

Increased Plasma Malondialdehyde in Patients with Viral Cirrhosis and Its Relationships to Plasma Nitric Oxide, Endotoxin, and Portal Pressure

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Abstract

Background and Aim Increased oxidative stress is involved in the development of portal hypertension in cirrhosis. Our study aimed to assess the relationship between oxidative stress and hemodynamic parameters in cirrhotic patients.

Methods Forty-two patients with viral cirrhosis and 24 normal controls were enrolled. Measurements of plasma levels of malondialdehyde (MDA), nitrite/nitrate (NO_x), endotoxin, and activities of superoxide dismutase (SOD) were carried out in all subjects. Systemic and splanchnic

hemodynamic measurements were carried out in cirrhotic patients.

Results Plasma levels of MDA, endotoxin, and NO_x were significantly higher in cirrhotic patients than in normal controls (900 ± 751 versus 226 ± 16 nM, $P < 0.01$; 62.0 ± 26.0 versus 14.8 ± 4.1 pg/mL, $P < 0.01$; 50.5 ± 22.6 versus 15.0 ± 9.2 nM, $P < 0.01$, respectively). Activities of SOD were significantly decreased in cirrhotic patients compared with in normal controls (2.62 ± 0.7 versus 6.8 ± 0.4 U/mL). Further, plasma levels of MDA in cirrhotic patients were significantly positively associated with hepatic venous pressure gradient (HVPG) ($r = 0.35$; $P = 0.025$), wedge hepatic venous pressure (WHVP) ($r = 0.42$; $P = 0.007$), and hepatic sinusoid resistance (HSR) ($r = 0.33$; $P = 0.033$). Plasma MDA levels also correlated positively with plasma endotoxin ($r = 0.71$, $P < 0.001$) and NO_x ($r = 0.55$, $P < 0.001$) levels in the cirrhotic patients. Multiregression analysis showed that the independent and strongest factors to predict HVPG, WHVP, and HSR are plasma levels of NO_x, MDA, and endotoxin, respectively.

Conclusion This study suggests a close interaction among MDA, endotoxin, and NO_x and that these substances are also associated with hemodynamic derangement in cirrhosis.

Keywords Cirrhosis · Endotoxin · Malondialdehyde · Nitric oxide · Portal hypertension · Superoxide dismutase

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Introduction

In aerobic life, oxygen is essential in many chemical reactions. During the utilization of the oxygen, oxidants such as aldehydic products of lipid peroxidation are

produced [1–3]. At the same time, antioxidants such as superoxide dismutases (SOD) are produced to counteract the generation of oxidants and to protect cells [4]. Increased oxidative stress is defined as an imbalance between the production of oxidants and antioxidants. Increased oxidative stress can stimulate lipid peroxidation reactions and result in DNA damage and posttranslational derangement of proteins [5].

Portal hypertension is a frequent and serious complication of chronic liver diseases. It is initiated by increased intrahepatic resistance and maintained by increased portal inflow [6]. It has been established that increased oxidative stress accelerates the progression of liver fibrosis during chronic liver injury of various etiologies [7]. Oxidative stress also contributes to hepatic endothelial dysfunction by modulating NO bioavailability in the intrahepatic microcirculation [4]. It has also been reported that a reduction in portal pressure and amelioration of hyperdynamic circulation were observed in portal hypertensive and bile-duct-ligated (BDL) rats receiving antioxidants [8–10]. Furthermore, patients receiving acute administration of an antioxidant had attenuation of the postprandial increase in hepatic venous pressure gradient [11]. Theoretically, increased oxidative stress is important for the development of portal hypertension and hyperdynamic circulation in cirrhosis.

Malondialdehyde (MDA), a typical aldehydic product of lipid peroxidation, has been found to be markedly increased in cirrhotic patients [12–15]. Further, chronic endotoxemia is a well-established phenomenon in cirrhotic patients [16]. Increased circulating NO, which positively correlated with endotoxemia, has been observed to be increased in cirrhotic patients [17, 18]. Therefore, the aim of our study was to examine the possible relationships among the increased oxidative stress, increased circulating NO, endotoxemia, and various hemodynamic parameters in cirrhotic patients.

