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Nitric oxide and renal functions in liver cirrhosis

Karaciğer sirozunda nitrik oksit ve renal fonksiyonlar

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Background/aims: *Nitric oxide, a potent vasodilating agent, has been proposed to play a role in pathogenesis of ascites and hepatorenal syndrome. The aim of this study was to evaluate the interaction between the plasma nitric oxide, nitric oxide synthetase levels and renal functions in patients with different degrees of chronic liver disease.* ***Methods:*** *The study population included 38 subjects: 14 patients with chronic hepatitis, 11 with preascitic cirrhosis and 13 with ascitic cirrhosis. Nitric oxide and nitric oxide synthetase were determined by colorometric assay. We calculated glomerular filtration rate and fractional sodium excretion.* ***Results:*** *Nitric oxide levels in groups were as follows: 79.28±24.86, 99.03±21.31, 197.05±49.61 µm, respectively. Nitric oxide synthetase levels were 2.64±0.56, 3.64±0.89, 7.75±2.46µmol/L/sec, respectively. Nitric oxide and nitric oxide synthetase levels in the ascitic cirrhotic group were significantly higher than in the others (p<0.05). When glomerular filtration rates were compared, the only significant difference was determined between the groups with chronic hepatitis and ascitic cirrhosis (92.31±25.21, 48.46±16.45, p<0.05). Fractional sodium excretion was significantly increased in the ascitic cirrhotic group (4.42±2.76, p<0.05).* ***Conclusions:*** *Nitric oxide and nitric oxide synthetase increased with progression of liver disease, especially in ascitic cirrhosis. We also showed that this increase negatively affects the renal tubular and glomerular functions.*

Key words: Nitric oxide, cirrhosis, renal functions

Amaç: *Nitrik oksit, potansiyel bir vazodilatatör ajan olup asit ve hepatorenal sendromun patogenezinde rol oynadığı düşünülmektedir. Bu çalışmanın amacı; değişik evrelerdeki kronik karaciğer hastalarında plazma nitrik oksit, nitrik oksit sentetaz düzeyleri ve renal fonksiyonlar arasındaki ilişkiyi değerlendirmektir.* ***Yöntem:*** *Çalışma grubundaki 38 hastanın 14'ü kronik hepatit, İVi preasitik siroz ve 13'ü asitik sirozlu hasta idi. Nitrik oksit, nitrik oksit sentetaz kolorimetrik yöntemle çalışıldı. Glomerüler filtrasyon hızı ve fraksiyonel sodyum atılımlarını hesapladık.* ***Bulgular:*** *Nitrik oksit seviyeleri gruplarda sırası ile; 79.28±24.86, 99.03±21.31, 197.05±49.61µmidi. Nitrik oksit sentetaz seviyeleri ise; 2.64±0.56, 3.64±0.89, 7.75±2.46 moll 11dk idi. Nitrik oksit, nitrik oksit sentetaz seviyeleri asitik sirozlu grupta diğer gruplara göre anlamlı ölçüde yüksekti (P<0.05). Glomerüler filtrasyon hızları karşılaştırıldığında sadece kronik hepatitli ve asitik sirozlu gruplar arasındaki fark anlamlı idi (92.31 ± 25.21, 48.46 ± 16.45, P < 0.08). Fraksiyonel sodyum atılımı asitik sirozlu grupta anlamlı ölçüde artmıştı (4.42±2.76, P<0.05).* ***Sonuç:*** *Nitrik oksit ve nitrik oksit sentetaz karaciğer hastalığının progresyonu ile özellikle asitik sirozlu hastalarda arttı. Aynı zamanda bu artışın renal tubular ve glomerüler fonksiyonları negatif yönde etkilediğini gösterdik.*

Anahtar kelimeler: Nitrik oksit, siroz, böbrek fonksiyonları

INTRODUCTION

Peripheral arterial vasodilation is an important event in the pathophysiology of ascites formation in patients with cirrhosis. The precise factors involved in the critical peripheral arterial vasodilation remain obscure (1, 2). Nitric oxide (NO) is a powerful vasodilating agent released from vascular smooth muscle. An increased release of NO has been proposed to play a role in the pathogenesis of vasodilation and vascular hypocontractility associated with portal hypertension (3).

Many studies in animals and humans with portal hypertension have provided evidence suggesting that NO has an important role in the hemodynamic abnormalities that characterize cirrhosis and the associated renal sodium and water retention, which lead to ascites formation (3-6).

The vasodilatory effects of portal hypertension do not appear to affect the renal vascular system in patients. When renal perfusion is compromised, renal function is sensitive to changes in blood vo-

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lume or toxins. This produces a decrease in glomerular filtration rate (GFR) that, along with activation of the renin-aldosterone system and vasopression, may intensify proximal tubular sodium resorption and reduce free water clearance, leading to hyponatremia. Furthermore, renal prostoglandin and NO production is important at this stage in diminishing the renal effect of vasoconstrictors. Eventually, vasoconstriction exceeds the vasodilatory influences, leading to progressive renal failure (7-9).

