

## Plasma Adrenomedullin among Patients with Chronic Liver Diseases

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### Abstract

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**Background and Aim:** Adrenomedullin (AM) is a potent vasodilator peptide and it has been implicated as one of the mediator of the vasodilatory state in liver cirrhosis. The aim of the study was to determine the level of AM in patients with chronic hepatitis and liver cirrhosis and comparing them with healthy controls.

**Methods:** Plasma concentration of adrenomedullin was measured by enzyme immunoassay in 11 patients with chronic hepatitis and 27 patients with liver cirrhosis and 16 subjects as controls. Patients were subjected to clinical, laboratory and ultrasonography examination.

**Results:** Of the 27 patients with liver cirrhosis; 12 patients had ascites and 13 patients had esophageal varices, and all patients had positive schistosoma antibodies. The mean levels of AM were higher in patients with chronic hepatitis and patients with liver cirrhosis compared to controls ( $0.571 \pm 0.197$  ng/ml,  $0.516 \pm 0.178$  ng/ml and  $0.303 \pm 0.065$  ng/ml, respectively;  $p < 0.001$ ). There was no significant difference in AM levels between patients with and without ascites ( $0.45 \pm 0.16$  ng/ml and  $0.56 \pm 0.19$  ng/ml respectively,  $p = 0.1$ ) and patients with and without esophageal varices ( $0.45 \pm 0.19$  ng/ml and  $0.57 \pm 0.17$  ng/ml respectively,  $p = 0.07$ ).

**Conclusion:** Chronic hepatitis and liver cirrhosis were associated with increase AM levels in blood. The increased AM in chronic hepatitis may denote that hemodynamic abnormalities start before the development of overt cirrhosis.

### Introduction

Adrenomedullin (AM) is a potent, long lasting vasodilator. Originally, it has been isolated from the extracts of human pheochromocytoma, it is expressed in adrenal glands, lungs, kidneys, smooth muscle cells and splanchnic organs [1]. Experimentally, acute administration of AM reduces the systemic arterial pressure and chronic infusion reduces the vascular resistance and increases the blood flow in the systemic and splanchnic circulation [2]. Liver cirrhosis is associated with circulatory

disturbances in the form of arterial hypotension, high cardiac output, and low vascular resistance. These circulatory disturbances are attributed to arterial vasodilatation that results from overproduction or reduced degradation of vasodilator substances and is most pronounced in the splanchnic area [3]. Several studies reported increase circulating AM in patients with liver cirrhosis and the increase is more pronounced in decompensated cirrhosis [1,4].

It has been found that AM is responsible for the

arteriolar vasodilatation and hyperdynamic circulation in liver cirrhosis. Also the increase in the renin-angiotensin-aldosterone and sympathetic nervous systems associated with functional renal impairment in liver cirrhosis is related to the increase of circulating plasma AM [5]. In this study, we aimed to determine the level of AM in patients with chronic hepatitis and liver cirrhosis and comparing them with healthy controls.

## Patients and Methods

A case control study was conducted in period from May to August 2008 at internal medicine and hepatology clinics of medical service unit in National Research Center of Egypt. The patients group consisted of 38 patients suffering from chronic liver diseases (32 males and 6 females, their ages ranged from 24 to 67 years, mean:  $47.9 \pm 10.3$  years). Sixteen healthy subjects (13 males and 3 females, their ages ranged from 23 to 61 years, mean:  $46.7 \pm 10.8$  years) served as controls.

All patients were subjected to full history taking including history of alcohol or drug abuse and past history of schistosomiasis infection. All patients were assessed clinically, laboratory including virological tests for hepatitis C virus (HCV) and hepatitis B virus (HBV) and schistosoma antibodies. Abdominal ultrasound and upper gastrointestinal endoscope were also done for all patients.

Liver cirrhosis was diagnosed clinically (encephalopathy, ascites) and/or radiological evidence of portal hypertension and/or endoscopic finding of esophageal varices (OV).

Patients with systemic hypertension, cardiopulmonary disease, renal disease or acute infection were excluded from the study.

All patients gave informed consent and the study was approved by the Institutional Ethical Committee.

