ENDOTHELIN-1 LEVELS IN CHILDHOOD LIVER DISEASE

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ABSTRACT

Objective: An in-patient with portal hypertension PH was used in a trial conducted to determine the levels of vasoconstrictors endothelin-1 (ET-1), Atrial natriuretic peptid (ANP), Angiotensin-II, aldosterone and vasopressin, and to investigate the correlation between ET-1 and the other hormones.

Methods: The trial included 40 patients with chronic active hepatitis B (CAH-B) (n=10), cirrhosis with portal hypertension (PH) (n=14), cirrhosis (n=6) and extrahepatic PH (n=10). The patients were followed up for one year in the pediatric clinic of the Cerrahpasa School of Medicine in Istanbul, Turkey. Nineteen of them were female and 21 of them were male, with a mean age ± Standard deviation (sd) SD of 10.2 ± 6.2 years. The control group included 10 healthy children, all of whom were male with a mean age of 9.2 ± 6 years. Plasma ET-1, ANP, Angiotensin-II, aldosterone and vasopressin levels were measured by radioimmunoassay (RIA) method in Istanbul University, Medical School, Experimental Research Institute (DETAM).

Results : Plasma ET-1 levels in chronic liver patients were higher than in the healthy control group according to One Way Analysis of Variance (ANOVA) (p=0.0001). In chronic liver disease patients, there was a positive correlation between ET-1 and aldosterone, ANP and ADH respectively r=0.45 (p=0.003), r=0.37 (p=0.003), r=0.40 (p=0.0001). In the same group, there was No correlation between ET-1 and angiotensin-II (r=0.22, p=0.07).

Conclusion: In conclusion, we found that ET-1 levels were higher in group I and IV than in the control group. Plasma ET-1 levels increase in patients diagnosed as having chronic liver disease with an undefined mechanism. This could be explained by either an increase in production or decrease in metabolic clearances or probably by both of these mechanisms.

Key Words: Chronic liver disease, Endothelin-1, Childhood

INTRODUCTION

In portal hypertension (PH), physiopathology of water and salt retention and hemodynamic changes have been a subject of investigation for many years. Although various theories have been proposed, this subject has not been

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completely explained yet (1,2). Many studies have been conducted on renin, angiotension, aldosterone (RAAS), vasopressin (ADH) and sympathetic system in patients with PH (3-10). The endothelins are recently described 21 amino-acid peptides. Endothelin-1 (ET-1) is produced and released from endothelial cells and powerful vasoconstrictor activity, about 10 times more powerful than angiotensin (11). It has been shown that in chronic liver disease with cirrhosis with or without ascites, the plasma endothelin level rises (12-14). In addition , in recent trials ET-1 was shown to be a potent vasoconstrictor peptide in sinusoidal and extrasinusoidal regions of liver microcirculation (15-17). Endothelin has a potent systemic and renal vasoconstrictor effect, increased atrial natriuretic peptid (ANP), water and salt retention properties and the modulating effects of RAAS were considered important factors in the pathophysiology of water and salt retention (18-22). These studies were mostly done in elderly age groups (12-14,23-25) and only one included children (25).

In the light of these information, we measured plasma ET-1, ANP, angiotensin-II, aldosterone and vasopressin levels which were of great significance in PH physiopathology in children with chronic liver disease. In addition, we investigated the correlation between ET-1 levels and the other vasoconstrictive hormones.

MATERIAL AND METHOD

The study included 40 patients who were followed in the Pediatric Gastroenterology Sub-department of Cerrahpasa School of Medicine in Istanbul, Turkey. Nineteen of these patients were female and 21 were male, with a mean age of 10.2 ± 6.2 years. A control group included 10 healthy children, all of whom were male with a mean age of 9.2 ± 6 years.

Patients were separated in five different groups. Group-I consisted of ten CAH-B cases of whom five were female and five were male with a mean age of 9±3.8 years. All of them had a histopathologically moderate degree of inflammatory activity and a moderate degree of fibrosis. According to Child-Turcotte classification seven cases were Child A, three cases were Child B. Group-II included 4 female and 10 male patients with cirrhosis and PH. The mean age was 11.84 ± 8.14 years. According to Child-Turcotte classification one case was Child A, 11 cases were Child B and two cases were Child C. Chronic active hepatitis –B was found in four cases, autoimmune CAH in two cases, cryptogenic cirrhosis in four cases, Wilson's disease in one case, operated biliary atresia in two cases and CAH-C in one case. Ascites was present in only two cases. Group –III consisted of six cases of cirrhosis all female without PH. The mean age was 11.36 ± 6.10 years, all the cases were Child A.