Methods

Study Patients

Between August 1998 and December 2002, 42 viral cirrhotic patients aged 20–80 years who were admitted to the Taipei Veterans General Hospital for diagnosis and evaluation of the severity of their liver disease and portal hypertension were finally enrolled. Diagnosis of cirrhosis was based on the imaging, clinical, and laboratory findings. Diagnosis of hepatitis B virus infection was made in the patients found seropositive for hepatitis B surface antigen (HBsAg; RIA kits, Abbott Laboratories, North Chicago, IL). Diagnosis of hepatitis C virus (HCV) infection was made in patients found to be seropositive for the antibody against HCV by a second-generation enzyme immunoassay

(Ortho-Clinical Diagnostics, Johnson-Johnson Company, Bucks, UK). Exclusion criteria included presence of hepatic encephalopathy, presence of hepatocellular carcinoma, history of previous operations for portal hypertension, active infection, history of esophageal variceal bleeding, use of vasoactive drugs within 1 week, use of antioxidants, history of heart diseases or renal disease, history of active alcoholism, and refusal to participate. The etiology of cirrhosis among the 42 enrolled patients was HBV related in 28 patients, HCV related in 8 patients, and HBV and HCV related in 6 patients. The severity of liver cirrhosis was established according to Pugh's modification of the Child classification and the model for end-stage liver disease (MELD) score [19]. All patients received abdominal ultrasound to assess ascites. The grading of ascites was as suggested by Gines et al. [20]. Briefly, ascites in an amount large enough to result in marked abdominal discomfort was considered as large volume; moderate-volume ascites was considered an amount causing moderate discomfort [20]. Upper gastrointestinal endoscopy was performed in each cirrhotic patient before enrollment. Written informed consent was obtained from each participant.

Hemodynamic Measurements

After overnight fasting, enrolled patients underwent hemodynamic evaluation. Briefly, under local anesthesia, catheterization was performed by 7-F Swan-Ganz thermodilution catheter (Gould, Cupertino, CA) as previously described [21]. By the Seldinger technique, the catheter was inserted percutaneously into the right femoral vein or right internal jugular vein. Then, it was advanced into the right hepatic vein, where the free hepatic venous pressure (FHVP) and wedge hepatic venous pressure (WHVP) were measured by inflation and deflation of the balloon with a multichannel recorder (model 78534C, Hewlett Packard, Palo Alto, CA). The hepatic venous pressure gradient (HVPG) was obtained by subtracting the FHVP from the WHVP. After hepatic vein catheterization, the catheter was turned into the right side of heart and the pulmonary artery for measurements of systemic hemodynamics: right arterial pressure (RAP), mean pulmonary arterial pressure (MPAP), and pulmonary capillary wedge pressure (PCWP). Mean arterial pressure (MAP) and heart rate were recorded with an external vital sign monitor (Dinamap 8100, Critikon, Tampa, FL). Cardiac output (CO) was measured by the thermodilution method. Systemic vascular resistance (SVR; dyne/s/cm^5) was calculated according to the following equation: $[(\text{MAP} - \text{RAP}) \times 80]/\text{CO}$. For measurement of hepatic blood flow (HBF), a dosage of 0.5 mg/kg indocyanine green (ICG) was infused via an antecubital vein over a period of 5 min and then a constant rate of 0.48 mg/min was maintained, as previously

described [21]. After reaching a steady state of ICG plasma concentration by waiting for 30 min, blood samples were sequentially obtained from the peripheral vein and the hepatic vein at 2 min intervals for 10 min. The hepatic sinusoid resistance ($\text{dyne}\cdot\text{S}\cdot\text{cm}^{-5}$, HSR) was estimated as $\text{HVPG (mmHg)} \times 80/\text{HBF (L/min)}$ [11]. During the hemodynamic study, peripheral blood was obtained for further laboratory investigations.

Laboratory Investigations

Blood samples were collected to measure plasma endotoxin and NOx (the sum of NO^{2-} and NO^{3-}). A pyrogen-free syringe containing ~ 75 units of heparin sodium was used. Then, the plasma obtained was stored at -70°C for subsequent analysis within 6 weeks. Enzyme-linked immunosorbent assay (ELISA) kits for measurement of plasma endotoxin and NOx were purchased from Cayman Chemical (Ann Arbor, MI). The serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), gamma glutamyl transpeptidase (GGT), and total bilirubin (TB) were measured by using a standard autoanalyzer technique (SMAC analyzer, Technicon Instrument Co., Tarrytown, NY).

The plasma activity of superoxide dismutase (SOD) was also measured. Three types of SODs were characterized according to their metal content: copper/zinc (Cu/Zn), manganese (Mn), and iron (Fe). In this study, total plasma SODs (Cu/Zn-, Mn-, and Fe-SOD) activities were measured by using a commercially available ELISA kit (Cayman Chemical, Ann Arbor, MI).

The plasma levels of malondialdehyde (MDA) were measured by a method based on the reaction of thiobarbituric acid with certain products of lipid peroxidation in an acidic environment at increased temperatures, as previous described [22]. The product formed was pink in color, which allowed for spectrophotometric determination. The procedure included addition of 250 μL distilled water, 500 μL 15% trichloroacetic acid (TCA), and 500 μL 0.37% thiobarbituric acid to a 250 μL homogenate. Thiobarbituric acid and TCA solutions were prepared in 0.25 M hydrochloric acid. The samples were heated in a boiling water bath for 10 min. Then, the samples were centrifuged at 4,500 rpm for 10 min after cooling. Absorbance of the sample was measured at 535 nm. MDA concentration was calculated according to the standard curve prepared from 1,1,3,3-tetraethoxypropane.

