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Correlation of Endothelin-1 Concentration and Angiotensin-Converting Enzyme Activity with the Staging of Liver Fibrosis

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A B S T R A C T

Increased serum angiotensin-converting enzyme (SACE) activity and serum concentration of endothelin-1 (ET-1) were found in liver cirrhosis. We investigated a correlation between the different stages of liver fibrosis and SACE activity and serum ET-1 concentration. Seventy patients with pathohistologically established chronic liver disease were divided in three groups according to Ishak criteria for liver fibrosis: minimal fibrosis (Ishak score 0–1, n=20), medium fibrosis (Ishak score 2–5, n=20) and cirrhosis (Ishak score 6, n=30). SACE activity and ET-1 concentration were determined using commercial ELISA kits. SACE activity and ET-1 concentrations were proportional to the severity of disease, the highest being in patients with liver cirrhosis. Maximal increase in SACE activity was found between minimal and medium fibrosis while maximal increase in ET-1 concentration was revealed between medium fibrosis and cirrhosis. The analysis of the Receiver Operating Characteristic (ROC) curve for SACE activity suggested a cut-off value to separate minimal from medium fibrosis at 59.00 U/L (sensitivity 100%, specificity 64.7%). The cut-off value for serum ET-1 concentration to separate medium fibrosis from cirrhosis was 12.4 pg/mL (sensitivity 96.8%, specificity 94.4%). A positive correlation between SACE activity and ET-1 concentration was registered (Spearman's ñ=0.438, p=0.004). Both SACE activity and ET-1 concentration were increased in all stages of liver fibrosis. Cut-off points for SACE activity and ET-1 concentration could be a biochemical marker for the progression of fibrosis. Positive correlation between SACE activity and ET-1 concentration might indicate their interaction in the development of liver cirrhosis.

Key words: *fibrosis, liver, cirrhosis, angiotensin converting enzyme, endothelin*

Introduction

Liver fibrosis is a common consequence of chronic liver injury regardless of its etiology. Advanced fibrosis disrupts the normal liver architecture, causing hepatocellular dysfunction and portal hypertension[1]. Hepatostellate cells (HSC) have an important role in the regulation and modulation of hepatic blood flow through growth factor secretion, retinoid storage and the release and production of extracellular matrix of space of Disse[1]. After liver injury, HSC undergo a phenotypic transformation to become myofibroblast-like with expression of á-smooth muscle actin an secretion of the extracellular matrix composed of various proteoglycans and proteins such as collagen type I[1]. Also, HSC activation includes increased expression of receptors for a variety of vasoconstrictor substances, such as angiotensin II (AT-II) and endothelin (ET-1)[2–4]. These afore mentioned changes are associated with the modulation of extracellular matrix production and fibrogenesis governed by HSC[2–4].

Previously, it has been observed that serum angiotensin-converting enzyme (SACE) activity, which mediates AT-II production, and serum ET-1 concentration were increased in the patients with chronic liver diseases, for example: chronic viral hepatitis B and C, non-alcoholic liver

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disease and liver cirrhosis[5–7]. Moreover, AT II and ET-1 have a leading role in the progression of chronic liver damage by up-regulation of extracellular matrix production and fibrogenesis[2–4]. However, to date, there are no studies about the activity of the mentioned parameters regarding the severity of liver fibrosis. The aim of this study was to analyze the SACE activity and serum ET-1 concentrations in the patients with chronic liver disease regarding the stages of liver fibrosis.

Patients and Methods

Study design

The prospective observational study was conducted from April 2002 to September 2004 in the Division of Gastroenterology and Hepatology, Dubrava University Hospital, Zagreb, Croatia. The 70 patients (42 men; mean age 52.00±16.46 years) with different stages of chronic liver disease were enrolled in the study. Patients suffering from the diseases in which SACE activity or ET-1 concentration might be changed (e.g. diabetes mellitus, hypertension, hyperthyroidism, sarcoidosis, silicosis, lung cancer, hepatocellular carcinoma, asthma, obstructive icterus, hypothyroidism, bacterial peritonitis, and other infectious diseases) were excluded from study. The study was conducted according to the principles of the Declaration of Helsinki and approved by the hospital ethics committee. All participants enrolled in the study gave informed consent.

Diagnosis of liver disease was established on the basis of clinical and biochemical means, ultrasonography and CT scan of the abdomen, and histological findings of liver bioptate. The stage of chronic liver disease was established through pathohistological examination of liver bioptate obtained under the ultrasonographic control.

Patients were divided in three groups according to Ishak criteria for fibrosis[8]: minimal fibrosis (Ishak score 0–1, n=20), medium fibrosis (Ishak score 2–5, n=20), and cirrhosis (Ishak score 6, n=30).

