

# Plasma Endothelin-1 Concentrations in Children With Cirrhosis and Their Relationship to Renal Function and the Severity of Portal Hypertension

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## ABSTRACT

**Background:** Plasma endothelin-1 (ET-1) is a potent vasoconstrictor peptide involved in the pathogenesis of several disorders. Endothelin-1 concentrations are increased in adult patients with cirrhosis. However, little is known about ET-1 concentrations in children with cirrhosis.

**Methods:** Radioimmuno assay was used to measure plasma ET-1 concentrations in 19 children with cirrhosis (8 patients with ascites, and 11 without ascites), and 11 age- and sex-matched healthy children. The plasma ET-1 concentrations were correlated with the mean blood pressure, creatinine clearance, and severity of portal hypertension, as measured by portal flow volume and portal flow velocity.

**Results:** Patients with cirrhosis and ascites had increased plasma ET-1 concentrations compared with patients who did not have ascites ( $6.8 \text{ pg/mL} \pm 0.62 \text{ pg/mL}$  vs.  $4.6 \text{ pg/mL} \pm 0.35 \text{ pg/mL}$ ; mean  $\pm$  SEM;  $P < 0.01$ ) and controls ( $3.6 \text{ pg/mL} \pm 0.27 \text{ pg/mL}$ ; mean  $\pm$  SEM;  $P < 0.0005$ ). Plasma ET-1 concentrations were higher in patients with cirrhosis who did not have ascites compared with controls ( $P < 0.005$ ). No significant differences

were observed between concentrations of the patients with cholestasis and those without cholestasis ( $5.4 \text{ pg/mL} \pm 0.52 \text{ pg/mL}$  vs.  $5.2 \pm 0.32 \text{ pg/mL}$ ; mean  $\pm$  SEM;  $P = 0.1$ ). Plasma ET-1 concentrations correlated positively with the mean blood pressure ( $r = 0.58$ ;  $P < 0.05$ ) and negatively with renal function, as measured by creatinine clearance ( $r = -0.7$ ;  $P < 0.005$ ). However, no correlation was detected between ET-1 concentrations and portal flow volume ( $r = -0.02$ ;  $P = 0.4$ ) or portal flow velocity ( $r = -0.16$ ;  $P = 0.4$ ).

**Conclusions:** Plasma ET-1 concentrations are increased in children with cirrhosis, with or without ascites, compared with controls. Patients with cirrhosis and ascites have increased ET-1 concentrations compared with those without ascites. The degree of increase does not relate to the severity of portal hypertension. This increase tends to maintain systemic blood pressure but is associated with a decrease in renal function. *JPGN 35:149–153, 2002.* **Key Words:** Cirrhosis—Endothelin—Ascites—Hemodynamics. © 2002 Lippincott Williams & Wilkins, Inc.

Endothelin-1 (ET-1) is a potent long-acting vasoconstrictor that may be involved in the pathogenesis of portal hypertension. This suggestion was based on the fact that plasma and hepatic ET-1 concentrations are in-

creased in adult cirrhotic patients and in rats with carbon tetrachloride-induced cirrhosis (1–4). Endothelin-1 is known to cause sinusoidal and presinusoidal constriction by acting on ET<sub>A</sub> and ET<sub>B</sub> receptors, and to increase perfusion pressure in healthy and cirrhotic rat liver (5,6).

Little is known about plasma ET-1 concentrations in children with cirrhosis. Nozue et al. (7) found that plasma ET-1 concentrations were increased in three patients with Byler disease and normal in five patients with biliary atresia after hepatoportoenterostomy. In the same study, plasma ET-1 concentrations correlated significantly with urinary concentration of N-acetyl- $\beta$ -D-glucosaminidase, a sensitive marker of renal injury. However, the study included a limited number of patients and none of the patients had biopsy-proven cirrhosis. In another study, Kobayashi et al. (8) measured ET concentrations in 19 children with biliary atresia after hepato-

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portoenterostomy and found that ET concentrations were higher than in controls. None of the patients had ascites or hepatorenal syndrome. In the same study, ET concentrations were also increased in the biliary atresia patients with unfavorable outcome compared with those who had favorable outcome. Endothelin production also increased in children with biliary atresia and cirrhosis who underwent living-related liver transplantation compared with healthy controls (9).

