RESEARCH COMMUNICATION

Elevated Renin Levels in Patients with Liver Cirrhosis and Hepatocellular Carcinoma

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Abstract

Liver fibrosis is the common consequence of chronic liver injury of any etiology, disrupting the normal architecture,and causing hepatocellular dysfunction and portal hypertension. Since the renin-angiotensin system (RAS) may be involved in chronic liver diseases, in the present study we assayed renin levels using ELISA in groups of Egyptian patients with liver cirrhosis (N=32) and hepatocellular carcinoma (HCC) (N=67), for comparison with twenty five healthy controls. The results showed significant differences between the control and liver cirrhosis patients (*P*<0.001) and also the controls and HCC patients (*P*<0.001), without significant variation between the patient groups. Furthermore, in HCC patients, it was found that the renin levels negatively correlated with serum albumin and prothrombin time (*P*=0.003 for each) and positively with α-fetoprotein (*P*=0.04). Thus, it is concluded that renin levels are elevated in patients with liver cirrhosis and HCC and suitable medical intervention should be placed for management of such alteration. Moreover, further studies are warranted to explore its prognostic significance.

Keywords: Renin - liver cirrhosis - hepatocellular carcinoma - Egypt

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Introduction

The renin-angiotensin System (RAS) is mainly composed of angiotensinogen, renin, angiotensin (Ang) I, angiotensin converting enzyme (ACE), Ang II, and Ang II receptors. This system has been categorized to systemic RAS and local RAS (de Gasparo et al., 1995). The former system regulates systemic circulation and body fluid homeostasis. On the other hand, local RAS is involved in the microcirculatory dynamics of the heart, kidney, and brain. Local RAS is considered to play an important role in regulating pathophysiology of ischemia and reperfusion (I/R) injury in these organs (Campbell, 1987; Zimmerman &Dunham, 1997). Renin of renal origin plays an important role in the control of blood pressure by cleaving angiotensinogen into angiotensin I. It is synthesized as a preprorenin that is converted to prorenin and then into active mature renin by proteolytic cleavage (Panthier et al., 1982). In nephrectomized rats, circulating active renin falls to undetectable levels (Menard & Catt, 1972), indicating that most or all of the circulating active renin is of renal origin. However, prorenin is still readily detectable and even increases in the plasma of nephrectomized rats (Doi et al., 1984). These data suggest that some extra-renal tissues also express the renin gene. Studies using renin activity assays of whole tissue extracts have detected renin-like activity in many different extra-renal tissues such as submaxillary glands, uterus, placenta, brain, anterior pituitary, testis, and adrenals (Gross et al., 1964; Ganten et al., 1971; Cohen et al., 1972; Hirose et al., 1978; Craven & Symonds 1979; Hirose et al., 1982; Naruse & Inagami, 1982; Warren et al., 1982; Naruse et al., 1983; 1984; Paul et al., 2006).

Liver fibrosis is the common consequence of chronic liver injury of any etiology (Albanis & Friedman, 2001). Advanced liver fibrosis disrupts the normal liver architecture, causing hepatocellular dysfunction and portal hypertension. The hepatic stellate cells (HSCs) are a major fibrogenic cell type in the liver (Bataller & Brenner, 2001). Following injury, HSCs are activated to myofibroblast-like cells, which promote collagen deposition. There is an evidence indicates that RAS may be an important mediator in liver fibrosis and a local renin angiotensin system is upregulated in experimental hepatic fibrogenesis (Asbert et al., 1992; Paizis et al., 2002). The response of repair by fibrosis is common to chronic disease processes in many organs, almost regardless of etiology, giving rise, for example, to cirrhosis in the liver, glomerulosclerosis in the kidney and interstitial fibrosis in the lung (Border & Noble, 1994; Weber, 1997). In the liver, the key event in the initiation of fibrosis is activation of the stellate cell, a process characterized by de novo expression of