In this study, we aimed to investigate the relationship between NO, NO synthetase (NOS) levels and renal functions in patients with different stages of chronic liver disease.

MATERIALS AND METHODS

The study population included 38 subjects: 14 patients with chronic hepatitis (group 1), 11 patients with preascitic liver cirrhosis (group 2) and 13 patients with ascitic cirrhosis (group 3). Diagnosis of chronic hepatitis and compensated liver cirrhosis was based on percutaneous liver biopsy and/or clinical, laboratory, ultrasonographic and endoscopic findings. None of the patients had heart failure, intrinsic renal disease, or infection or had received nephrotoxic drugs or diuretics. Their ages ranged from 25 to 72 years. All of the preascitic cirrhotic patients belonged to class A according to Child-Pugh classification, and eosophageal varices were seen in five. Four ascitic patients belonged to class B and the others to class C. All of the ascitic cirrhotic patients had eosophageal varice.

The nitrate/nitrite colorimetric assay 780001 kit, purchased from Cayman Chemical CO., USA was used for NO and NOS activation measurements. Venous blood samples were taken after obtaining informed consent. Blood was drawn after 12 hours fasting from subjects who had been in supine position for at least 15 min. The heparinized blood samples were immediately cooled and centrifuged. The serum was separated from erythrocytes and frozen at -70°C until NO and NOS analysis. Colorimetric reading was made by Triturus automatic ELISA.

Glomerular filtration rate (GFR) was calculated from Cockcroft-Gault formula (10).

Fractional sodium excretion (FeNa) was calculated by the formula:

100X Urine Na X plasma creatinine/Urine creatinine X plasma Na

Statistical Analyses: One-way ANOVA, Mann-Whitney U and Kruskal Wallis tests were used for comparing the groups. $P<0.01$ was accepted as significant. Results are expressed as mean±SD. Correlation coefficients were derived by using Pearson's correlation test.

RESULTS

Clinical and laboratory findings of the patients are reported in (Table 1).

There were no significant differences between the groups when compared for age and sex.

Plasma levels of NO, NOS and renal function tests of the groups are shown in (Table 2).

No massive or moderate proteinuria or hematuria was seen in urine analysis of the groups.

Statistical Results

Nitric oxide and NOS levels in the ascitic cirrhotic group were significantly higher than in the others ($P<0.05$). Although NO was lower in the chronic hepatitis group compared to the preascitic cirrho-

Table 1. Clinical and laboratory data of patients with chronic liver disease

	Chronic Hepatits n=14	Preascitic Cirrhosis n=ll	Ascitic Cirrhosis n=13
Sex (male/female)	8/6	7/4	7/6
Age (year)	52 (25-72)	57 (42-64)	55 (40-71)
Etiology			
HBV	8	7	7
HDV	1		
HCV	4	4	5
Alcoholic	1		1
ALT (IU/L)	92.25±24.2	98.76±33.6	36.2±8.3
AST (IU/L)	81.7±18.7	54.7 ±18.5	59.4+16.1
GGT **(U/L)**	72±21.9	59.2+18.23	112.4+49.6
Serum bilirubin (mg/dl)	0.96±0.13	1.36+0.42	3.76±1.68
Platelet count (cell/mm^3)	198 200±43.800	134 000±27 100	84700±57800
Prothrombin time (%)	92±7	78±9	61+6

Table 2. NO, NOS and renal function tests of patients with chronic liver disease

	Chronic Hepatits n=14	Preascitic Cirrhosis n=ll	Ascitic Cirrhosis n=13
NO (μm)	79.28±24.86	99.03±21.31	197.05+49.61
NOS (μol/L/sec)	2.64±0.56	3.64+0.89	7.75±2.46
GFR (ml/sec)	92.31±25.21	68.36±23.60	48.46±16.45
FeNa (%)	1.05+0.41	1.21±0.45	4.42±2.76

NO: Nitric oxide, NOS: nitric oxide synthetase, GRF: glomerular filtration rate, FeNa: fractional sodium excretion

tic group, the difference was not significant (P>0.05). NOS levels were not significantly different between groups 1 and 2 (P>0.05).

When we compared GFR in groups, the only significant difference was determined between the chronic hepatitis and ascitic cirrhotic groups (92.31±25.21, 48.46+16.45) (P<0.05). FeNa was significantly increased in the ascitic cirrhotic group (P<0.05). There was no difference between groups 1 and 2 when compared for FeNa (1.05±0.41, 1.21±0.45) (P>0.05).