Therefore, we measured plasma ET-1 concentrations in patients with cirrhosis, with and without ascites, and correlated these concentrations with mean blood pressure, creatinine clearance as an indicator of renal function, and severity of portal hypertension as measured by portal flow volume and portal flow velocity. We also correlated ET-1 concentrations with the portal vein diameter and the hepatic artery resistance index (HARI).

## PATIENTS AND METHODS

Our study included 19 children with biopsy-proven cirrhosis (8 with chronic hepatitis B, 5 with biliary atresia after hepatoportoenterostomy, and 6 with Byler disease). Eight patients had ascites based on clinical examination and ultrasonography, whereas 11 had no ascites. Eleven healthy age- and sex-matched children attending the general pediatrics outpatient clinic for routine physical examination were recruited to serve as controls (Table 1). Parents and controls gave informed consent before being enrolled in the study.

After the patients have been recumbent for 30 minutes, venous samples were collected in glass tubes containing aprotinin and ethyldiaminetetraacetic acid. Plasma was separated by centrifugation at 4°C and stored at -70°C until assayed. After extraction from the plasma using organic solvent (methanol), and after loading the plasma on SepPak C18 cartridges, the radioimmunoassay was performed. Endothelin-1 concentra-

tions were measured by competitive immunoassay kit (Peninsula Laboratories Inc., Belmont, CA, U.S.A.) using a previously described method (10). The coefficient of variation was 12%. The cross-reactivity of the antiserum with ET-3 and Big ET was 5% and 10% respectively. The intraassay and interassay coefficients of variation were 9% and 12%, respectively.

Blood pressure was measured in all subjects in triplicate, and results were averaged. Creatinine clearance was calculated using plasma creatinine and 24-hour urinary creatinine excretion.

The morning after an overnight fast, all patients and controls underwent Doppler examination of the portal and hepatic circulation by the same well-trained operator. The examiner was masked to all clinical and laboratory information related to the study. Each parameter was averaged from four readings. The examinations were performed with an ATL Ultramark 9 HDI color and pulsed Doppler unit with a 3.5 MHz probe and a low-value high-pass filter. The main portal vein was sampled at the crossing of the hepatic artery. The sample volume included the whole lumen of the portal vein. The Doppler angle between the long axis of the vessel and the incident Doppler beam was between 30 and 60 degrees. Time-averaged mean blood velocity, in cm/s, of the portal vein was automatically calculated using samples of more than 4 seconds each. The cross sectional area of the portal vein was calculated by directly measuring its diameter, assuming a circular configuration. Portal flow volume (mL/min) was calculated as time-averaged mean blood velocity × cross sectional area. The hepatic artery was also sampled at its crossing over the portal vein. The peak systolic (S) and end diastolic (D) flow velocities were measured directly on the time frequency Doppler wave forms. The HARI was then calculated:  $HARI = (S - D) \div S$ .

One-way analysis of variance with the Tukey student range test was used to determine the significance of difference between groups. Pearson correlation coefficients were used to determine the association between plasma ET-1 concentrations and mean blood pressure, creatinine clearance, portal flow volume, portal flow velocity, portal vein diameter, and HARI. Data were expressed as mean ± SEM unless otherwise indicated. Results were considered significant if *P* was less than 0.05.

**TABLE 1.** Characteristics of the study population (data are expressed as mean ± SEM)

Characteristics	Patients with cirrhosis		Controls
	Ascites	No ascites	
Age (years)	9.2 ± 0.9	10.5 ± 1.0	10.3 ± 1.3
Sex (male/female)	4/4	6/5	5/6
AST	59 ± 6*††	41 ± 4*	20 ± 3
ALT	68 ± 7*††	44 ± 5*	21 ± 3
Total bilirubin (mg/dL)	2.4 ± 0.3*††	1.1 ± 0.1†	0.6 ± 0.07
Prothrombin activity (%)	62 ± 5.4 <sup>NS,§</sup>	78 ± 6.3†	83 ± 4.9
Albumin (g/dl)	2.5 ± 0.4*††	3.4 ± 0.5†	3.9 ± 0.6
Mean blood pressure (mm Hg)	79.62 ± 1.8†,§	87.6 ± 2.6†	89.9 ± 2.5
Creatinine clearance (mL/min/1.73 m <sup>2</sup> )	107.9 ± 3.9*§	117.1 ± 4.8†	126.8 ± 2.6
Time-averaged mean velocity (cm/s)	3.9 ± 0.3*††	5.1 ± 0.5†	5.4 ± 0.6
Portal flow volume (mL/min)	230 ± 21*††	290 ± 29†	315 ± 32
Portal vein diameter (mm)	13 ± 1*§	13.8 ± 0.8*	9 ± 0.4
Hepatic artery resistance index	0.63 ± 0.13*§	0.69 ± 0.02*	0.47 ± 0.01

\* Significantly different from control values.

