



## Diagnostical significance of dimethylarginine in the development of hepatorenal syndrome in patients with alcoholic liver cirrhosis

Dijagnostički značaj dimetilarginina u razvoju hepatorenalnog sindroma kod bolesnika sa alkoholnom cirozom jetre

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### Abstract

**Background/Aim.** Chronic consumption of alcohol during a longer period of time leads to the development of cirrhosis with the reduction in metabolic liver function and disorders in arginine metabolism. Hepatorenal syndrome (HRS) is the most severe complication of alcoholic liver cirrhosis. The aim of the study was to analyze disorders in arginine metabolism by monitoring concentrations of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) in patients with liver cirrhosis and HRS. **Methods.** The study included three groups of subjects: a group of patients with cirrhosis and HRS (24 patients), a group of patients with cirrhosis without HRS (18 patients) and a control group composed of 42 healthy voluntary blood donors. Concentrations of ADMA, SDMA and L-arginine in plasma were measured in all groups using the high pressure liquid chromatography (HPLC) method. **Results.** The concentration of SDMA was significantly higher in the patients with HRS compared to the patients without HRS and it was also higher than the values obtained from the healthy participants ( $1.76 \pm 0.3 \mu\text{mol/L}$ ;  $1.01 \pm 0.32$  and  $0.520 \pm 0.18$

$\mu\text{mol/L}$ , respectively;  $p < 0.01$ ). The concentrations of ADMA were higher in the cirrhotic patients with HRS than in those without this serious complication of cirrhosis. The concentration of ADMA in all the examined cirrhotic patients was higher than those obtained from healthy volunteers ( $1.35 \pm 0.27 \mu\text{mol/L}$ ,  $1.05 \pm 0.35 \mu\text{mol/L}$  and  $0.76 \pm 0.21 \mu\text{mol/L}$ , respectively). In the patients with terminal alcoholic liver cirrhosis, the concentrations of ADMA and SDMA correlated with the progress of cirrhosis as well as with the development of cirrhosis complications. In the patients with HRS there was a positive correlation between creatinine and SDMA in plasma ( $r^2 = 0.0756$ ,  $p < 0.001$ ) which was not found between creatinine and ADMA. **Conclusion.** The obtained results demonstrate that the increase in SDMA concentration is proportionate to the progression of chronic damage of the liver and kidneys. Increased ADMA concentration can be a causative agent of renal insufficiency in patients with cirrhosis.

**Key words:** liver cirrhosis, alcoholic; hepatorenal syndrome; diagnosis; prognosis; arginine; chromatography, high pressure liquid.

### Apstrakt

**Uvod/Cilj.** Hroničnim konzumiranjem alkohola u dužem vremenskom periodu razvija se ciroza jetre sa smanjenjem metaboličke funkcije jetre i poremećajima metabolizma arginina. Hepatorenalni sindrom (HRS) je najteža komplikacija ciroze jetre. Cilj ove studije bio je da se analiziraju poremećaji metabolizma arginina praćenjem koncentracija asimetričnog dimetilarginina (ADMA) i simetričnog dimetilarginina (SDMA) kod bolesnika sa cirozom jetre i HRS. **Metode.** Istraživanjem su bile obuhvaćene tri grupe ispitanika: grupa bolesnika sa cirozom i HRS (24 bolesnika), grupu bolesnika sa cirozom bez HRS (18 bolesnika) i kontrolna

grupa od 42 zdrava dobrovoljna davaoca krvi. Svim ispitanicima merene su koncentracije ADMA, SDMA, i L-arginina u plazmi, korišćenjem metode tečna hromatografija pod visokim pritiskom (HPLC). **Rezultati.** Koncentracija SDMA bila je znatno viša, kod bolesnika sa HRS, u odnosu na bolesnike bez HRS, i viša nego kod zdravih ispitanika ( $1.76 \pm 0.3 \mu\text{mol/L}$ ;  $1.01 \pm 0.32 \mu\text{mol/L}$  i  $0.52 \pm 0.18 \mu\text{mol/L}$ , respektivno;  $p < 0.01$ ). Koncentracija ADMA bila je viša kod bolesnika sa HRS nego kod bolesnika bez HRS. Koncentracije ADMA kod bolesnika sa i bez HRS bile su više nego kod zdravih ispitanika ( $1.35 \pm 0.27 \mu\text{mol/L}$ ,  $1.05 \pm 0.35 \mu\text{mol/L}$  i  $0.76 \pm 0.21 \mu\text{mol/L}$ , respektivno,  $p < 0.01$ ). Kod bolesnika u terminalnoj fazi alkoholne ciroze jetre, koncentracije ADMA i SDMA korelirale su sa progresom ciroze jetre kao i sa razvojem komplikacija ciroze. Kod bolesnika sa HRS postojala je pozitivna korelacija između kreatinina i SDMA u plazmi ( $r^2 = 0.0756$ ,  $p < 0.001$ ) koja nije pronađena između kreatinina i ADMA. **Zaključak.** Dobiveni rezultati demonstriraju da povećanje koncentracije SDMA u plazmi je proporcionalno progresiji hronične štete jetre i bubrega. Povećanje koncentracije ADMA može biti uzročnik bubrežne insuficijencije kod bolesnika sa cirozom jetre.

