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Arteriosclerosis and Vascular Endothelial Function in Chronic Liver Disease

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Abstract **Background:** Patients with chronic liver disease (CLD) are known to have a low incidence of cardiovascular events. However, the extent of arteriosclerosis in CLD has not been studied well. **Aims:** To assess arteriosclerosis in CLD, and to assess and examine vascular endothelial function. **Methods:** The subjects were 17 patients with chronic hepatitis (CH), 48 patients with liver cirrhosis (LC), and 18 non-CLD patients (control) with no history of cardiovascular events (coronary artery disease: CAD). Arteriosclerosis was assessed by ultrasonic determination of intima-media complex thickness (IMT) and stiffness parameter (β value) of the common carotid artery. Vascular endothelial function was assessed by determining flow-mediated dilatation (FMD) of the brachial artery. Nitrogen oxides (NOx), angiotensin II (AG II), and endothelin-1 (ET-1) were measured as vasoactive substances in the serum of all subjects. The concentration of adiponectin, which has antiatherogenic action, was also measured in the serum of all subjects. **Results:** No significant differences in IMT or β value were found between the groups. The FMD in CH (5.8 ± 1.9%) was significantly higher compared to the control (3.4 ± 1.4%) (F=3.3, p=0.015). The serum concentrations of ET-1 and adiponectin in CLD patients were greater the more advanced the disease. **Conclusions:** There were no apparent differences in the extent of arteriosclerosis between CLD and the control, while vascular endothelial function in CLD was better preserved compared to the control, which may be attributable to the antioxidant action of nitric oxide (NO) and/or the antiatherogenic action of adiponectin.

Key words: arteriosclerosis, vascular endothelial function, chronic liver disease

Introduction

Based on reports to date, it is widely known that only a low percentage of patients with chronic liver disease (CLD) also have arteriosclerotic disorders such as ischemic heart disease. The risk of cardiovascular events is reportedly low, even when patients with liver cirrhosis (LC) also have diabetes, which is a risk factor for arteriosclerosis.[1] However, the results of studies on arteriosclerosis in patients with CLD have not been reported very much. No conclusions have yet been reached, as some reports state that hepatitis virus infection is a risk factor for carotid artery plaque,[2][3] while other reports state there is no correlation between hepatitis B virus (HBV) infection and coronary arteriosclerosis.[4] There are also reports that the development of arteriosclerosis is inhibited in diabetic patients with LC.[5] Although reasons that have been proposed include low cholesterol or platelets, this has not been adequately studied.

Hyperdynamic circulation is a hemodynamic

state that occurs following elevated portal blood pressure in liver cirrhosis. Animal experiments have confirmed increased vascular endothelial nitric oxide synthase (eNOS) in the serum of rats with portal hypertension.[6] The following cycle has been contemplated as the mechanism leading to hyperdynamic circulation: as first portal pressure elevates, secondly the peripheral vascular resistance decreases and the cardiac output increases, thirdly shear stress increases, and finally nitric oxide (NO) increases and peripheral vascular resistance further decreases.[7] However, no research evaluating endothelial function in portal hypertension has thus far been reported.

Recently, flow-mediated dilatation (FMD) of the brachial artery has been widely used as a non-invasive test to assess endothelial dysfunction, which is an early sign of arteriosclerosis.[8-11] In this study, FMD was used to assess vascular endothelial function in all subjects. Nitrogen oxides (NOx), angiotensin II (AG II), and endothelin-1 (ET-1) were also measured as vasoactive substances. We also measured adiponectin, which has antiatherogenic action, anti-inflammatory action, and anti-diabetic action, in particular among the adipocytokines secreted by adipocytes.[12][13] The purpose of this study was primarily to assess the extent of arteriosclerosis in CLD patients with hepatitis C virus (HCV) infection, and to elucidate the cause of the low incidence of cardiovascular events in CLD patients.

Subject and methods

Subjects

The subjects were 83 patients randomly selected from patients examined at Yamaguchi University Hospital over a two year period from 2006 to 2007 (17 chronic hepatitis (CH) patients (33 to 75 years of age), 48 LC patients (42 to 77 years of age), and 18 non-chronic liver disease patients with no history of cardiovascular events (51 to 76 years of age)). The LC group was divided according to the Child-Pugh classification into a Child A group (49 to 77 years of age), Child B group (44 to 74 years of age), and Child C group (42 to 68 years of age). This study protocol complied with the Declaration of Helsinki and was approved by ethics committee of Yamaguchi University Hospital (project registration number H18-64). Informed consent was obtained from all patients before the study.