Measurement of serum ET-1 concentration

The plasma ET-1 concentration was determined with enzyme linked immunosorbent assay (ELISA) using commercial kit »*Endothelin ELISA*« (Biomedica, Vienna, Austria) and ELx800 universal Plate Reader, Bio-Tek Instruments Inc. This is a sandwich assay which recognizes following epitopes on ET-1: coating-antibody recognizes amino acids 16–21, while detection-antibody recognizes amino acids 8–16. Detection-antibody is conjugated with peroxidase which catalyzes degradation of tetramethylbenzidine, and this reaction is accompanied by color change detec± on an ELISA reader. The amount of developed color is directly proportional to the amount of ET-1 in the sample, expressed in pg/mL.

Measurement of SACE

The SACE activity was determined with enzyme linked immunosorbent assay (ELISA) using commercial kit »*Infinity™ ACE Liquid Stable Agent*« (Thermo Electron Corporation, New York, USA). This kit utilizes the catalytic activity of ACE in the following reaction: furylacryloylphenylalanineglyclglycine (FAPGG) → furylacryloylphenylalanine (FAP) + glyclglycine (GG). Hydrolysis of

TABLE 1
EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF THE STUDY POPULATION REGARDING THE STAGE OF LIVER FIBROSIS

Characteristics	Stage of disease		
	Minimal fibrosis N=20	Medium fibrosis N=20	Cirrhosis N=30
Ishak score	0–1	2–5	6
Female/Male (No. of pts)	9/11	8/12	11/19
Age (years)	52 (19–69)	52 (33–75)	52 (24–72)
Etiology (No. of pts.)	Steatosis (18) Minimal fibrosis (2)	Hepatitis B (6) Hepatitis C (4) Hepatitis B and C (1) Alcoholic hepatitis (6) Primary biliary cirrhosis (3)	Alcoholic hepatic cirrhosis (30)
Ascites (No. of pts)	0	0	0
Sodium (mmol/L)	142 (126–151)	140 (132–145)	138 (128–143)
Potassium (mmol/L)	4.3 (3.4–5.2)	4.2 (3.6–5.2)	4.2 (2.4–7.2)
Amoniac (μmol/L)	12 (3–71)	21 (11–99)	61 (12–110)
Bilirubine (μmol/L)	11.9 (7.8–61.3)	19.1 (5.2–198.1)	63.3 (8.8–518.6)
BUN (mmol/L)	5.8 (3.6–11.3)	5.3 (3.4–11.9)	7.5 (3.6–22.1)
Creatinin (μmol/L)	78 (24–94)	80 (43–167)	83 (52–277)
Albumins (g/L)	49 (35–53)	47 (32–53)	41 (23–51)
Prothrombin time(%)	91 (37–106)	69 (42–97)	54 (10–99)

FAPGG results in decrease in absorbance at 340 nm. The SACE activity in the sample is determined by comparing the sample reaction rate to that obtained with the ACE Calibrator, and the results were expressed in IU/L. All the measurements were performed on Olympus AU--CONNECTOR 27000 (Olympus, Japan).

Statistics

Since Kolmogorov-Smirnov test did not show normal distribution of the variables, SACE activity and serum concentration of ET-1 in three different groups were summarized by median and range. Cut-off values of SACE activity and serum concentration of ET-1 between groups were determined by the Receiver Operating Characteristic (ROC) curve analysis. Correlation between SACE activity and serum concentration of ET-1 was analyzed through Spearman's test of rank correlation, followed by linear regression analysis. All statistical analyses were performed using MedCalc for Windows (v.7.5. Frank Schoonjans Inc., Mariakerke, Belgium).

Results

Demographic and clinical variables in study population are presented in Table 1. The values of ammonia and bilirubin were progressively increased with regard to the stage of liver disease, the highest being in patients with liver cirrhosis. On the contrary, the value of prothrombin time was gradually decreased with regard to the progression of liver disease, the lowest being in patients with liver cirrhosis. There were no differences in the other demographic and clinical variables.