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α-smooth muscle actin and transformation to a cell of myofibroblastic phenotype that produces a range of prosclerotic cytokines and matrix proteins (Friedman, 1993). With progressive fibrosis, there are changes in both the quantity and quality of hepatic extracellular matrix with increased formation of fibrillar, scar associated matrix proteins such as type I collagen. The generation of Ang II by a local, as distinct from the systemic RAS (Deschepper et al., 1986; Dzau et al., 1987), may be an integral part of the general response of tissues to injury (Gilbert et al., 1999). The finding that activated human stellate cells possess Ang II type 1 receptors and proliferate and contract in vitro in response to Ang II (Bataller et al., 2000) provides support to the hypothesis that in the liver, as in other organs, Ang II may play an important role in fibrogenesis (Naruse et al., 1983), acting via Ang II type 1 receptors on stellate cells. In the current study, we aimed to assay the renin levels using ELISA in Egyptian patients with liver cirrhosis and hepatocellular carcinoma.

Materials and Methods

Study subjects

The present study covered 32 liver cirrhosis and 67 hepatocellular carcinoma patients from the Gastroenterology Surgery Centre, Mansoura University, Mansoura, Egypt. Twenty five healthy controls were included for comparison purposes. Patients with RAS related pathological syndromes such as primary hyperaldosteronism, secondary hyperaldosteronism, Addison's disease, essential hypertension, hemorrhage, hypokalemia, malignant hypertension, renin-producing renal tumors (Bartter's syndrome), renovascular hypertension, salt-retaining steroid therapy, ADH therapy, and pregnancy were excluded.

All patients and controls were subjected to thorough history-taking; complete clinical examination; abdominal ultrasound; and laboratory investigations, including total bilirubin (TB), direct bilirubin (DB), total protein (TP), serum albumin (S. Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum creatinine. Hepatitis B surface antigen (HBsAg), anti-HCV antibodies were detected by ELISA (Diasorium kit; Diasorium SR, Italy) and RT-PCR for HCV RNA (Amplicor PCR; Roche Molecular Systems, Inc., Pleasanton, Calif., USA). Liver cirrhosis and HCC patients were strictly positive for anti-HCV and HCV RNA and/or HBV. On the other hand, subjects of the control group were free of both HCV and HBV. Liver biopsy was performed for the patient groups only. Two pathologists did the histopathological assessment separately, with no awareness of clinical data, and then a consensus between them was made on discordant assessments.

The study protocol respected the most recent Declaration of Helsinki, and all the patients or their guardians gave consent to the use of their samples and clinical data for research purposes after being informed about the nature of the study.

Estimation of renin level using ELISA

Blood for renin level determination was obtained between 8 and 10 AM after at least 0.5h of sitting or standing; it was prepared and tested using enzyme linked immunosorbant assay (ELISA). Patients were advised to avoid highly salted foods for five days preceding specimen collection. Thereafter, no dietary advice preceded collection (Alderman et al., 2004). The method described by us earlier (Abdel-Aziz et al., 2009) was followed. After optimization of reaction conditions, polystyrene microtiter plates were coated with 50 ul/well of each serum sample diluted 1:1000 in carbonate/bicarbonate buffer (pH 9.6). The plates were incubated overnight at room temperature and washed three times using 0.05% (v/v) PBS-T20 (pH 7.2) and then incubated for one hour at room temperature with 200 ul/well of 0.2% (w/v) non-fat milk in carbonate/bicarbonate buffer (pH 9.6). After washing, 50 ul/well of rabbit anti-human renin (Kind gift from Prof. Dr. E.G. Brandt, Saint Louis University, USA) diluted 1:1000 were added and incubated at 37°C for two hour. After washing, 50 ul/well of anti-human IgG alkaline phosphatase conjugate (Sigma), diluted 1:250 in PBS-T20, was added and incubated at 37°C for one hour. Excess conjugate was removed by extensive washing and the amount of coupled conjugate was determined by incubation with 50 ul/well p-nitrophenyl phosphate (Sigma) for 30 min at 37 °C. The reaction stopped using 25 ul/well of 3M NaOH and absorbance was read at 405 nm using microplate autoreader (Bio-Tek Instruments. WI, USA).

Statistical analysis

Statistical comparisons between the different groups were made by the method of Student T test or ANOVA as appropriate using the SPSS V18 software on a Windows platform (SPSS Inc., Chicago, IL) and P-values < 0.05 were deemed statistically significant.