tracije ADMA i SDMA korelisale su sa stepenom progresije ciroze, kao i sa razvojem komplikacija ciroze. Kod bolesnika sa HRS postojala je pozitivna korelacija između kreatinina i SDMA u plazmi ( $r^2 = 0,0756$ ,  $p < 0,001$ ), ali ne i između kreatinina i ADMA. **Zaključak.** Dobijeni rezultati pokazuju da je povećanje koncentracije SDMA srazmerno progresiji hroničnog oštećenja jetre i bubrega. Porast koncentracije

ADMA može biti uzročni faktor razvoja bubrežne insuficijencije kod bolesnika sa cirozom jetre.

#### **Ključne reči:**

**jetra, bolesti izazvane alkoholom; hepatorenalni sindrom; dijagnoza; prognoza; amino kiseline, esencijalne; hromatografija, tečna, pod vp.**

### **Introduction**

Hepatorenal syndrome (HRS) is a potentially reversible syndrome which develops in conditions of chronic insufficiency and liver cirrhosis. It is characterized by renal dysfunction and severe changes in systemic circulation. Reduction in renal function is a consequence of reduction in blood circulation through the kidneys. It is manifested by the reduction in glomerular filtration. Renal failure is caused by the activation of specific vasoconstrictor systems involving activation of the sympathetic system, renin-angiotensin system and vasopressin<sup>1</sup>. Based on the new consensus concerning the definition, diagnosis and treatment of HRS, the International Ascites Club has established a new criteria for the diagnosis of HRS<sup>2</sup>.

Dimethylarginins are formed by transmethylation modification, *via* reaction of enzyme protein methyltransferase (PRMT) on the remainder of arginine. During transmethylation, S-adenosylmethionine, which is a methyl group donor, transforms into S-adenosylhomocysteine. The methylated arginine remainder forms asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) by proteolysis. The main metabolic pathway of ADMA takes place in the liver. ADMA is hydrolyzed by the action of the enzyme dimethylarginine dimethylaminohydrolase (DDAH) on citrulline and dimethylamine. A small part of ADMA enters the circulation and is removed as such through the kidneys<sup>3</sup>. ADMA is a direct inhibitor of the enzyme nitric oxide (NO) synthase, which participates in NO synthesis. NO participates in the maintenance of vascular tonus. Increased concentration of ADMA in blood of patients with decompensated liver cirrhosis reduces the synthesis of NO, whereby intrahepatic vascular resistance is increased<sup>4</sup>. Compared to ADMA, SDMA has indirect inhibitory effect on NO synthase. SDMA can disturb the synthesis by competing in the transport against L-arginine on the level of cell membrane<sup>5-7</sup>.

Some studies<sup>8</sup> have demonstrated that increased ADMA level in blood of patients with decompensated liver cirrhosis is probably the result of DDAH enzyme activity exhaustion. Increased level of ADMA has a causative role in the development of HRS. Accumulation of ADMA in patients with liver cirrhosis causes liver damage. Accumulation of ADMA inhibits NO synthase thereby causing vasoconstriction of the kidney blood vessels. Thus, blood flow through the kidney is interrupted, in other words, glomerular filtration is reduced and SDMA is retained in the kidney. Compared to ADMA, SDMA is not broken down by the action of DDAH enzyme but is excreted as such through the kidneys<sup>9,10</sup>.

In patients with alcoholic cirrhosis increased level of ADMA is strongly correlated with the severity of liver disease, in accordance with the reduction of its metabolic function. However, the level of SDMA in plasma is within normal values in patients with alcoholic cirrhosis. The levels of dimethylarginine in plasma in HRS are not known. Therefore, the aim of this study was to estimate the level of dimethylarginine (ADMA and SDMA) in plasma, in patients with cirrhosis and HRS, as well as in patients with cirrhosis without HRS.