Healthy Controls

Twenty-four healthy controls who were age- and sex-matched with the cirrhotic patients were enrolled for measurement of general blood tests, plasma levels of MDA, endotoxin, and

NOx, and activities of SOD. None of these subjects had a history of major systemic diseases. All subjects agreed to participate in the study and were aware of its content.

Statistical Analysis

Statistical analysis was performed by using the SPSS 15.0 statistical package (SPSS Inc., Chicago, IL). All results are expressed as mean \pm standard deviation (SD). Statistical significance of differences between groups was analyzed by using Student's *t*-test or the Mann–Whitney *U*-test. Correlations between continuous parameters were assessed by using Pearson's correlation analysis. Stepwise multiregression analysis was performed to determine which marker (MDA, endotoxin or NOx) was of greatest and independent significance for prediction of splanchnic hemodynamic parameters. To adjust for the confounding effect of severity of cirrhosis on correlations between plasma markers and splanchnic hemodynamic parameters, partial correlations were run using Child-Pugh and MELD score as the controlling factors. Receiver operating characteristic (ROC) curve analyses were used to determine the diagnostic sensitivity and specificity of MDA, SOD, NO, and endotoxin for distinguishing patients with cirrhosis from healthy subjects. Results were considered statistically significant at a *P* value of less than 0.05.

Results

Demographic and clinical data are summarized in Table 1. The cirrhotic patients had significant lower levels of serum albumin, hemoglobin, platelet count, and plasma activities of SOD than the healthy controls. Further, significantly higher levels of blood urea nitrogen, total bilirubin, MDA, NOx, and endotoxin were observed in the cirrhotic patients than in the normal controls. The results of hemodynamic parameters for the cirrhotic patients are summarized in Table 2.

In the current study, the plasma levels of MDA correlated positively with HVPG ($r = 0.35$, $P = 0.025$) (Fig. 1a), WHVP ($r = 0.42$, $P = 0.007$) (Fig. 1b), and HSR ($r = 0.33$, $P = 0.033$) (Fig. 1c). Additionally, partial correlations between MDA and hemodynamic parameters were also evaluated after adjusting for the effect of MELD and Child-Pugh scores. Interestingly, partial correlation between MDA and WHVP ($r = 0.373$, $P = 0.018$) or between MDA and HVPG ($r = 0.34$, $P = 0.033$) was still significant. Meanwhile, a trend of positive correlation was still noted between MDA and HSR ($r = 0.31$, $P = 0.055$). Plasma endotoxin level did not correlate with HVPG ($r = 0.188$, $P = 0.227$) (Fig. 1d), but had association with WHVP ($r = 0.324$, $P = 0.036$) (Fig. 1e) and HSR ($r = 0.375$, $P = 0.015$) (Fig. 1f). However, partial correlation of plasma endotoxin

Table 1 Demographic and clinical data

	Control (<i>n</i> = 24)	Cirrhosis (<i>n</i> = 42)
Sex (male:female)	16:8	32:10
Age (years)	49 ± 11	54 ± 9
Body mass index (kg/m ²)	23 ± 3	24 ± 3
Hepatitis B virus-related cirrhosis	–	28
Hepatitis C virus-related cirrhosis	–	8
HBV + HCV-related cirrhosis	–	6
Child (A:B:C)	–	22:14:6
MELD score	–	11.6 ± 3.3
Ascites volume (absent:small:moderate:large)	–	30:4:8:0
Esophageal varices (absence:present)	–	12:30
Gastric varices (absence:present)	–	38:4
Albumin (g/dL)	4.7 ± 0.3	3.3 ± 0.6 [#]
Total bilirubin (mg/dL)	1.1 ± 0.5	2.1 ± 1.6 [#]
Blood urea nitrogen (mg/dL)	12.7 ± 2.9	15.9 ± 7.0*
Serum creatinine (mg/dL)	0.9 ± 0.1	1.1 ± 0.2
Serum sodium (mEq/L)	140 ± 1.1	139 ± 3
Alanine aminotransferase (U/L)	35 ± 6	56 ± 21
Aspartate aminotransferase (U/L)	32 ± 8	50 ± 14
Hemoglobin (g/dL)	14.1 ± 1.7	11.5 ± 1.6 [#]
Platelet (×10 ³ /cumm)	253 ± 74	79 ± 40 [#]
INR for prothrombin time	1.0 ± 0.1	1.2 ± 0.2
Malondialdehyde (nM)	226 ± 16	900 ± 745 [#]
Superoxide dismutase (U/mL)	6.8 ± 0.4	2.6 ± 0.6 [#]
Endotoxin (pg/mL)	14.8 ± 4.1	62.0 ± 26.0 [#]
Nitrite/nitrate (NOx, nM)	15.0 ± 9.2	50.5 ± 22.6 [#]