† Not significantly different from control values.

†† Significantly different from patients without ascites values.

§ Not significantly different from patients without ascites values.

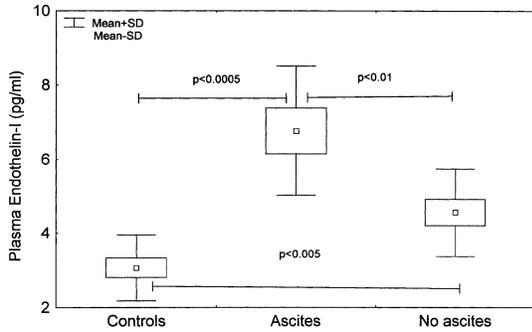


FIG. 1. Plasma endothelin-1 in controls, patients with ascites, and patients without ascites.

RESULTS

Patients with cirrhosis and ascites had increased ET-1 concentrations compared with patients without ascites (6.8 pg/mL ± 0.62 pg/mL vs. 4.6 pg/mL ± 0.35 pg/mL; mean ± SEM; *P* < 0.01) and controls (3.6 pg/mL ± 0.27 pg/mL; mean ± SEM; *P* < 0.0005) (Fig. 1). Plasma ET-1 concentrations were increased in patients with cirrhosis who did not have ascites compared with controls (*P* < 0.005). We found no significant difference in ET-1 concentrations between the patients with cholestasis and those without cholestasis (5.4 pg/mL ± 0.52 pg/mL vs. 5.2 pg/mL ± 0.32 pg/mL; mean ± SEM; *P* = 0.1).

Plasma ET-1 concentrations correlated positively with the mean blood pressure (*r* = 0.58; *P* < 0.05) (Fig. 2) and negatively with creatinine clearance (*r* = -0.7; *P* < 0.005) (Fig. 3). However, no correlation was detected between ET-1 concentrations and portal flow volume, portal flow velocity, portal vein diameter, or HARI (Table 2).

DISCUSSION

We measured plasma ET-1 concentrations in children with cirrhosis and showed that they are increased in patients with cirrhosis compared with controls. Plasma ET-1 concentrations were increased in patients with as-

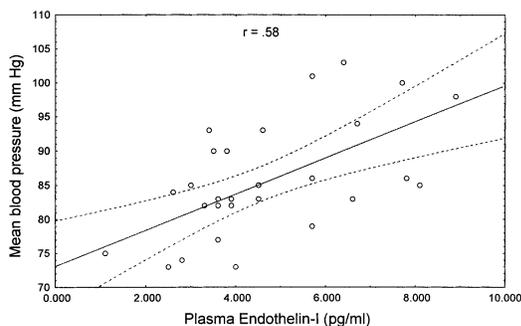


FIG. 2. Correlation between plasma endothelin-1 and mean blood pressure.

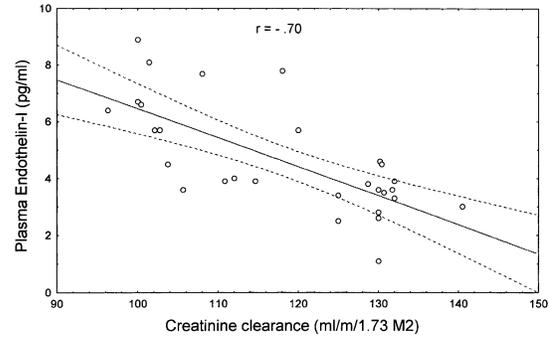


FIG. 3. Correlation between plasma endothelin-1 concentrations and creatinine clearance.

cites compared with those who did not have ascites. However, the fact that the patients with ascites were receiving diuretic therapy, which can cause hypovolemia with subsequent activation of the vasopressor systems, may limit our study.

The increase in plasma ET-1 concentrations also was seen in patients without ascites compared with controls. This is consistent with two other studies in children. Kobayashi et al. (8) reported increased plasma ET-1 concentrations in children with biliary atresia without ascites compared with healthy controls. In another study, plasma ET-1 concentrations were increased in children with biliary atresia and Byler disease who did not have ascites compared with healthy children (7).