Autoimmune CAH was found in three cases, cryptogenic cirrhosis in two cases and one was CAH-B.

Group-IV covered 10 cases of extrahepatic PH consisting of four females and six males. Histopathology revealed no liver damage in all the cases. The mean age was 10.84 ± 2.67 years. The control group, called group V included three females and seven males consisting of in-patients admitted to the outpatient clinic for minor complaints and found to have no chronic illness at examination. The mean age was 9.2 ± 6 years.

Cases with a diagnosis of cirrhosis were evaluated according to Child-Turcotte classification representing levels of total bilirubin, albumin, presence of ascites, encephalopathy and nutritional status.

The presence of ascites and hepatomegaly were detected by physical examination and ultrasonography. Abdominal ultrasonography was carried out in the pediatric radiology section of Cerrahpasa School of Medicine, Istanbul, Turkey.

Upper gastrointestinal system endoscopy was done with "Olympus XP 20" a pediatric gastroscope. Patients were premedicated with a rectal application of midazolam used as 0.5 mg/kg, given half an hour before endoscopy.

Study Protocol

The cases included in this study were put on a restricted sodium diet one week prior to their hospitalization. Although there was no fluid intake restriction, diuretic and/or antihypertensive

drugs were stopped three days prior to the study. Bacterial infection, gastrointestinal bleeding and hepatic encephalopathy were not observed in any of these patients. On the seventh day of the trial, patients collected their 24 hours urine specimen. After an over night's fast in the morning of the eighth day, at eight o'clock, after having rested for two hours, 20 ml blood was drawn from the antecubital vein of each patient to test for serum electrolytes, aldosterone, angiotensin-II, vasopressin, ANP and ET-1. A serum aldosterone sample was collected in a dry tube and blood samples for angiotensin-II, ANP, ET-1 and vasopressin were collected in EDTA containing tubes and subsequently immersed in icy water. Meanwhile all patients underwent regular examinations of their heart beat per minute, arterial blood pressure and pulse per minute.

ANP, ET-1, angiotensin-II and vasopressin blood samples were centrifuged for 15 minutes, at 2000 cycle at +2°C in the pediatric metabolism laboratory of our Medical School and the plasma samples were put in separate Eppendorf tubes and were kept at -20°C till they were processed. One hundred microliters of aprotinin (Trasylol BAYER) was added only to ANP plasma samples.

Serum Endothelin (ET-1)

ET-1 analysis was made by Radioimmunoassay (RIA) method (Euro-Diagnostica-Sweden) in the Istanbul University, Medical School, Experimental Research Institute (DETAM). The ET-1 sediment was extracted from plasma samples that were kept in Eppendorf tubes frozen to-20°C using Sep-Pak C18 columns (Waters, Millipore Intertech Corporation, P.O Box 255 Bedford M.M USA). A plasma sample of one ml was used for analysis. This plasma sample was taken into a polystren tube placed in an ice bath. Ten microliters of trifluoroacetic acid diluted in 1/10 proportion was added. The mixture was carefully intermingled with Vortex and afterwards centrifuged at +8°C for 10 minutes at 1500 RPM. Sep-Pak C18 columns were separately placed in vacuum connectors. After consecutive use of 10 ml TFA: H20: Methanol (0.50: 99.5: 400), ml TFA: H20 (0.50:500) and 2 ml TFA: H2O (0.50: 500) they were washed and left to dry. All centrifuged plasma samples were processed from the columns. Endothelin kept in columns was washed with a solution consisting of 4 ml TFA:H2O: Methanol mixture (at a rate of 4-8 ml per minute) and was placed in glass tubes. In the warming bath, nitrogen gas was used and all the liquid part was left to evaporate at + 45°C. In experimental conditions, recovery control of ET-1 was found to be 75.3%.

The remaining dry substance was dissolved with 0.5 ml of tamponade solution, shaken for 10 minutes and prepared for RIA process. Seven standard solutions were prepared from the solution containing 125 pmol synthetic human endothelin-1 per liter. 500 microliter of rabbit anti endothelin-1 antiserum was added to all the samples and mixed consecutively with this tamponade solution; 500 microliters of tamponade solution was also added and incubated at +2°C for 24 hours. Anti-rabbit immunoglobulin solution was added to each tube as 100 microliters, shaken with vortex, and incubated at +2°C for an hour.Afterwards it was centrifuged for 15 minutes at +4°C 2000 RPM. The liquid part was powered out and counted at the gamma counter for two minutes.