### **Methods**

The study included two groups of subjects the target and control group. The group with cirrhosis consisted of 42 patients classified according to the presence of HRS. The patients were all male, aged 25 to 70 years, with an average age of  $53.13 \pm 23$  and with history of more than ten years of alcohol abuse. All patients were in terminal stage of alcoholic cirrhosis with moderate to severe ascites. HRS was diagnosed in 24 patients with cirrhosis while 18 patients had no HRS. The control group consisted of 42 healthy examinees who were voluntary blood donors. All examinees in the control group were males, average age  $50.76 \pm 9.7$  years with normal laboratory findings.

The diagnosis of cirrhosis was established in all the patients based on clinical, biochemical and ultrasound findings as well as liver biopsy. All the patients had moderate to severe ascites. The presence of ascites was confirmed by diagnostic paracentesis. HRS was diagnosed in accordance with the latest criteria, suggested by the International Ascites Club<sup>3</sup>. The criteria included: cirrhosis with ascites, low glomerular filtration rate, serum creatinine above  $133 \mu\text{mol/L}$  (above  $1.5 \text{ mg/dL}$ ), proteinuria below  $500 \text{ mg/day}$ , the absence of shock, the absence of bacterial infection, loss of fluid, poor kidney function after discontinuing diuretic treatment (serum creatinine remains at  $\geq 133 \mu\text{mol/L}$  after at least 48 hours following the application of albumin dose of  $1$  to  $100 \text{ g/kg}$  a day), treatment without nephrotoxic drugs, the absence of parenchymal kidney disease (patient has no proteinuria  $> 500 \text{ mg/day}$ , no microhematuria  $> 50$  erythrocytes, as well as pathologic findings from echosonographic examination of the kidneys).

The study was conducted in accordance with the ethical standards of the Committee on Human Experiments or with the Declaration of Helsinki from 1975, revised in 1983. The study was prospective, in accordance with the Ethics Committee and it was conducted after obtaining consent from all the patients.

General biochemical parameters were obtained from patients' serum using standard biochemical methods of the International Federation of Clinical Chemistry (kinetic spectrophotometric methods performed on multichannel biochemical analyzer OLYMPUS AU680). Arginine and dimethylarginine were determined in all three groups using high pressure liquid chromatography (HPLC) method<sup>11</sup>. The method was performed in the following way: 50  $\mu$ L of monomethylarginine as an internal standard (IS) was added to 0.2 mL plasma. This mixture was used in various phases in SPE cartridges with previous activation with 1 mL of methanol and 2 mL, of trichloroacetic acid (TCA) 2%. After washing cartridges (1 mL TCA 2%; 1 mL phosphate buffer – 150 mmol/L, pH 8.0; 1 mL methanol), amino acids were eluted with 1.2 mL of 2% triethylamine dissolved in 70 : 30 solution of methanol / water. Eluates were dried in nitrogen, afterwards an aliquot of 0.4 mL buffer was added to dry resi-

sion analysis and goodness of fit analysis, as well as Pearson correlation coefficient.

## Results

Liver damage was more severe in the HRS group. This is manifested by de Ritis coefficient which was significantly greater in HRS patients, compared to patients without HRS and healthy examinees. Synthetic liver function, measured by albumin concentration, was significantly decreased in patients with cirrhosis without HRS. Excretory liver function, measured by bilirubin concentration, was significantly reduced in the patients with HRS as compared to the patients without HRS and healthy examinees. Kidney function parameters were considerably increased in the patients with HRS as compared to the patients without HRS and the healthy control (Table 1).

Table 1

Basic demographic, clinical and biochemical characteristics of the patients and healthy examinees