Ultrasonography

Vascular endothelial function: The subjects rested in the supine position for at least 15 minutes in a quiet temperature- and humidity-controlled room. The resting vascular diameter of the right brachial artery was measured using an ultrasonograph equipped with a 13.5 MHz high frequency ultrasonic probe (SSD-6500, Aloka, Mitaka, Japan). A pressure equal to systolic blood pressure (SBP) + 50 mmHg was applied for 5 minutes with a manchette for blood pressure measurement wrapped around the right forearm. The change in vascular diameter after the pressure had been released was measured continuously for 3 minutes. The maximum dilation in vascular diameter after the pressure release relative to the vascular diameter at rest was calculated as FMD.

Echocardiography: An ultrasonograph equipped with a 3.5 MHz was used. The cardiac output (CO) was calculated by the following equation using the left ventricular outflow tract diameter (LVOTd) in the parasternal long axis view and the velocity time integral (VTI) of the left ventricular outflow measured in the apical long axis view.

$$CO\ (l/min) = \pi(LVOT\ d/2)^2 \times VTI$$

Carotid artery ultrasonography: A 13.5 MHz high frequency ultrasonic probe was used to measure the left and right common carotid artery intima-media complex thickness (IMT) in 3 locations at 1 cm intervals, and the mean was used as the mean IMT. IMT greater than 11 mm in any location was considered to be plaque (+). The common carotid artery diastolic diameter (Dmin) and systolic diameter (Dmax) were measured, and the systolic blood pressure (SBP) and diastolic blood pressure (DPB) were used to calculate the stiffness parameter (β) by the following equation.

$$\beta = \ln(SBP/DBP)/\{Dmax/(Dmin-Dmax)\}$$

Congestion index (CI): A 3.5MHz convex probe was used to measure the diameter (D) and mean velocity of the main trunk of the portal vein in patients breathing quietly at rest in the supine position, and the CI was calculated by the following equation.[14]

$$CI = \{\pi(D/2)^2\}/\ mean\ velocity$$

Table 1 Clinical characteristics of study subjects

	Control	CH	Child A	Child B	Child C	
	n=18	n=17	n=19	n=19	n=10	p Value
Age, yrs	64±2	60±2	63±2	61±2	60±3	0.45
Male, %	72.2	35.3	57.9	63.2	70.0	0.21
BMI, kg/m2	21.3±6.3	22.9±3.1	24.3±2.2	24.4±2.8	23.7±1.8	0.09
Viral infection (HBV/HCV)		1/16	2/15	4/12	1/7	
Hypertension, %	37.5	35.7	31.6	38.9	10.0	0.49
Diabetes mellitus, %	26.7	25.0	17.7	31.6	30.0	0.90
Smoker, %	27.8	23.5	15.8	10.5	50	0.18
Systolic pressure, mmHg	115±4	123±4	119±4	113±4	108±5	0.10
Diastolic pressure, mmHg	61±9	66±9	64±9	62±7	61±13	0.40
Fasting glucose, mg/dl	117±36	99±18	107±20	108±23	108±39	0.41
Total cholesterol, mg/dl	193±8	169±8	174±8	148±8	108±11	<0.0001
Triglycerides, mg/dl	121±61	98±31	89±28	70±26	57±18	0.002
Albumin, g/l	3.9±1.0	3.9±0.4	3.7±0.5	3.0±0.3	2.5±0.4	<0.0001
Bilirubin, mg/dl	0.8±0.3	0.8±0.3	1.2±0.5	1.4±0.6	3.2±1.0	<0.0001
Platelets, ×104/μl	20.9±3.9	15.8±4.5	12.8±6.2	8.7±5.6	7.5±2.7	<0.0001
CRP, mg/dl	0.1±0.0	0.1±0.1	0.2±0.0	0.1±0.0	0.3±0.0	0.02
Cardiac output, l/min	3.2±0.9	3.9±1.4	4.5±1.6	4.6±1.4	5.6±2.2	0.02

Data expressed as mean ± SD. p values from analysis of variance (ANOVA).

All ultrasonography was performed by a trained tester. No details on the condition of subjects were disclosed to the tester.

Assays of vasoactive substances and adiponectin

Serum samples were collected from all subjects after ultrasonography, and stored at -80℃ until subsequent assay. Serum nitrite and nitrate concentrations were determined using a procedure based on the Griess reaction. ET-1 and AGII levels were measured using radioommunoassay methods in which antibody-binding parameters were optimized. Serum adiponectin level was evaluated using a sandwich ELISA kit (Human-adiponectin ELISA kit; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan).