Statistical Evaluation

In patient and control groups mean and standard deviation (mean±Sd) was calculated for each parameter. The Unpaired t-test and the One Way ANOVA test was used to compare groups. For F-statistics when $P<0.05$ was found, the Tukey-HSD multiple comparisons test was used for pairwise comparisons. The correlation coefficient was calculated and tested. The SPSS (10.0 windows) was used for statistical analysis.

The study was approved by the Pediatric Gastroenterology Sub-department of Cerrahpasa School of Medicine.

Informed consent was obtained from the parents of all children. Healthy children were excluded from the performance of a percutaneous liver biopsy and the analysis of sera for testing.

RESULTS

In the group of cirrhosis with PH only two cases had ascites. According to Child-Turcotte

Table I: Plasma concentrations of ET-1, Angiotension-II, ANP, Aldosterone and ADH in study and control groups.

M±SD	Chronic Liver disease (GI-IV) n=30	Control Groups (Group V) n=10	P*
Endothelin-1	16.9 ± 2.1	9.7 ± 1	0.000
Angiotension II	11.7 ± 9.8	4.5 ± 2.5	0.006
ANP	14.6 ± 6.7	5.7 ± 1.1	0.004
ADH	4.8 ± 4.5	2.5 ± 1.6	0.003
Aldosterone	17.0 ± 10.2	4.6 ± 3.8	0.000

p*: shows the differences between ET-1, Angiotension-II, ANP, ADH and aldosterone levels in chronic liver disease (Group I-IV) and the control group

ANP : Atrial natriuretic peptid
ADH : Vasopressin

classification 14 of the cases were considered Child A, 14 of the cases Child B and two of them Child C. ET-1, Angiotension-II, ANP, ADH and aldosterone values according to groups are shown in Table-I. Individual results of ET-1 in patients are reported in Table-II. No significant difference in ET-1 levels was observed between groups (p>0.05).

No correlation was found between ET-1 and angiotensin-II in chronic liver patients (r:0.22, p:0.07) (Fig.1). A positive correlation was found between ET-1, aldosterone, ADH and ANP in

Table II: ET-1 levels in chronic active hepatitis (G-I) cirrhosis with PH (G-II), cirrhosis (G-III) and extrahepatic cases (G-IV).

M±SD	Group - I n = 10	Group - II n = 14	Group - III n = 6	Group - IV n = 10	GI - II	GI - III	GI - IV	GII - III	GII - IV	GIII - IV
Endothelin - 1	16.8 ± 2.1	18.1 ± 1.7	16.1 ± 1.2	15.6 ± 2.6	NS	NS	NS	NS	p<0.05	NS

p*: No differences were found between groups (p>0.05) except for Group II-IV (p<0.05).
NS: Non-significant

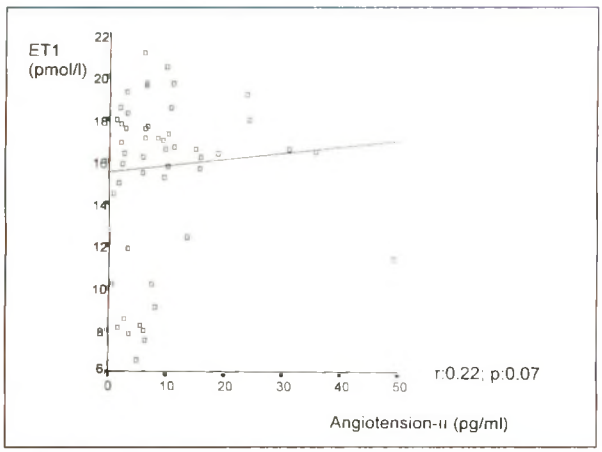

Fig.1: Correlation of ET-1 and Angiotensin-II in chronic liver patients (Group I-IV)