Parameters	Cirrhosis with HRS	Cirrhosis without HRS	Healthy controls
Males/females (n)	18/ 0	24/ 0	42/ 0
Age (years)	55.83 $\pm$ 12.41	50.58 $\pm$ 11.08	50.76 $\pm$ 9.7
AST/ALT (U/L)	3 $\pm$ 1.4*	1.8 $\pm$ 0.6*	1.0 $\pm$ 0.2
Albumine (g/L)	25 $\pm$ 5.6*	27.1 $\pm$ 6.6*	40.9 $\pm$ 4.2
Total bilirubine ( $\mu$ mol/L)	115.8 $\pm$ 81.1*	54.7 $\pm$ 35.8*	5.7 $\pm$ 1.9
Indirect bilirubine ( $\mu$ mol/L)	61.0 $\pm$ 46.0*	29.9 $\pm$ 16.6*	5.1 $\pm$ 1.5
Direct bilirubine ( $\mu$ mol/L)	54.7 $\pm$ 38.4*	24.8 $\pm$ 21.2*	0.6 $\pm$ 0.5
Urea (mmol/L)	15.05 $\pm$ 7.8*	6.7 $\pm$ 3.2*	4.58 $\pm$ 1.5
Creatinine (mmol/L)	218.6 $\pm$ 109.6*	94.5 $\pm$ 17.2	84.4 $\pm$ 15.2

Data are presented as n/n or mean $\pm$ SD; \* $p$  < 0.01 vs. other groups  
HRS – hepatorenal syndrome; AST – aspartate aminotransferase; ALT – alanine aminotransferase

due (mobile phase A). Analysis was followed by derivatization with ortho-phthalaldehyde (0.1 mL, 2 min). For performing the HPLC method, a fluorescent detector ( $\lambda_{ex}$  340 nm,  $\lambda_{em}$  445 nm; photomultiplier (PMT) is 12 between 2.60 and 4.20 min.) was necessary as well as Zorbax SB-C18 column (150  $\times$  4.6 mm, 3.5  $\mu$ m). The basic separation of methylarginine was achieved by gradient between mobile phase A (sodium phosphate buffer- 40 mmol/L, pH 6.2) and phase B (methanol). The analysis began with 32% phase B for 4.0 minutes. It was then followed by mobile phase B which grew linearly up to 100% in the next 0.5 min. The whole process of washing a column lasted up to 7.5 min. After that, the content of the mobile phase returned to starting conditions. The whole process lasted for 9 minutes. Analysis of L-arginine was also performed within the process.

Readings for arginine were 2.90 min, for IS 3.50 min, for ADMA 3.85 min and SDMA 4.10 min. The concentrations of L-arginine, ADMA and SDMA in the samples were determined in comparison with the standards.

Data were entered in MS Excel. For analysis of data the statistical program SPSS 17.0 was used. The data were represented as mean values  $\pm$  SD [95% confidence interval (CI) for medium]. The data were compared among the groups using the ANOVA test. Post hoc analysis by using Dunnett's T3 and Tuckey test was also used. Statistically significant difference was accepted with  $p$  < 0.01 risk. The ratio between the tested variables was determined by linear regres-

sion analysis and goodness of fit analysis, as well as Pearson correlation coefficient. In cirrhosis with reduced kidney function the level of ADMA in serum rose with the increase in creatinine level (Pearson correlation coefficient,  $C = 0.45$ ). With the manifested renal insufficiency in HRS this correlation was lost and the level of ADMA was relatively constant as compared to increase in creatinine (Figure 1).

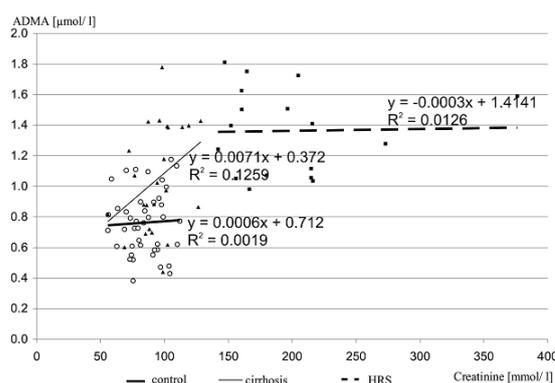
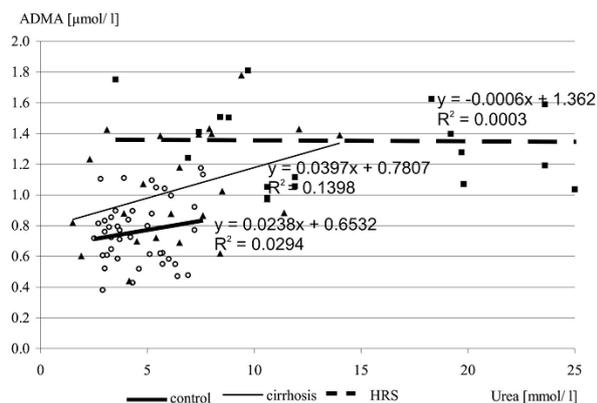