The SACE activity was increased in proportion with liver fibrosis (Figure 1). The lowest SACE activity was found in the patients with minimal disease (median 57 IU/L, range 16–94 IU/L), while the highest activity was observed in the patients with liver cirrhosis (median 89, range 65–139 IU/L). Serum concentrations of ET-1 showed a similar trend (Figure 2). Namely, the lowest ET-1 concentrations (median 6.25, range 2.38–11.80 pg/mL) were observed in patients with minimal disease, while in

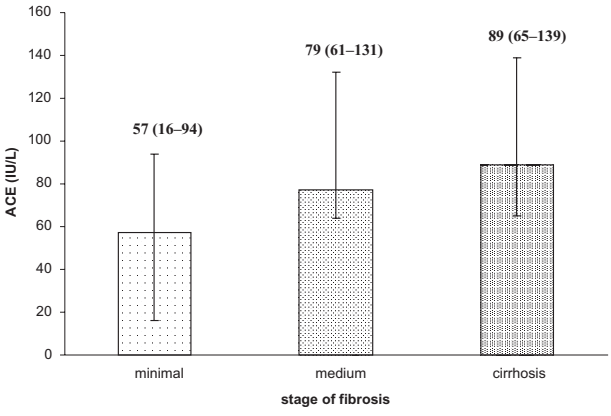

Fig. 1. Serum angiotenzin-converting enzyme (ace) activity in patients with minimal fibrosis, medium fibrosis and cirrhosis.

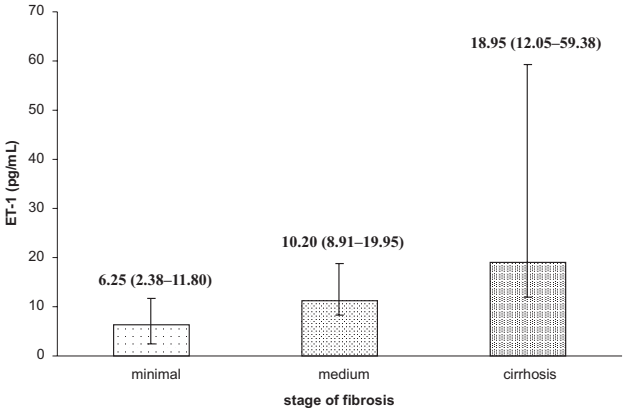

Fig. 2. Serum concentration of endothelin-1 (et-1) in patients with minimal fibrosis, medium fibrosis and cirrhosis.

patients with cirrhosis, the highest ET-1 concentrations (median 18.95, range 12.05–59.38 pg/mL) were registered. In addition, there was the positive correlation between SACE activity and serum concentration of ET-1 (Spearman's p=0.438, p=0.004; Figure 3).

Since the elevation of SACE activity and ET-1 concentrations did not follow the progression of liver fibrosis in a linear manner. The highest increase in SACE was observed in the progression from minimal to moderate fibrosis, while the highest increase in serum ET-1 concentration was observed during the progression from medium fibrosis to liver cirrhosis. Therefore, we tried to determine cut-off values for both variables using ROC curve analysis. The analysis of the ROC curve for SACE activity determined a cut-off value to separate minimal fibrosis from medium fibrosis at 59.00 U/L, with a sensitivity of 100%, and a specificity of 64.7% (Figure 4). The cut-off value for serum ET-1 concentration to separate medium fibrosis from cirrhosis was 12.4 pg/mL, with a sensitivity of 96.8% and a specificity of 94.4% (Figure 5).

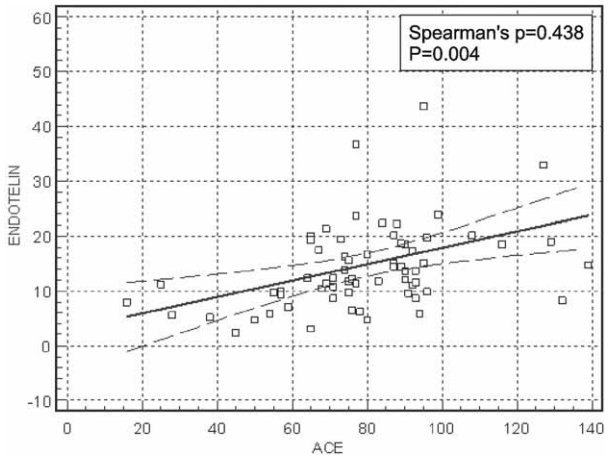

Fig. 3. Dissociation between serum angiotezin-convertin enzyme (ace) activity and serum concentration of endothelin -1 in patients with minimal fibrosis, medium fibrosis and cirrhosis.