All data expressed
as mean ± SD

MELD, model of end-stage liver
disease; INR, international ratio

* *P* < 0.05 versus normal
controls

[#] *P* < 0.01 versus normal
controls

Table 2 Hemodynamic data of cirrhotic patients

Parameter	Result
Wedge hepatic venous pressure (WHVP, mmHg)	22.4 ± 5.1
Free hepatic venous pressure (FHVP, mmHg)	5.0 ± 2.9
Hepatic venous pressure gradient (HVPG, mmHg)	17.4 ± 5.1
Hepatic blood flow (HBF, mL/min)	808 ± 397
Hepatic sinusoid resistance (HSR, dyne.s.cm ⁻⁵)	1,955 ± 766
Right atrial pressure (RAP, mmHg)	2.5 ± 2.3
Mean pulmonary arterial pressure (MPAP, mmHg)	13.3 ± 1.6
Pulmonary capillary wedge pressure (PCWP, mmHg)	7.0 ± 3.0
Cardiac output (L/min)	6.4 ± 1.8
Systemic vascular resistance (SVR, dyne.s.cm ⁻⁵)	1,206 ± 361
Mean arterial pressure (MAP, mmHg)	94 ± 12
Heart rate (beats/min)	73 ± 13

All data expressed as mean ± SD

with WHVP (*r* = 0.210, *P* = 0.193) or HSR (*r* = 0.30, *P* = 0.056) was not significant after adjusting for the effect of MELD and Child-Pugh scores. Plasma level of NOx was significantly positively associated with HVPG (*r* = 0.382, *P* = 0.013) (Fig. 1g) and WHVP (*r* = 0.335, *P* = 0.032)

(Fig. 1h) but not HSR (*r* = 0.220, *P* = 0.167) (Fig. 1i). The partial correlation of NOx with HVPG was significant (*r* = 0.34, *P* = 0.029) but it was insignificant between NOx and WHVP (*r* = 0.219, *P* = 0.181) after adjusting for the effect of MELD and Child-Pugh scores.

Further, stepwise multiregression analysis was performed to identify which factor (from MDA, endotoxin, NO, Child-Pugh score, and MELD score) independently predicted the three hemodynamic parameters in cirrhotic patients. We found that plasma MDA level is the most significantly independent factor to predict the level of WHVP (standardized coefficients β = 0.42, *P* = 0.007). Furthermore, plasma NO level is the only significantly independent factor to predict the level of HVPG (β = 0.382, *P* = 0.013). In addition, plasma endotoxin level is the only significantly independent factor to predict the level of HSR (β = 0.375, *P* = 0.015).

The plasma levels of MDA were also significantly correlated with the plasma levels of endotoxin and NOx (*r* = 0.71, *P* < 0.001; *r* = 0.55, *P* < 0.001, respectively) (Fig. 2a, b). Additionally, a significant positive correlation was noted between the plasma levels of NOx and endotoxin in the cirrhotic patients (*r* = 0.59, *P* < 0.001) (Fig. 2c).