## Detection of Adrenomedullin

Blood samples for AM-like immunoreactivity were collected into the vacutainer tubes, which contained EDTA. Blood was transferred from the vacutainer tubes to centrifuge tubes containing aprotinin (0.6 TIU/ml of blood) and gently rocked for several times to inhibit the activity of proteinases. Blood was centrifuged at 1 600 G for 15 min at 4°C. AM-like immunoreactivity concentration was measured by enzyme immunoassay (Phoenix Pharmaceuticals Inc. Harbor Boulevard, Belmont, California

94002) after extraction through the Sep-pak C-18 column supplied by the manufacturer [6]. Plasma in both parameters was immediately frozen and stored at -80°C until assayed.

## Statistical Methods

Data are presented as means  $\pm$  SD and percentages. The compiled data were computerized and analyzed by EPI Info version 6.2 produced through the collaboration between CDC/WHO and by SPSS PC+, version 14. The following tests of significance were used: analysis of variance (ANOVA) test between more than two means with post hoc test LSD to compare mean values between each two groups after ANOVA, t test between means to analyze mean difference. A level of significance with  $p < 0.05$  was considered significant,  $p < 0.001$  was considered highly significant and  $p > 0.05$  was considered insignificant. Multiple correlation coefficients  $\{r\}$  were used to determine the correlation of the studied parameters to each other.

## Results

In the current study, it was found that the cause of liver disease was hepatitis C virus in 34 patients (89.5%) and hepatitis B in four patients (10.5%). All patients were positive for schistosoma antibodies.

**Table 1: General characteristics of the studied patients.**

Variable	Value
Age, years (mean $\pm$ SD)	47.9 $\pm$ 10.3
Sex (male, female)	32.6
Cause of liver disease: HCV, HBV	34.4
Liver cirrhosis n (%)	27 (71.1)
Ascites n (%)	12 (31.6))
Esophageal varices, n	13 (34.2)
Grade II, n	9
Grade III, n	3
Grade IV, n	1

HCV = hepatitis C virus; HBV = hepatitis B virus.

Table 1 shows the demographic and clinical characteristics of the studied patients. Table 2 shows the biochemical characteristics of the studied patients.

There was no significant correlation between adrenomedullin and haemoglobin,

Platelets, liver function tests as aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, serum albumin and prothrombin concentration

**Table 2: Biochemical characteristics of the studied patients.**

Variables	Studied patient N=38
Haemoglobin (g/dl)	11.9 ± 1.9
Platelets (thousands /cc)	209.792 ± 109.307
AST (IU/L)	64.1 ± 12.0
ALT (IU/L)	63.7 ± 31.5
Serum bilirubin (mg/dl)	1.6 ± 0.72
Serum albumin (g/dl)	3.2 ± 0.85
INR (mean ± SD)	0.94 ± 0.24

AST = aspartate aminotransferase; ALT = alanine aminotransferase; INR = international randomized ratio.

The mean values of adrenomedullin were significantly higher among patients with liver cirrhosis (0.516 ± 0.178 ng/ml) and patients with chronic hepatitis (0.571 ± 0.197 ng/ml) compared to controls (0.303 ± 0.065 ng/ml), F=12.1, p<0.001 and no significant difference between chronic hepatitis and cirrhosis (Figure 1).

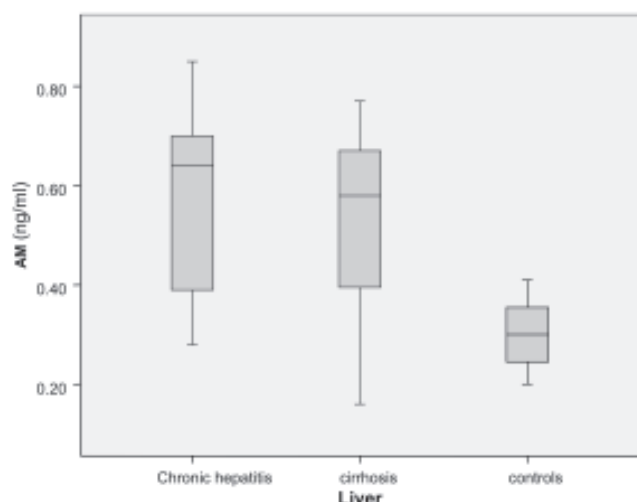


Figure1: Mean values of adrenomedullin in patients and controls.