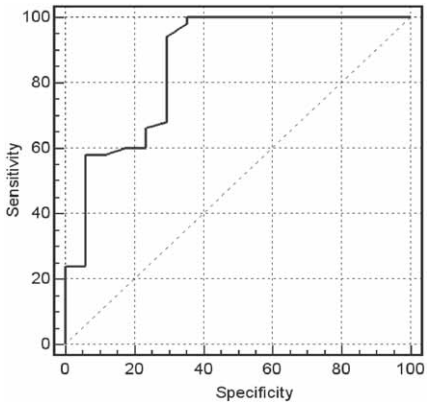

Fig. 4. The analysis of the roc curve for serum angiotenzin-converting enzyme (sace) activity in patients with minimal fibrosis, medium fibrosis and cirrhosis. A cut-off value determined to separate early from medium fibrosis was 59.00 U/L, with the sensitivity of 100%, and specificity of 64.7%.

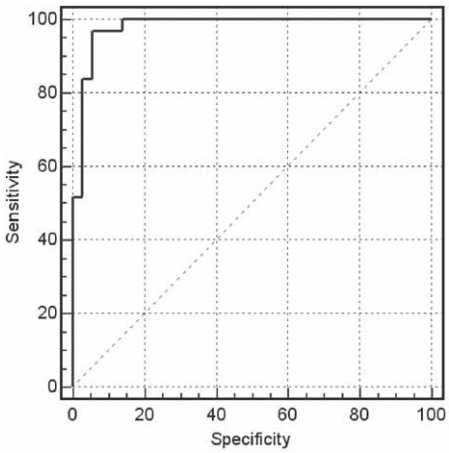

Fig. 5. The analysis of the roc curve for serum concentration of endothelin-1 (et-1) in patients with minimal fibrosis, medium fibrosis and cirrhosis. A cut-off value determined to separate medium fibrosis from cirrhosis was 12.4 pg/mL, with the sensitivity of 96.8%, and specificity of 94.4%.

Discussion and Conclusion

The main findings of our study are: 1) both SACE activity and serum concentration of ET-1 are increased in patients with chronic liver disease in proportion with the stage of liver fibrosis, 2) there is positive correlation between SACE activity and ET-1 concentration in this group of patients, and 3) SACE activity and ET-1 serum concentration could be valuable laboratory markers for determining progression of liver disease from mild fibrosis into cirrhosis.

Previously, it has been observed that SACE activity was increased in the patients with chronic liver diseases – e.g. chronic viral hepatitis B and C, non-alcoholic liver disease and liver cirrhosis[5–7]. The exact mechanism of the increase of the SACE activity found in these patients is not clear yet. The possible causes could be decreased inactivation of this enzyme caused by diminished lung function in cirrhosis, ACE hyper production in the spleen, hypoxia and electrolyte disbalans caused by hemodynamic changes[9–11]. Additionally, these patients show elevated concentrations of histamine, a substance that increases the secretion of ACE from blood vessel endothelial cells[10,11]. On the other hand, using the model of chronic liver damage caused by the ligation of biliary ducts, Paizis et al. showed increased ACE secretion from activated HSC, phagocytes, and proliferating epithelial cells of biliary ducts[12].

Increased activation of tissue and intracellular renin--angiotensin-aldosterone system (RAAS) in chronically damaged liver, as well as the increased ACE activity in local circulation, lead to increased serum concentration of AT-II[13–16]. Bataller et al. demonstrated that activated HSC expressed AT-1 subtype of AT II receptors[17]. They also showed that binding of AT-II to these receptors induced contraction and proliferation of HSC[17]. This study implies that AT-II is a mitogenic factor for activated HSC, which could play a role in the pathogenesis of intrahepatic portal hypertension due to its effects on HSC[17]. It has been reported that AT-II induced the secretion of the vascular endothelial growth factor (VEGF) in a dose-dependent manner[18]. The hepatic VEGF level in chronic liver diseases has been shown to be increased with disease progression, and it stimulated HSC activation and sinusoidal capillarization[18]. Besides its other effects, AT II is a powerful simulator of ET-1 production in chronic liver disease, which could be a possible explanation of the positive correlation between SACE activity and ET-1 concentration observed in our study[19].

On the other hand, the elevation of serum ET-1 concentration that we found in all our patients is probably due to the diminished catabolism and elimination of ET-1, or increased synthesis and secretion that occur in chronic liver damage. The lungs have an important role in the clearance of ET-1[20]. Therefore, it is possible that in liver cirrhosis intrahepatic shunts cause the diminishment of lung clearance of ET-1[20]. Additionally, the kidney clearance of ET-1 is also changed, mainly as the consequence of lower activity of the enzymes that metabolize it, such as neural endopeptidase[21,22]. On the other side, increased production of ET-1 in the splanchnic pool in the patients with liver cirrhosis represents an additional load for already slowed liver elimination (due to reduced functional liver tissue by fibrosis) [23]. In liver cirrhosis, there is a significantly increased production of ET-1 in the endothelial and smooth muscle cells of splanchnic blood vessels, as well as in the intestinal mucosal cells[23,24]. This is a response to hypoxia, oxidative stress, bacterial endotoxemia, and increased activation of systemic RAAS/AT II[25,26]. Since the endotoxin levels are proportionally elevated in more severe liver damage, especially in cirrhosis, endotoxemia could be another mechanism of ET-1 increment in liver cirrhosis[23].