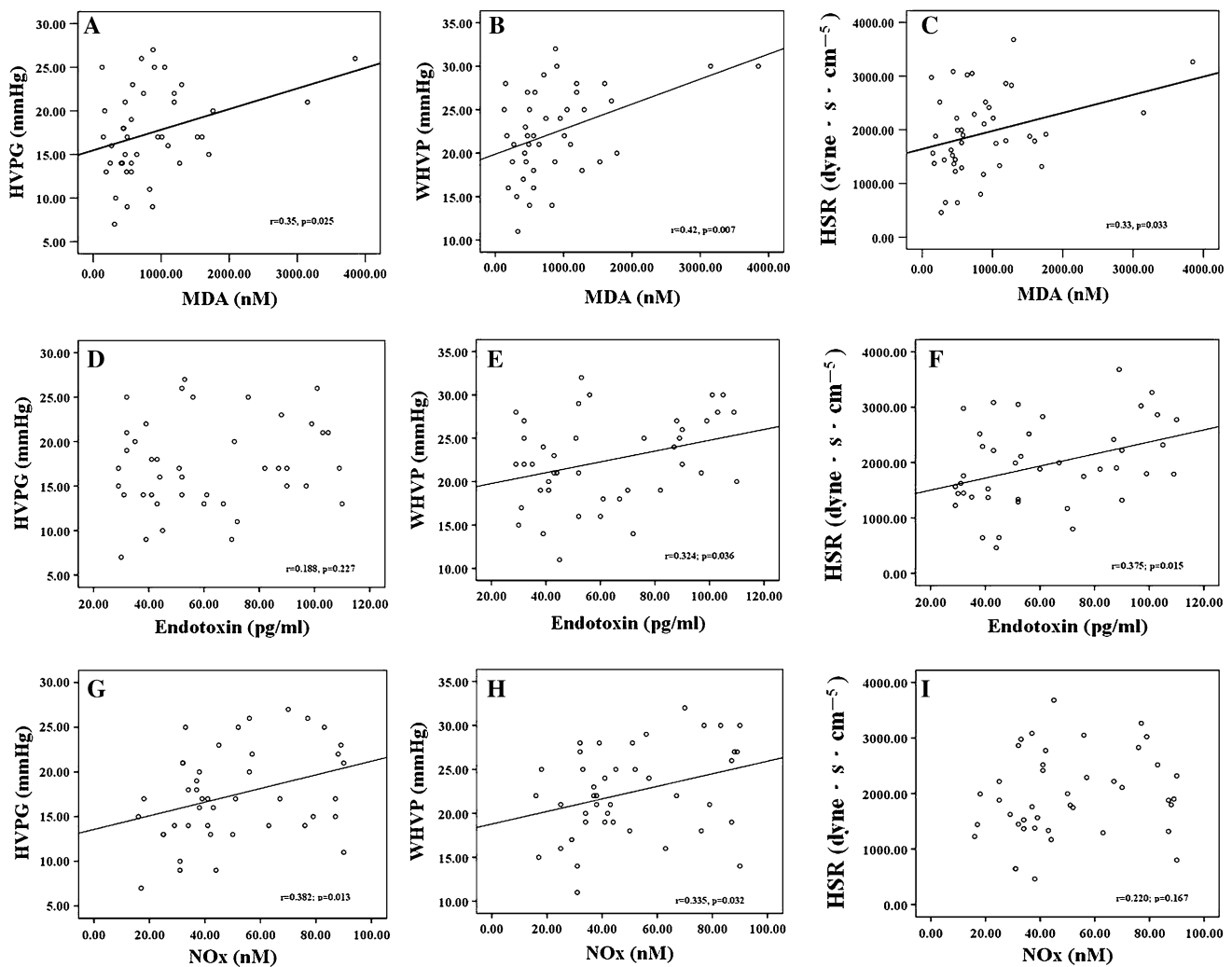


Fig. 1 Correlation of plasma levels of malondialdehyde (MDA) with **a** hepatic venous pressure gradient (HVPG), **b** wedge hepatic venous pressure (WHVP), and **c** hepatic sinusoid resistance (HSR).

Correlation of plasma levels of endotoxin with **d** HVPG, **e** WHVP, and **f** HSR. Correlation of plasma levels of nitrite/nitrate (NOx) with **g** HVPG, **h** WHVP, and **i** HSR

However, the plasma levels of MDA did not correlate with other measured hemodynamic parameters, including CO, SVR, and MAP. There was no significant correlation between plasma MDA level and age ($r = -0.14, P = 0.38$) or body mass index ($r = -0.15, P = 0.34$) of the cirrhotic patients. In contrast, the activities of SOD had no significant correlation with any of the hemodynamic parameters, clinical data, or plasma levels of endotoxin and NOx.

In addition, plasma MDA, endotoxin, and NOx levels increased with the severity of liver cirrhosis (Table 3). Plasma MDA, endotoxin, and NOx levels were significantly higher in patients with Child B + C cirrhosis than in those with Child A cirrhosis (Table 3). The cirrhotic patients with high (≥ 14) MELD scores had significantly higher plasma MDA and endotoxin levels than those with low (< 14) MELD scores (Table 3). Further, cirrhotic patients with ascites had significantly higher endotoxin and

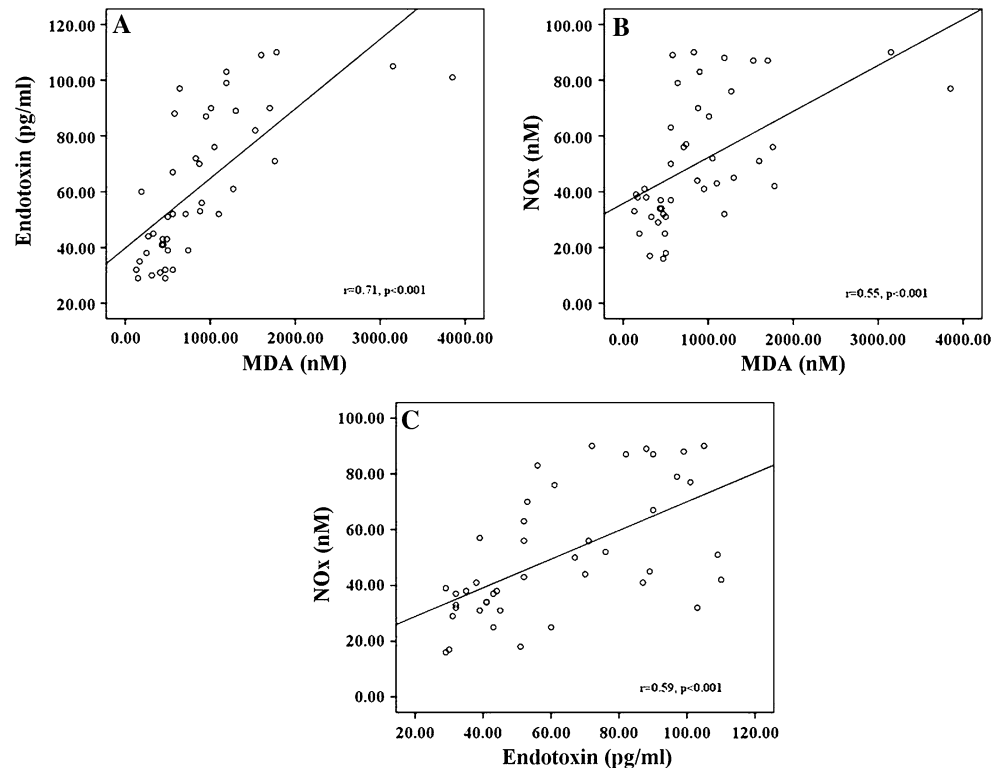
NO levels than those without ascites (Table 3). Plasma MDA in cirrhotic patients with ascites was only slightly elevated compared with those without ascites ($P = 0.19$).

