrand factor level in patients with liver cirrhosis scoring less than 9 in the Child-Pugh classification was 311.000 ± 110.468 %, which was, by statistics, significantly higher than the level in patients scoring over 9 (Table 9). The results of this study were in line with those found by the previous researcher. From the data above, we could see an average increase in the level of von Willebrand factor in patients with liver cirrhosis, and that the increase was in line with the severity of disease according to the Child-Pugh classification. In his study, Albornoz et al found a higher von Willebrand factor level in cirrhotic patients with ascites compared to those without, but the exact mechanism causing this condition is still unclear.⁸

In this study, out of the 35 patients with cirrhosis, there were 23 patients with ascites and 12 without ascites. The average von Willebrand factor level in those with ascites was 455.087 ± 176.076 %, while the average von Willebrand factor level in those without ascites was 293.000 ± 118.991 %. The average von Willebrand factor level in ascites was higher than in those without ascites, which demonstrated a statistically significant difference (Table 10). From this data, we can

see that the presence of ascites can influence the increase in von Willebrand factor in patients with liver cirrhosis, which was in accordance to the findings of previous researchers. In his study, Albornoz also found a linear correlation between NO levels and von Willebrand factor levels in patients with liver cirrhosis.⁸

In this study, we found a higher average level of NO and von Willebrand factor in patients with cirrhosis compared to healthy subjects, which after analysis with Pearson correlation resulted in an r value of -0.053 and a p of over 0.05 . This means that there was no linear correlation between NO levels and von Willebrand factor levels in patients with liver cirrhosis. This finding was unlike those of previous researchers, possibly due to long-standing cirrhosis, the influence of drugs such as antibiotics, vasodilators (which the patients had previously received, but was not found during history-taking, since the patients themselves often do not know what drugs they have consumed), which might have influenced the levels of one of the endothelial factors.

CONCLUSION

1. There is an increase in the levels of NO and von Willebrand factor in patients with liver cirrhosis compared to healthy subjects.
2. The increase in NO in liver cirrhosis was in line with the severity of disease, while the increase in von Willebrand factor level was in line with the severity of disease according to the Child-Pugh criteria. Ascites does not have an influence on the level of NO in patients with liver cirrhosis, but influences the level of von Willebrand factor. There was no linear correlation between the levels of NO and von Willebrand factor in liver cirrhosis.

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