Statistical analysis

The measured results were expressed as the mean ± standard deviation (SD). The measured results were compared between groups by one-way layout analysis of variance, and the χ^2 test was used for category analysis.

Results

Clinical characteristics of study subjects

Table 1 presents the clinical data of the groups. There were no significant differences between the groups in terms of age, gender ratio, BMI, systolic blood pressure, diastolic blood pressure, or fasting glucose. Albumin, platelets, T-cholesterol, and triglycerides tended to be lower for the greater severity in chronic liver disease, while bilirubin and CO tended to be higher for the greater severity. CO also showed a significant inverse relationship to cholesterol, albumin, platelets, and PT%, while there was a significant positive relationship to bilirubin, portal diameter, and congestion index (Table 2).

Table 2 Correlation of cardiac output and other parameters

	r	r^2	p Value
Total cholesterol	-0.25	0.06	0.048
Triglycerides	-0.09	0.01	NS
Albumin	-0.25	0.06	0.046
Bilirubin	0.34	0.11	0.007
Platelets	-0.34	0.11	0.008
CRP	0.18	0.03	NS
PT%	-0.30	0.09	0.019
Portal diameter	0.46	0.21	0.001
Portal blood flow	0.10	0.06	NS
Congestion index	0.34	0.12	0.017

NS: Not Significant

Carotid artery ultrasonography

Table 3 presents the results of carotid artery ultrasonography. There were no significant differences between the groups in terms of mean IMT, stiffness parameter, or common carotid artery diameter.

Vascular endothelial function test

The FMD in the CH group (5.79 ± 0.50%) was significantly higher than in the control group (3.44 ± 0.48%), Child A group (4.20 ± 0.46%), and Child B group (3.84 ± 0.47%) (Fig. 1A). There was also a inverse relationship between FMD and CO in the LC group (r= -0.44, p= 0.01) (Fig. 2), but not in the CH group (r=0.14, p=0.68).

Vasoactive substances

Among the vasoactive substances produced by vascular endothelial cells, vasodilator NOx were significantly higher in the CH group (69.2 ± 43.7 μmol/L) and Child C group (85.8 ± 49.0 μmol/L) compared to the control group (25.8 ± 25.2 μmol/L) (Fig. 1B). The vasoconstrictor ET-1 was significantly higher in the Child B group (6.6 ± 2.4 pg/mL) and Child C group (9.8 ± 2.8 pg/mL), compared to the control group (1.5 ± 0.5 pg/mL), and tended to be higher for the greater severity in CLD patients (Fig. 1C).

Table 3 Parameters associated with carotid atherosclerosis

	Control	CH	Child A	Child B	Child C	p Value
Mean IMT, mm	0.9±0.4	0.7±0.2	0.8±0.3	0.7±0.2	0.6±0.1	0.10
Plaque (+), %	44.4	17.7	26.3	21.1	10.0	0.25
Stiffness parameter (β)	16.2±6.0	14.9±4.2	15.8±5.2	14.9±4.1	14.7±4.5	0.87
Carotid diameter, mm	7.5±1.0	7.7±1.0	7.8±1.1	7.7±0.9	7.5±0.7	0.86