The distributions of plasma levels of MDA, endotoxin, and NOx in both studied groups are shown as Fig. 3. The ROC curve analyses are shown in Fig. 4 (sensitivity versus 1-specificity). The cutoff values of plasma MDA, endotoxin, and NOx to separate cirrhotic patients from healthy subjects were 426 nM, 21.5 pg/mL, and 27.5 nM, respectively.

Discussion

It has been shown that systemic overproduction of NO contributes to peripheral vasodilatation and hyperdynamic circulation in cirrhosis [18]. Endotoxin, the lipopolysaccharide component of the cell wall of Gram-

Fig. 2 Correlation of plasma levels of malondialdehyde (MDA) with **a** endotoxin and **b** nitrite/nitrate (NOx) in cirrhotic patients. **c** Correlation between plasma levels of endotoxin and NOx in cirrhotic patients



negative bacteria, stimulates NO synthesis [23]. In cirrhotic patients, elevated plasma levels of endotoxin have been found to be associated with increased circulating NO [17]. Similarly, a significant positive correlation was noted between the increased plasma level of endotoxin and NO in the cirrhotic patients of this study.

In our study, plasma levels of MDA were positively correlated with plasma levels of endotoxin in the patients with cirrhosis. It has been shown that cirrhotic patients exhibit increased translocation of gut-derived endotoxin, which can reach portal and systemic circulation due to increased intestinal permeability [17, 24]. In carbon tetrachloride-induced cirrhotic rats, an increase in the generation of oxygen free radicals may damage intestinal mucosa and increase production of intestinal MDA [25]. The increased oxidative-stress-related damage of enterocytes in cirrhotic rats leads to increased intestinal permeability [25]. Accordingly, the increase in intestinal oxidative stress and MDA may aggravate the preexistent endotoxemia in cirrhosis. Further, in bile-duct-ligated (BDL) rats, increased bacterial translocation was also associated with increased intestinal mucosal concentrations of MDA, and both intestinal mucosal MDA and bacterial translocation were reduced by chronic administration of antioxidants [26]. On the other hand, rats receiving injection of endotoxin exhibited increased production of MDA in various tissues,

Table 3 Plasma levels of MDA, endotoxin, and NOx in cirrhotic patients

	MDA (nM)	Endotoxin (pg/mL)	NOx (nM)
Child			
A	743 ± 764	52.6 ± 20.3	43.1 ± 19.2
B	850 ± 541	66.9 ± 30.9	50.1 ± 21.3
C	1,368 ± 964 ^a	83.7 ± 21.5 ^{b,c}	69.7 ± 25.3 ^{a,d}
B + C	1,072 ± 716 ^a	71.9 ± 28.2 ^a	57.1 ± 23.2 ^a
Ascites volume			
Absent	807 ± 707	54.5 ± 21.8	43.2 ± 18.0
Small	890 ± 491	75.8 ± 22.1	70.0 ± 24.1*
Moderate	1,254 ± 976	74.8 ± 30.9	64.0 ± 24.6*
Present	1,133 ± 839	75.1 ± 27.5*	66.0 ± 23.5**
MELD score			
<14	809 ± 801	55.2 ± 23.2	46.8 ± 21.0
≥14	1,057 ± 497 [#]	82.9 ± 25.9 [#]	61.6 ± 26.0

All data expressed as mean ± SD

MDA, malondialdehyde; NOx, nitrite/nitrate; MELD, model of end-stage liver disease

* $P < 0.05$ versus the group without ascites, ** $P < 0.01$ versus the group without ascites