LSD test showed high significant differences between cirrhotic patients and control, p<0.001 and between chronic hepatitis patients and control, p<0.001.

**Table 3: AM in patients with and without ascites and with and without esophageal varices (OV).**

Group	Adrenomedullin	P value
Patients with ascites (n=12)	0.45 ± 0.16 ng/ml	0.10
Patients without ascites (n=26)	0.56 ± 0.19 ng/ml	
Patients with OV (n=13)	0.45 ± 0.19 ng/ml	0.07
Patients without OV (n=25)	0.57 ± 0.17 ng/ml	

The presence of ascites or esophageal varices did not affect the mean values of AM as shown in Table 3.

## Discussion

Low systemic vascular resistance due to arterial vasodilatation is a hemodynamic feature of human and experimental liver cirrhosis. This vasodilation has been suggested to be a major pathogenic mechanism for hyperdynamic circulation characterized by arterial hypotension, hypervolemia, and high cardiac output [7]. Adrenomedullin is a potent vasodilator peptide derived from vascular endothelium and smooth muscle cells; it has been implicated as one of the mediator of the vasodilatory state in liver cirrhosis. Adrenomedullin elicits its vasodilator effects through two different mechanisms: a direct effect on vascular smooth muscle cells to increase intracellular cAMP by stimulating AM receptors and adenylate cyclase activity and an indirect effect on vascular endothelial cells by stimulating Ca<sup>2</sup> mobilization to increase endothelium-derived nitric oxide release via the stimulation of constitutive nitric oxide synthase [8]. Several previous studies reported increase circulating AM in patients with liver cirrhosis compared to controls [1,4].

In the present study, AM was found to be elevated in patients with chronic hepatitis and liver cirrhosis compared to healthy controls. However there was no significant difference between chronic hepatitis and liver cirrhosis. To the best of our knowledge, this is the first study that assessed the level of AM in patients with chronic hepatitis before the development of cirrhosis. The increase levels of AM in chronic hepatitis, indicates that the hemodynamic abnormalities associated with liver cirrhosis start earlier before overt cirrhosis develops. This may have important implication in the treatment of patients with chronic hepatitis.

AM is overproduced by various stimuli including endotoxin, cytokines, vasoactive substances and/or shear stress [9,10], which are enhanced in liver cirrhosis [11,12].

It was suggested that increased cytokines such as TNF-alfa and IL6 in liver cirrhosis is the stimulus for the production of AM by vascular smooth muscle and endothelial cells [13]. Human hepatic stellate cells (HSC), a perisinusoidal pericytes, secrete AM under basal conditions and this secretion is markedly increased by cytokines, also HSCs have functional receptors for AM, the stimulation of which blunts the contractile effect of endothelin-1 [14]. In liver cirrhosis, subclinical endotoxemia

may occur in portosystemic shunts that allow microorganisms and endotoxins from the intestine to bypass liver kupffer cells and enter systemic circulation and endotoxins increase plasma levels of AM [10,15]. The initial cause of portal hypertension in liver cirrhosis is increased hepatic outflow resistance which stimulates shear stress. Previous study reported that AM mRNA expression in endothelial cells was markedly increased by shear stress [16].

In the present study the mean values of AM were not related to presence or absence of ascites or of esophageal varices. This is in contrast to several previous studies that reported that AM is higher in patients with liver cirrhosis and ascites than in those without ascites [1,5]. The contradictory results between our study and the previous studies may be due to schistosoma infection in our patients. Schistosoma affects blood vessels through granuloma formation around radicals of portal veins causing perisinusoidal portal hypertension without affecting liver parenchyma [17].

## Conclusion

Chronic hepatitis and liver cirrhosis are associated with increase AM levels in blood. The increased AM in chronic hepatitis indicate that hemodynamic abnormalities start before the development of overt cirrhosis.

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