However, Uchihara et al. (1) have shown that plasma ET-1 is increased in adult patients with ascites and cirrhosis. However, in patients with cirrhosis without ascites and patients with chronic hepatitis, no significant difference was observed. Asbert et al. (3) reported that plasma ET-1 concentration was higher in adult patients with cirrhosis (with and without ascites) compared with controls. However, the increase seen in patients without ascites did not reach statistical significance. The cause of cirrhosis may explain this discrepancy. The cause of cirrhosis in most of the adult studies is alcoholic, primary sclerosing cholangitis, or primary biliary cirrhosis, which differs from the cause of cirrhosis in the pediatric age group

Studies have shown that bile acids are endothelium-independent vasorelaxants (11-14), which can lead to compensatory activation of the endothelin system. However, in our study, we found no significant difference in

TABLE 2. Correlation between plasma endothelin-1 levels and portal flow volume (PFV), time-averaged mean blood velocity (TAMV), diameter of portal vein (DVP), and hepatic artery resistance index (HARI)

	PFV		TAMV		DVP		HARI	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>P</i>
Endothelin-1	-0.02	0.4	-0.16	0.4	0.03	0.9	0.03	0.09

plasma ET-1 concentrations between the patients with cirrhosis who had cholestasis and those who did not have cholestasis, and we found no correlation between plasma ET-1 and bilirubin concentrations. Sarac et al. (15) reported increased serum ET-1 concentrations after common bile duct obstruction in rats, and that the increase could be reversed by ET<sub>A</sub> and ET<sub>B</sub> receptor blockers. However, they found no correlation between plasma ET-1 concentrations and bilirubin. The lack of correlation between plasma ET-1 concentrations and bilirubin was also reported in another study (16).

Increased plasma concentrations of endothelin have been reported in patients and in experimental animals with heart failure, arterial hypertension, and cardiogenic shock (17–19). Administering endothelin at doses that increase plasma concentrations twofold to eightfold is associated with a significant increase in arterial pressure and peripheral vascular resistance (20,21). Liver cirrhosis is associated with increased concentrations of vasodilator substances such as nitric oxide and PGI<sub>2</sub>. Therefore, stimulating ET-1 production could be a homeostatic response to counteract the action of these vasodilators to maintain arterial pressure at normal concentrations (22,23). This may explain the positive correlation observed in our patients between plasma ET-1 concentrations and mean arterial pressure.

In our study, plasma ET-1 concentration correlated negatively with creatinine clearance. A report by Uchihara et al. (1) supports this. They suggest that increased ET-1 concentrations may play a pathogenic role in the renal functional changes associated with cirrhosis. Impaired kidney function in patients with cirrhosis is not the cause of increased ET-1 concentrations found in these patients because decreased glomerular filtration rate does not play a role in the clearance of endothelin (24).

Taourel et al. (25) demonstrated that in patients with alcoholic cirrhosis, only portal vein blood velocity and flow, but neither HARI nor portal vein diameter, correlate to the severity of portal hypertension and to the severity of liver failure. In another study, children with portal hypertension had decreased velocity and volume of blood flow compared with healthy children, whereas no difference was observed in the size of the portal vein or in HARI (26).

Kardorff et al. (27) found that loss of hepatic vein flow undulation, decreased maximum portal blood flow velocity, and increased HARI related significantly to the severity of hepatic derangement in children with extrahepatic biliary atresia. We found no significant correlation between plasma ET-1 concentrations and portal flow volume, portal flow velocity, portal vein diameter, or HARI. This lack of correlation may be because of the complex interaction among different vasoactive peptides. For example, it has been reported that ET-1 can induce vasoconstriction by interacting with ET<sub>A</sub> receptors, but it can also interact with ET<sub>B</sub> receptors increasing the re-

lease of vasodilators, such as nitric oxide and prostacyclin (28,29).

Strong positive correlation has been reported between the plasma concentrations of ET-1 and its precursor Big ET-1 (30). However, plasma concentration of Big ET-1 must be measured in future studies to confirm that increased ET-1 concentrations in children with cirrhosis is caused by increased production rather than decreased clearance.

In conclusion, our study demonstrates that plasma ET-1 concentrations are increased in children with cirrhosis, with or without ascites. The degree of increase does not relate to the severity of portal hypertension. This increase correlates with the mean blood pressure but is associated with decreased renal function.

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