[#] $P < 0.05$ versus the group with MELD score < 14 (Mann–Whitney U test)

^a $P < 0.05$ versus Child A group, ^b $P < 0.01$ versus Child A group,

^c $P < 0.05$ versus Child B group, ^d $P < 0.01$ versus Child B group

Fig. 3 The distribution of plasma levels of **a** malondialdehyde (MDA), **b** endotoxin, and **c** nitrite/nitrate (NOx) in cirrhotic patients and normal subjects

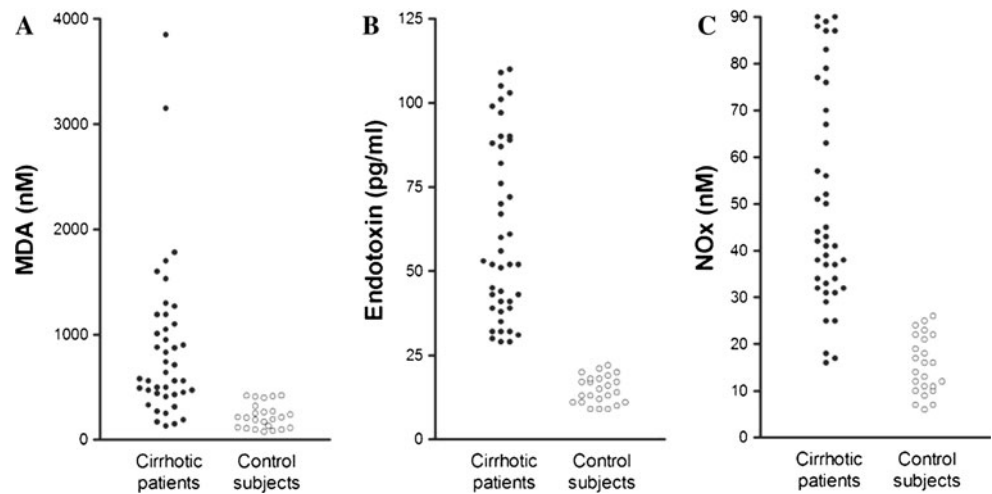
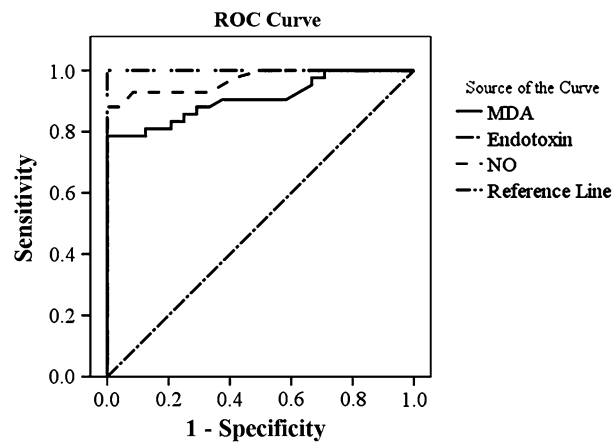


Fig. 4 Receiver operating characteristic (ROC) curve and optimal cutoff levels of malondialdehyde (MDA), endotoxin, and nitrite/nitrate (NOx) for distinguishing cirrhotic patients from healthy controls. AUC area under the curve



Parameter	AUC	Standard error	95% confidence interval	Sensitivity (%)	Specificity (%)	Optimal cut-off
MDA	0.908	0.035	[0.839; 0.977]	78.6	100	426.5 (nM)
Endotoxin	1.000	0.000	[1.000; 1.000]	100	95.8	21.5 (pg/ml)
NOx	0.968	0.018	[0.932; 1.004]	88	100	27.5 (nM)

including liver [27–29]. Taken together, MDA and endotoxin were caught in a vicious circle.

Meanwhile, a significant correlation between plasma MDA and NO levels in the cirrhotic patients was observed in this study. It has been demonstrated that overproduction of NO increased lipid peroxidation and oxidative stress in cells [30, 31]. Theoretically, increased circulating NO may also lead to the increased plasma levels of MDA in our cirrhotic patients. In brief, the current study suggests interactions among endotoxin, NO, and MDA in cirrhosis.

Interestingly, plasma MDA levels were noted to have positive associations with HVPG and WHVP in the cirrhotic patients in this study. It has been demonstrated that MDA inhibits the endothelium-dependent vasorelaxation to acetylcholine in the tail artery of normal rats [32]. Additionally, NO also plays a major key role in the development

of hyperdynamic circulation and portal hypertension in cirrhosis [18]. Multiregression analysis showed that the level of plasma MDA was an independent factor to predict the value of WHVP, whereas the level of plasma NOx was the strongest variable to predict HVPG. Therefore, the significant association of plasma MDA levels and HVPG may be caused by the interaction between NO and MDA in cirrhotic patients in this study.