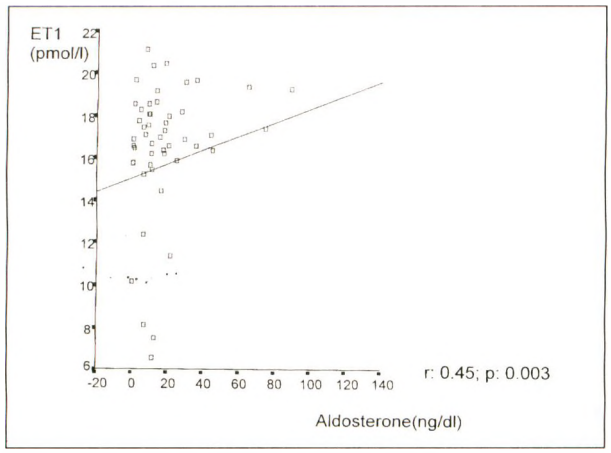

Fig.2: Correlation of ET-1 and Aldosterone in chronic liver patients

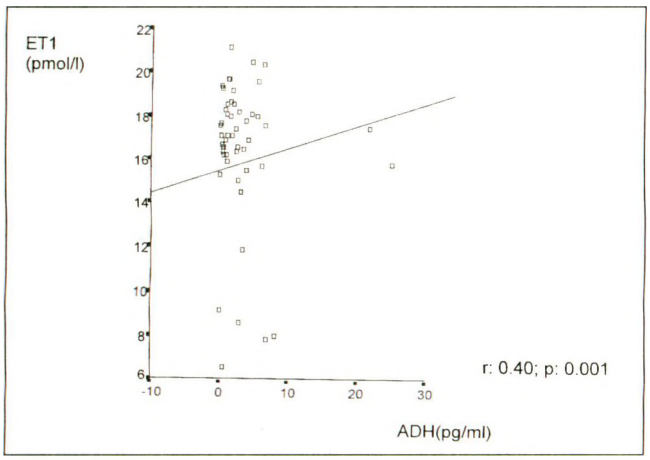

Fig.3: Correlation of ET-1 and ADH in chronic liver patients

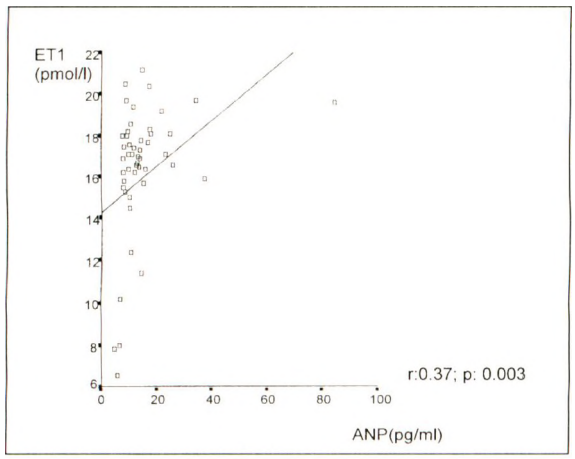

Fig.4: Correlation of ET-1 and ANP in chronic liver patients

chronic liver patients (respectively r:0.45, p:0.003; r:0.40, p:0.001; r:0.37, p:0.003) (Figs-2,3,4).

DISCUSSION

In recent years trials have been conducted regarding the role of ET-1 in portal hypertension physiopathology (16,26,27). Mechanisms responsible for the increase in plasma ET-1 level are increased production of ET-1 and decreased excretion from liver, kidney or lungs (28,29). Endothelin-1 synthesis in endothelium increase is probably a compensatory mechanism in order to antagonize the effect of vasodilator factors of endothelial origin. In decompensated cirrhotic patients with ascites, Lerman et al have found plasma ET-1 levels to be normal before transplantation and high after transplantation (12). In 1992 Veglio et al observed low plasma endothelin levels in cirrhotic patients with ascites (30); in the same years Uchihara et al found high plasma ET-1 levels among cirrhotic patients with ascites and normal plasma ET-1 levels in cirrhotic patients without ascites (13). Again in these years, Moore et al observed approximately 3 to 9 times higher plasma ET-1 levels in chronic liver disease and in hepatorenal syndrome patients compared to healthy control group (24). At the same time they investigated two groups of chronic and acute renal failure patients and found no significant difference in plasma ET-1 levels between these two groups (24). However, they noticed that the plasma ET-1 levels of chronic liver disease patients was twice as high as those of chronic renal failure patients (24). In 1993 Asbert et al compared cirrhotic patients with ascites, renal failure patients with cirrhosis and ascites to a healthy control group (14). The plasma ET-1 levels were found high in cirrhotic cases, and there was a five fold increase in ET-1 value in cirrhotic patients with ascites. Matsumato et al (31) and Hartleb et al (32) found a higher plasma ET-1 level in chronic liver patients than in controls. Gerbes et al (33) and Moller et al (34) investigated ET-1 and ET-3 levels in cirrhotic patients and also found that both these parameters were obviously higher in cirrhotic patients than in controls.