Fig. 1 – Analysis of the correlation and linear regression equation between asymmetric dimethylarginine (ADMA) and serum creatinine in the tested groups

HRS – hepatorenal syndrome

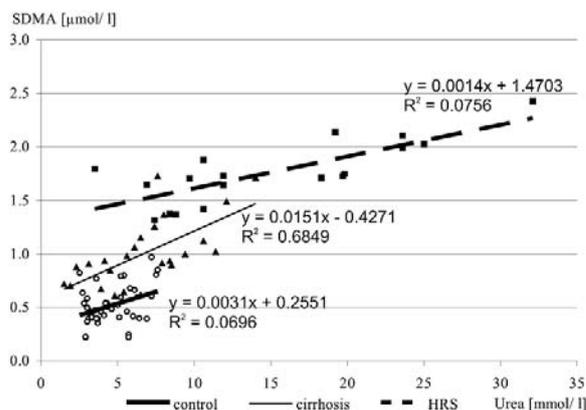
In the group with cirrhosis, the increase in ADMA was followed by the increase in urea which was in accordance with the reduced metabolic and detoxification liver function

(C = 0.36). Further on, the level of ADMA was relatively constant (Figure 2).



**Fig. 2 – Analysis of the correlation and linear regression equation between asymmetric dimethylarginine (ADMA) and serum urea in the tested groups**  
HRS – hepatorenal syndrome

In cirrhosis, the level of SDMA in serum rose with the increase in creatinine level (C = 0.83). With manifested renal insufficiency in HRS this correlation continued (C = 0.43,  $p < 0.05$ ) (Figure 3).



**Fig. 4 – Analysis of the correlation and linear regression equation between symmetric dimethylarginine (SDMA) and serum urea in the tested groups**  
HRS – hepatorenal syndrome

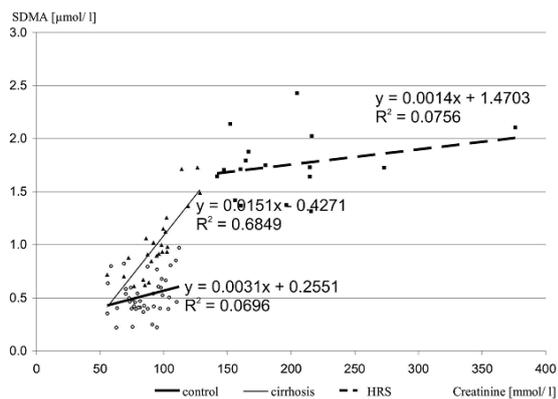
The lowest values of arginine were obtained in the group with cirrhosis and HRS. ADMA and SDMA were the highest in patients belonging to the groups with cirrhosis with and without HRS (Table 2).

**Table 2**

Arginine and dimethylarginine in tested groups			
Parameters	Cirrhosis with HRS	Cirrhosis without HRS	Healthy controls
Arginine (µmol/L)	60.69 ± 16.8*	79.17 ± 21.7	91.98 ± 27.6
ADMA (µmol/L)	1.35 ± 0.27*	1.05 ± 0.35*	0.76 ± 0.21
SDMA (µmol/L)	1.76 ± 0.3*	1.01 ± 0.32*	0.52 ± 0.18
ADMA/ SDMA	0.79 ± 0.2*	1.09 ± 0.36*	1.58 ± 0.48

Data are presented as mean ± SD; \* $p < 0.01$  vs. other groups

HRS – hepatorenal syndrome; ADMA – asymmetric dimethylarginine; SDMA – symmetric dimethylarginine



**Fig. 3 – Analysis of the correlation and linear regression equation between symmetric dimethylarginine (SDMA) and serum creatinine in the tested groups**  
HRS – hepatorenal syndrome

In the group with cirrhosis, with and without HRS, the level of SDMA was increased with increased urea level (C = 0.66 and C = 0.78, respectively;  $p < 0.01$ ). The increase was more intense in the group with HRS (Figure 4).

### Discussion

ADMA and SDMA are arginine metabolites which have great influence on liver and kidneys damage in chronic alcoholism. In order to prevent the progress of damage and development of HRS, it is important to maintain their levels within normal. The liver has an important role in ADMA metabolism. Great amounts of ADMA from systemic circulation are broken down in the liver by the effect of DDAH enzyme. In this study, in the group of patients with cirrhosis, high values of ADMA were obtained as compared to values in control group. High levels of ADMA appear to be caused by the reduction in metabolic function of the liver. Thereby, ADMA may represent a marker for the degree of liver damage<sup>12, 13</sup>. Some studies have shown that increase in the level of ADMA is followed by increase in liver damage. Some results have shown that two groups of patients with different clinical pictures and similar pathologic conditions of the liver have different levels of ADMA. By direct inhibition of NO synthase, ADMA influences the NO deficiency. This leads to vasoconstriction in liver sinuses which causes the develop-

ment of ischemia in the liver and destruction of hepatocytes<sup>13</sup>.