Elevated ET-1 serum concentration found in the patients with progressive liver damage has a vasoconstrictive effect in the sinusoidal endothelial cells and activated HSC. This significantly worsens the hemodynamic condition in the damaged liver. At the same time, in activated HSC ET-1 induces production of the extracellular matrix significantly more than AT-II. ET-1 enhances the development of liver fibrosis and its progression into cirrhosis[27,28].

In the presented study both SACE activity and ET-1 serum concentration were increased in all groups of patients with liver disease. Moreover, values of both parameters were proportional to the severity of the disease, the highest being in patients with liver cirrhosis. However, the highest increase of SACE activity was accompanied with the progression from minimal to moderate fibrosis, while the highest increase in serum ET-1 concentration was observed during the progression from moderate fibrosis to cirrhosis. In that context, the analysis of the ROC curve set 59.00 U/L to be a cut-off value for SACE activity which could separate minimal from medium fibrosis, while the same analysis pointed out serum ET-1 concentration of 12.4 pg/mL as a cut-off value which indicates the progression from medium fibrosis into cirrhosis. The positive correlation of SACE activity and serum ET-1 concentration observed in our study suggests the possibility that the increase of serum ET-1 concentration partially might be the result of the increased ET-1 production in HSC and endothelial sinusoidal cells stimulated with AT II. Thus, it could be speculated that suppression of the SACE activity (e.g. with using ACE inhibitors) may result in decreases of serum ET-1 concentration and slower progression of liver fibrosis into cirrhosis.

In conclusion, both SACE activity and serum concentration of ET-1 are increased in patients with chronic liver disease in proportion with the stage of liver fibrosis. SACE and serum ET-1 concentration could be additional serum markers in the non-invasive »staging« of chronic liver disease. Further studies are needed to elucidate the possible correlation between staging of liver fibrosis, SACE activity and ET-1 concentrations and changes in liver blood flow, which could imply therapeutic interventions aimed at the modulation of the effect of vasoactive substances.

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KONCENTRACIJA ENDOTELINA-1 I AKTIVNOST ANGIOTENZIN-KONVERTIRAJUĆEG ENZIMA U ODNOSU NA STUPANJ JETRENE FIBROZE

Povećana aktivnost angiotenzin-konvertirajućeg enzima (SACE) i serumska koncentracija endotelina-1 (ET-1) zabilježene su u bolesnika s cirozom jetre. U našem smo istraživanju korelirali aktivnost SACE i serumsku koncentraciju ET-1 s različitim stadijima jetrene fibroze. Sedamdeset bolesnika s patohistološki potvrđenom kroničnom bolešću jetre podijeljeno je u tri skupine prema Ishakovim kriterijima za fibrozu jetre: minimalna fibroza (Ishakov skor 0–1, n=20), umjerena fibroza (Ishakov skor 2–5, n=20) i ciroza (Ishakov skor 6, n=30). Aktivnost SACE i koncentracija ET-1 određene su primjenom komercijalnih ELISA kitova. Aktivnost SACE i koncentracija ET-1 bile su proporcionalne težini bolesti; najveće vrijednosti zabilježene su u bolesnika s cirozom jetre. Najveći porast aktivnosti SACE je zabilježen na prijelazu minimalne u umjerenu fibrozu, dok je najveći porast koncentracije ET-1 zabilježen na prijelazu iz umjerene fibroze u cirozu. Analiza ROC krivulje za aktivnost SACE sugerira vrijednost od 59,00 u/l kao razdjelnicu između minimalne i umjerene fibroze (osjetljivost 100%, specifičnost 64,7%). Vrijednost koncentracije serumskog ET-1 od 12,4 pg/mL sugerirana je kao razdjelnica umjerene fibroze i ciroze jetre (osjetljivost 96,8%, specifičnost 94,4%). Zabilježena je pozitivna korelacija između aktivnosti SACE i koncentracije ET-1 (Spearmanov koeficijent korelacije p=0,438, p=0,004). Aktivnost SACE i koncentracija ET-1 mogu poslužiti kao biokemijski biljezi za praćenje napredovanja fibroze jetre. Pozitivna korelacija između aktivnosti SACE i koncentracije ET-1 može ukazivati na njihovu interakciju u razvoju ciroze jetre.