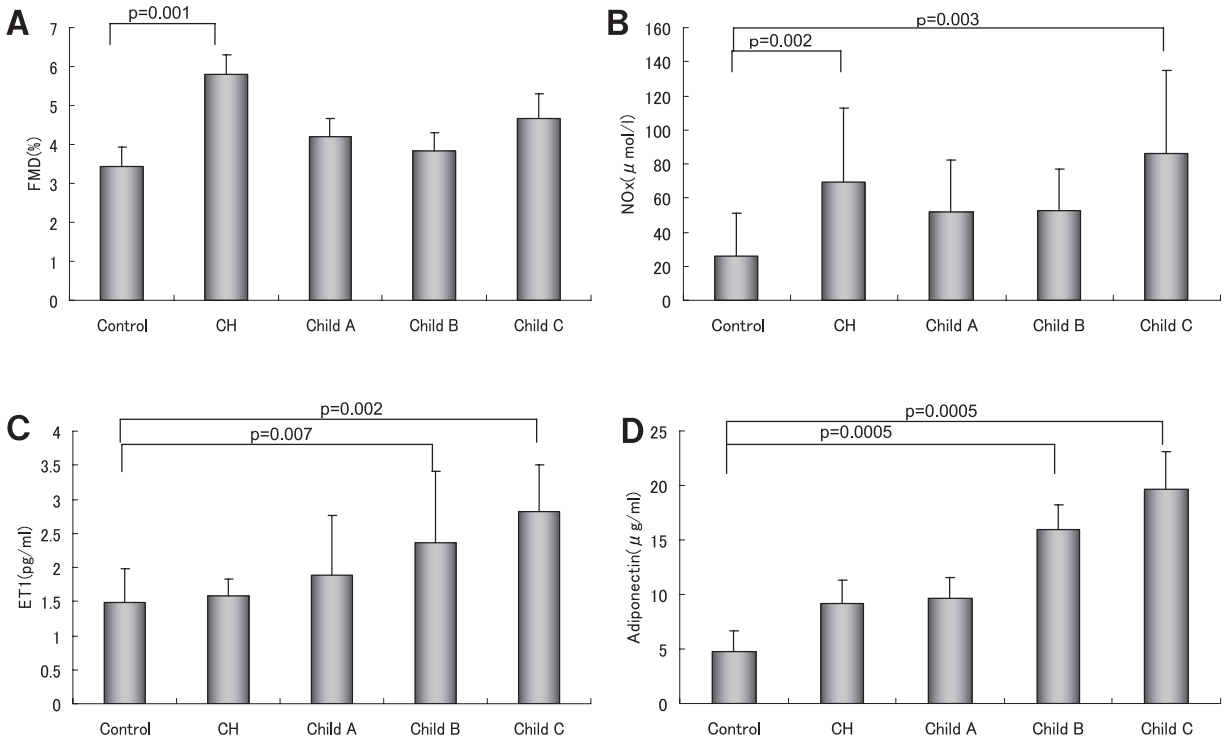

Fig. 1 A: FMD in the disease group: FMD was significantly higher in the CH than in the control group. B: NOx in the disease group: The NOx level was significantly higher in the CH and Child C than in the control group. C: ET-1 in the disease group: The ET-1 level was significantly higher in the Child B and C than in the control group. D: Adiponectin in the disease group: The adiponectin level was significantly higher in the Child B and C than in the control group.

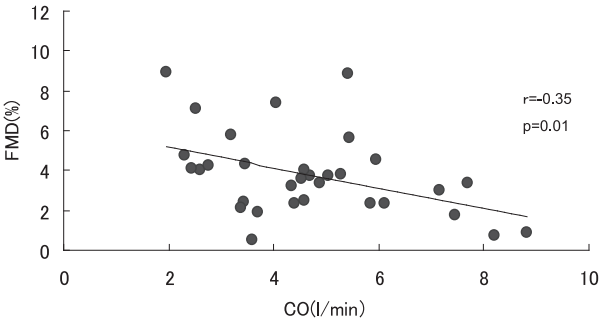

Fig. 2 Relationship between FMD and cardiac output: FMD and CO were significantly negatively correlated in the LC group.

There were no significant differences in AG II between the control (16.6 ± 5.3 pg/mL), CH (14.1 ± 5.7 pg/mL), Child A (28.0 ± 5.9 pg/mL), Child B (12.9 ± 6.6 pg/mL), and Child C (31.8 ± 9.8 pg/mL) groups.

Adiponectin

Adiponectin was significantly higher in the Child A (9.7 ± 1.9 μg/mL), Child B (15.9 ± 2.3 μg/mL), and Child C (19.6 ± 3.5 μg/mL) LC groups compared to the control group (4.8 ± 1.9 μg/mL), and tended to be higher for the greater severity in CLD patients (Fig. 1D).

Discussion

Although patients with CLD are known to have a low incidence of cardiovascular events, few studies have evaluated arteriosclerosis in very much detail in CLD patients. In this study, arteriosclerosis in CLD patients was assessed using artery wall thickening and stiffness as indicators. Endothelial dysfunction, which has been noted as an early sign of arteriosclerosis, was also studied in conjunction with the concentration of vasoactive substances in blood.

Being a carrier of the hepatitis B virus or hepatitis C virus, which cause the majority of CLD, has been reported to be an independent risk factor for IMT thickening and carotid artery plaque positivity,[2)3)] and chronic infection caused by *Chlamydia* or *Helicobacter pylori* has been reported to be a risk factor for IMT thickening.[15-19)] In this view, in other words, the incidence of arteriosclerosis increases in hepatitis viral infection, because chronic inflammation caused by viral infection is a potential risk factor for IMT thickening or carotid artery plaque. However, this point of view is not supported by the results of our study. In our study of arteriosclerosis using IMT or stiffness parameters and the prevalence of plaque as indicators, the results were within normal range in CLD patients, thus suggesting a lack of arteriosclerosis progression in CLD patients. The most significant result was that FMD levels were significantly higher in CH than in the control, whereas there were no significant differences between LC and the control, and that the FMD levels in CLD patients overall were nearly the same as the FMD levels in normal individuals reported thus far. These results indicate better vascular endothelial function in CH than in the control, and the absence of endothelial dysfunction in LC.