We found a negative correlation between NO and GFR (r=-0.49, p=0.002), a significant positive correlation between NO and FeNa (r=0.63) (p=0.000) (Figure 1), a significant positive correlation between NOS and FeNa (r=0.50, P=0.001), and a negative correlation between NOS and GFR (r=-0.54, P=0.000)

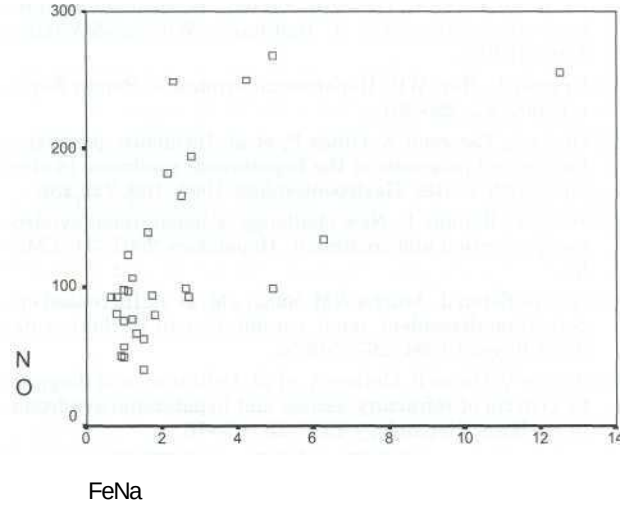

Figure I. Positive correlation between nitric oxide and fractional sodium excretion

DISCUSSION

There is no explanation that throughly describes the complex relationship between the liver and kidney in either physiological or pathological conditions (11). In a large prospective follow-up study of cirrhotic patients with ascitis, development of renal failure occurred in 18% and 39% of the patients at one year and five years, respectively (12).

With progression of cirrhosis, a gradual increase in splanchnic blood flow occurs and is followed by systemic vasodilation. NO has been proposed to be an important effector responsible for splanchnic vasodilation in cirrhosis. The activity of NO may change from one vascular territory to another. It may be increased in the splanchnic and in the renal circulation and decreased in the intrahepatic circulation (7, 11, 13). The secondary hormonal and hemodynamic responses in the setting of ascites produce renal arterial vasoconstriction that is progressive. At this stage local vasodilator prostoglandins and NO are important in maintenance of renal perfusion (2, 9). In animal studies it is shown that the renal microcirculation in rats with cirrhosis is less sensitive to the effects of nonspecific blockade of nitric oxide synthetase than seen in normal rats because of differences in the location or type of NOS (or both) (2, 5, 6). But in contrast, NOS inhibition in cirrhotic rats with ascites does not affect renal blood flow or function, despite an increased systemic pressor effect. Furthermore, renal vasoconstriction may occur in the presence of increased glomerular nitrite production. This suggests that renal microcirculation in ascites is less sensitive to nitric oxide (2,14). We found that NOS and NO increased significantly with progression of liver disease especially in the decompensated cirrhotic group. This result supports that NO is important in the progression of cirrhosis.

One of the most diffucult issues in the clinical evaluation of patients with cirrhosis is how to assess renal function because of the standard methods used like blood urea nitrogen (BUN) and creatinine. BUN levels may be lower than expected in patients with liver disease because of reduced hepatic synthesis. On the other hand, BUN may increase because of gastrointestinal hemorrhage or catabolic states. And endogen creatinine production

may decrease because of protein malnutrition. Finally the sensitivity of creatinine clearance is probably higher than BUN or serum creatinine levels (15). For this reason we used creatinine clearance in evaluating the glomerular function. The mean GFR of ascitic cirrhotic patients was significantly lower than the mean GFR of the patients with chronic hepatitis in our study. The negative correlation found between NO, NOS and GFR may reflect the help of NO in vasoconstriction of renal perfusion.

It is reported that sodium retention, impaired free-water excretion and decreased renal perfusion and GFR are the main renal function abnormalities in cirrhosis (8, 11). When cirrhosis is still compensated, subtle abnormalities in renal sodium metabolism can be detected. With the progress of the disease, the impairment in sodium metabolism increases, at which point patients are unable to excrete the sodium intake (8, 11, 15). It is believed that tubular functions are usually preserved and that low urinary sodium concentration is a characteristic finding in cirrhosis. On the contrary, relatively high urinary sodium concentrations have been reported even in patients with hepatorenal syndrome (16, 17). In cirrhotic patients, prolonged renal hypoperfusion, renal medullary hypoxia and high frequency of infectious complications ultimately contribute to renal tubular damage (11). Our increased FeNa data in the decompensated cirrhotic group may indicate the tubulary damage. This can be explained by recalling that most of our patients in this group were Child C according to Child-Pugh classification, so complications like infection and gastrointestinal hemorrhage were frequent in these patients. Diuretic therapy may also contribute to this process.

As a result, with an increase in NO and NOS with progression of the liver disease, especially in ascitic cirrhosis, renal tubular and glomerular functions are negatively affected. Modulation of NO synthesis may help in the management of circulatory and renal dysfunction in these patients. Further studies are needed regarding this subject.

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