The results obtained in this study, show that in the group of patients with cirrhosis, along with increase in urea, the level of ADMA increased as well. This was confirmed by the reduction in detoxification function of the liver. In this group, the level of ADMA was increased along with the increase in creatinine level which means that ADMA level can be a marker for impending kidney insufficiency. Some studies have shown that high concentration of ADMA in plasma, developed in cirrhosis, can be biologically effective in blood vessels of the kidneys<sup>14, 15</sup>. It is therefore believed that increased concentration of ADMA in liver dysfunction may have an important role in the development of kidney insufficiency in patients with cirrhosis. Some researches<sup>16</sup> have shown that increased concentration of ADMA can cause vasoconstriction effects in the kidney as well as cerebral arteries. Besides, by direct inhibition of NO synthase in the endothelium of renal blood vessels, ADMA causes vasoconstriction in the kidney. This leads to the reduction in glomerular filtration and damage in kidney function. Damage in kidney function causes the retention of SDMA<sup>17-19</sup>. The results of this study show no correlation between urea and ADMA, as well as creatinine and ADMA in the group of patients with cirrhosis with HRS. The level of ADMA is relatively constant which points out that the kidney has no significant effect on ADMA catabolism. The results also show a considerable increase in concentration of ADMA in the groups with cirrhosis with and without HRS. Therefore, ADMA may represent a marker for kidney and liver damage.

Compared to ADMA, SDMA is not broken down in the liver. It was catabolized in the kidney tissue and eliminated by urine. In the group of patients with cirrhosis, a significant correlation between SDMA level and urea may suggest a reduced liver detoxification function. Some studies have shown that in patients with cirrhosis but with normal range kidney function, SDMA in plasma does not correlate with clinical signs of liver damage. The values of SDMA also remain within normal range in patients with terminal phase of liver damage before liver transplantation<sup>20</sup>. The results of this study show a significant positive correlation between SDMA and creatinine in patients with cirrhosis. This shows that SDMA may be a marker for impending kidney insufficiency. In the group of patients with cirrhosis and HRS a significant correlation between SDMA and urea was demonstrated. This may point out to the severity of kidney insufficiency. In this group there was also a continued positive cor-

relation between creatinine and SDMA, which may demonstrate the significant role of kidney tissue in the catabolism of SDMA. The results demonstrate that in the group of patients with cirrhosis, the concentration of SDMA is approximately two times higher than in the control group. In the group of patients with cirrhosis and HRS, the concentration of SDMA is approximately three times higher as compared to the control group. These results may point to the role of kidneys in SDMA catabolism. Similar results were obtained in other studies and they confirm the role of kidneys in the catabolism and elimination of SDMA<sup>21</sup>. It has also been demonstrated that the concentration of SDMA was returned to a normal value after kidney transplantation<sup>22</sup>. Some studies<sup>20</sup> have demonstrated that the increased level of SDMA may not be in correlation with clinical course of kidney damage in patients with terminal phase of liver cirrhosis before liver transplantation.

The obtained results demonstrated that the concentration of arginine decreases, which was followed by an increase in the concentration of ADMA and SDMA. In the group of patients with cirrhosis and HRS, the concentration of arginine had the lowest values, which may point to a significantly reduced liver function and severe kidney insufficiency.

### Conclusion

In patients with alcoholic cirrhosis, in line with reduced metabolic liver function, ADMA may be a marker for the degree of chronic alcoholic liver damage.

In patients with cirrhosis, ADMA, as well as SDMA could be markers for kidney insufficiency development. Accumulation of ADMA in plasma causes kidney vasoconstriction and thereby retention of SDMA.

Considering that ADMA has several damaging effects, it can be concluded that modulation of the activity of enzyme which participates in ADMA catabolism may represent a new therapeutic goal which is intended to reduce the progress of liver and kidney damage and thus the development of HRS.

Further research should be directed toward establishing the referential values of ADMA and SDMA for liver and kidney damage.

### Aknowledgments

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