In our study, ET-1 levels of the group consisting of CAH-B patients, cirrhotic and extrahepatic PH patients were 1.5 times higher than in the healthy control group and plasma ET-1 level of cirrhotic and PH patients group was 2 times higher than healthy control group (Table-I) . However, since we had few cases with cirrhosis and ascites and no case with hepatorenal syndrome, we found lower ET-1 levels than stated in the literature. At the same time, we did not find any difference in ET-1 levels sampling different groups of chronic liver disease (Table-II). We can explain this by minor differences between our groups in terms of liver damage. In cirrhotic patients, plasma angiotensin-II, aldosteron and ADH levels increase when the effective blood volume decreases. Schroeder et al have shown that renin angiotensin activity increases in patients with cirrhosis whose kidney function is impaired (4). Rosoff et al (5), Wong et al (6), Bernardi et al (9) found the plasma renin and aldosteron level of patients with cirrhosis to be higher than the healthy control group independent of ascites. In our study, we found the angiotension-II and aldosteron level in chronic liver patients to be 3 times higher than in the healthy group. We can explain this by aldosteron being discharged due to the effective volume decrease and impaired liver function in patients with cirrhosis. Although the systemic effect of endothelin is not completely known, it is thought that it regulates arteriolar vascular tonus and stimulates vasoactive hormone secretion (35,36). Endothelin decreases renal blood flow and increases the secretion of renin, aldosteron, ADH and ANP (35,37,38).

Although the influence of ET-1 over RAAS has not been fully explained in experimental animal studies, after ET-1 administration plasma renin activity was found unchanged (39), increased (35,40) or decreased (22,40). In 1993 Asbert et al showed that in cirrhotic patients, high ET-1 level supresses plasma renin activity (14). In 1994 Matsumato et al pointed out the dual increase of angiotensin-II and ET-1 in cirrhotic patients (31). At the same time both investigator groups found out that ET-1 levels increase simultaneously with increasing plasma aldosteron. In our trial, no correlation was found between ET-1 and angiotensin-II in chronic liver patients (Fig.1). We can explain this by no more severe liver damage in our patients and the fluid electrolyte balance and systemic renal hemodynamic not yet deteriorated. In the same

manner we found a rising positive correlation between aldosterone and ET-1 (Fig.2). This can be explained by a continuous stimulation of ET-1 secreting cells by activated RAAS due to low effective plasma volume in liver cirrhosis.

In cirrhosis, plasma ADH level increases and it was shown that the plasma ADH level was higher in decompensated cirrhotic patients than in the cirrhotic patients (10). In our cases we found a high ADH level and a positive correlation between ADH and ET-1 (Fig.3). This stituation can be interpreted in advanced liver patients to be associated with inappropriate ADH hypersecretion .

Although it has been shown that plasma ANP level is increased in cirrhotic patients, its mechanism is not so clear (41,42). Stimulation of ANP secretion could be due to an increase in arterial pressure and ET-1 direct influence over atrial myosites (38). It has been shown that when ET-1 is administered to experimentally decompensated cirrhotic rats, renin secretion is inhibited and aldosterone, ANP secretions are increased (43). While Asbert et al (14) found a positive correlation between ANP and ET-1, Uchihara et al (13) did not find any correlation. We also found a positive correlation between plasma ET-1 and ANP values among our chronic liver patients (Fig.4). We can explain this by an increase of RAAS in cirrhotic patients and over production in the myocardium.

In conclusion we found out that ET-1 levels were higher in patients with chronic liver disease than in the control group. Plasma ET-1 levels were not significantly different from other chronic liver diseases. We explained this by the low number of patients with cirrhosis and ascites in Group II and the other groups. Plasma ET-1 levels were increased in Group II patients. We thought the reason for the elevation of plasma levels of ET-1 may be either the increase of ET-1 production or the decreased metabolic clearance rate of ET-1, or both. However, when plasma ET-1 levels within groups are considered, we can suggest that ET-1 which is an autocrine and paracrine hormone, plays an important role in cirrhosis and portal hyperdynamic circulation.

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