It has been reported that there is no relationship between HBV infection and coronary arteriosclerosis in patients who have undergone coronary angiography,[4)] and that carotid artery plaque positivity is lower for patients with the higher serum bilirubin level.[20)] In other words, this supports the view that a low percentage of patients with cardiovascular events have hepatitis viral infection, that carotid artery plaque positivity is lower for the greater severity of CLD, and that furthermore the progression of arteriosclerosis is inhibited in LC. The results of our study are consistent with this view.

Several factors may be considered as reasons for why good vascular endothelial function was preserved, with no progression of arteriosclerosis, in CH, despite the fact that it is a viral infection. It has thus far been assumed that the risk of cardiovascular events in CLD was low because of low levels of cholesterol or platelets which influence arteriosclerosis in LC, or because of high levels of bilirubin which supposedly has antioxidant action.[21)22)] However, despite the lack of significant differences in platelets, cholesterol, or bilirubin in CH compared to the control, the vascular endothelial function was clearly good. Since the levels of NOx having antioxidant action were more than two times greater than in the control, and the levels of adiponectins, which have recently attracted attention as having an-

tiatherogenic action, were markedly higher, it is possible that the antioxidant action of NOx and/or the antiatherogenic action of adiponectins in protecting vascular endothelial function may have had a significant effect. In other words, it is possible that NOx or adiponectin production was already markedly elevated in CLD caused by viral infection as well as in CH, and that vascular endothelial function was well preserved by the antioxidant action or antiatherogenic action. It is also possible that NOx and adiponectin production are further stimulated as the severity of LC increases, thereby further preventing the progression of arteriosclerosis. As a result, a low incidence of cardiovascular events may be expected in patients with CLD.

The concentrations of ET-1 or NOx in blood are already known to increase as the severity of CLD worsens.[23)24)] The results of the present study also showed that these concentrations increased as the severity of CLD worsened. ET-1 is a vasoactive substance with vasoconstricting action that is well known to have action in bringing about endothelial dysfunction and promoting arteriosclerosis. However, it has thus far been reported that since hyperdynamic circulation is facilitated[6)] by peripheral vasodilation due to increases in NOx from greater shear stress, or decreases in arterial response to ET-1 in LC,[25)] the significant impact on vasodilation by NOx despite the increase in ET-1 is not contradictory. Although many points about the increases in NOx in LC remain unclear, the potential involvement of tetrahydrobiopterin which increases eNOS activity, has been pointed out to increase NO synthesis due to greater shear stress.[26)27)] In light of the markedly elevated NOx in CH, which is not characterized by hyperdynamic circulation, it may well be possible that increases in NOx result from factors other than shear stress. In view of the above, it appears that increases in the severity of CLD may have been accompanied by increases in ET-1 or NOx, resulting in vasodilation.

The results of the present study showed that increases in the severity of CLD resulted in greater CO. These results raise the hypothesis that increases in the severity of CLD result in greater CO and a trend toward higher levels of FMD. The negative correlation between CO and FMD found in our study would at first glance seem to contradict such a hypothesis. However, several mechanisms are involved in FMD. The results of reactive hyperemic vasodilation response relative to resting vascular diameter are calculated as a relative percentage. Therefore, if blood vessels already tend to be dilated while at rest, the vasodilating response to shear stress would be minimal, and the vascular endothelial function may be underestimated. In fact, the results of our study indicate that ET-1, NOx, and CO tend to increase as the severity of CLD increases, and it would be easy to surmise an accompanying increase in resting vascular diameter.

It is noteworthy that the FMD effect may be underestimated in patients with LC, which is characterized by hyperdynamic circulation with increased CO. Although there were no apparently significant differences in FMD from the control, the possibility cannot be ruled out that vascular endothelial function would actually be evaluated as being better than the control in the same manner as in CH by avoiding the underestimation of FMD due to the impact of increased CO. It is possible that vasodilation cannot be properly assessed while CO is elevated.

In the future, for a more increased sample size, the changes in shear stress during FMD measurement (at rest and during reactive hyperemia) should be studied in detail in order to clarify the relationship between CO and FMD levels in CLD patients, and to clarify the reason for the low incidence of cardiovascular events in CLD. Comparison of vasoactive substances in LC with and without elevated CO may also allow us to understand the mechanism involved in the onset of arteriosclerosis and LC prognosis.

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