On the other hand, plasma MDA levels further correlated with HSR in cirrhotic patients in our study. It is conceivable that oxidative stress may play a contributory role in enhanced intrahepatic resistance in cirrhosis [4]. A previous study demonstrated that the cirrhotic patients with high plasma MDA levels had marked postprandial elevation of portal pressure, which represented increased intrahepatic resistance [11]. That study also showed that acute administration

of high doses of ascorbic acid reduced the plasma MDA levels and blunted the postprandial increase in portal pressure of cirrhotic patients [11]. Moreover, a concordant increase of hepatic and plasma MDA was demonstrated in cirrhotic rats [33]. Accordingly, the elevation of calculated hepatic sinusoid resistance (HSR), which can estimate the increased intrahepatic resistance indirectly, may be explained by the increased plasma level of MDA in cirrhotic patients of our study. Nevertheless, endotoxemia, which can enhance release of MDA in cirrhosis, has been also found to increase intrahepatic resistance and subsequently elevate portal pressure [16, 25, 34, 35]. In this study, multiregression analysis demonstrated that the plasma level of endotoxin was the strongest factor to predict HSR. Therefore, the significant association between plasma MDA and HSR may be the result of close interaction between endotoxin and MDA in these cirrhotic patients.

Increased oxidative stress, which is indicated by increased oxidants and decreased antioxidants, has been observed in cirrhotic patients with various etiologies [12–15, 36–38]. Our study found significantly higher plasma levels of oxidant MDA and significantly lower plasma activities of antioxidant SOD in the patients with viral cirrhosis than in the healthy controls. This result further supports the existence of increased oxidative stress in cirrhotic patients [37, 38]. Oxidative stress not only contributes to the derangement of hemodynamics mentioned above in cirrhosis, but also promotes liver fibrosis [7]. In BDL rats, the increased plasma MDA level was found to correlate positively to collagen deposition in liver [33]. Further, it was also reported that higher MDA level was associated with more advanced stage of liver fibrosis in patients with chronic liver disease [39]. Moreover, our study found that the cirrhotic patients with Child B + C or high (≥ 14) MELD score had significantly higher plasma MDA levels. Similarly, a previous study showed that plasma MDA levels were positively correlated with Child-Pugh scores of cirrhotic patients [36]. These results indicate that plasma MDA levels in cirrhotic patients might increase in concordance with the progression of the severity of cirrhosis and fibrosis.

In this study, plasma MDA was associated with the hemodynamic parameters and also the severity of cirrhosis. The previous study had demonstrated that the severity of cirrhosis correlated with HVPG [19]. To avoid the confounding effect of the severity of cirrhosis on the association between plasma MDA and hemodynamic parameters, we performed partial correlation analysis using Child-Pugh score and MELD score as controlling factors. Interestingly, significant associations were still noted between plasma MDA and hemodynamic changes (HVPG and WHVP). These results suggested that these associations of MDA with hemodynamic derangement may be independent of the severity of cirrhosis. However, the sample size of this study

was relatively small, so a contribution of the severity of cirrhosis to the association between MDA and hemodynamic changes could not be totally excluded.

Further, investigators have tried to find more noninvasive biomarkers for cirrhotic patients for years [40]. We observed good abilities of plasma MDA, endotoxin, and NOx levels to distinguish cirrhotic patients from healthy subjects, with good sensitivities and specificities by ROC analysis. Additionally, these parameters also were found to be elevated in concordance with the severity of cirrhosis. Thereby, it is possible that plasma levels of MDA, endotoxin, and NOx could be evaluated as candidate biomarkers for initial and long-term assessment of liver cirrhosis in our clinical practice.

In the present study, we found significantly decreased plasma SOD activity in cirrhotic patients as compared with normal volunteers. This result is similar to the finding of a previous study [13]. Further, Nalini et al. [13] found that alcoholic cirrhotic patients had significantly lower SOD activities in the hemolysate than those in nonalcoholic cirrhotic ones. In contrast, another previous study demonstrated higher serum SOD activity in alcoholic cirrhotic patients than in normal subjects [41]. Moreover, SOD is the enzyme to catalyze O_2^- to hydrogen peroxide (H_2O_2) [4]. Hydrogen peroxide has been demonstrated to be an endothelium-derived hyperpolarizing factor (EDHF) in human mesenteric arteries [42] and therefore may induce splanchnic vasodilation which would contribute to cirrhotic portal hypertension [18]. However, the release of EDHF was inhibited by NO [43], which is significantly elevated in cirrhotic systemic circulation [17]. This may explain why no significant correlation was observed between SOD and hemodynamic parameters in cirrhotic patients in this study.

In conclusion, systemic oxidative stress was significantly increased in patients with viral cirrhosis. This study suggests close interaction among increased endogenous oxidant MDA, endotoxin, and NOx and that these substances are associated with hemodynamic derangement in cirrhotic patients.

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