Results

The results showed that the renin was elevated significantly in the patients with liver cirrhosis and hepatocellular carcinoma (P<0.001 for each) versus the normal control group. A non significant difference was found between the cirrhotic and the HCC groups (P>0.05). The mean renin levels were (0.23±0.04) vs. (0.39±0.08) vs. (0.41±0.12) in normal control, liver cirrhosis and hepatocellular carcinoma respectively (Figures 1&2).

In HCC patients, it was found that the renin level is elevated from Child-Pugh A to Child-Pugh B to Child-Pugh C. The mean renin levels were (0.38±0.12), (0.43±0.10), (0.49±0.12) in Child-Pugh A, Child-Pugh B, Child-Pugh C respectively. A significant difference was existed between Child-Pugh A and Child-Pugh C only (P=0.007). On the other hand, no significant difference was found between Child-Pugh A and B (P=0.1) and between Child-Pugh B and C (P=0.15) (Figures 3&4).

In HCC patients, it was found that the renin levels is negatively correlated with serum albumin and prothrombin time (P=0.003 for each) and positively correlated with the levels of α-fetoprotein (P=0.04). On the other hand no positive or negative correlation was existed between the renin levels with total protein, total bilirubin, direct bilirubin and the tumor size (P>0.05) (Table 1).

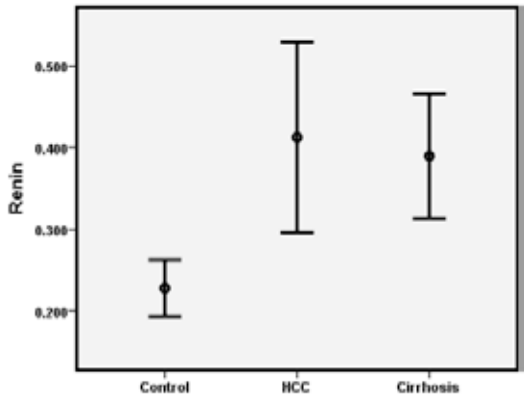

Figure 1. Mean ± SD (and error bars) for Renin in Hepatocellular Carcinoma and Liver Cirrhosis Patients Versus the Normal Healthy Controls

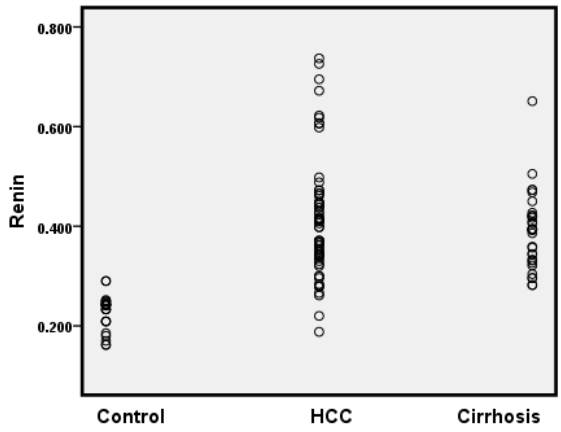

Figure 2. Scatter Plot of Individual Renin Values

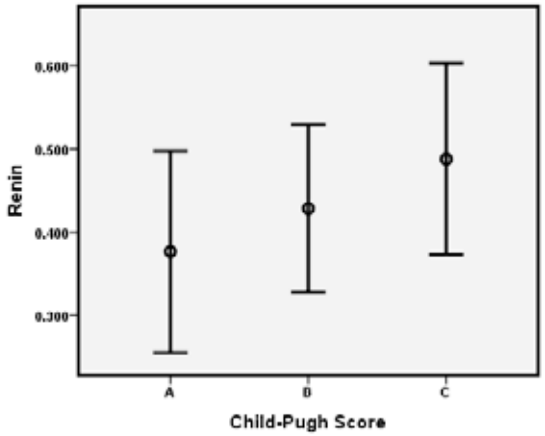

Figure 3. Mean ± SD of Renin in Hepatocellular Carcinoma Patients with Different Child-Pugh Scores

Discussion

Renin, an aspartyl proteinase involved in the regulation of cardiovascular homeostasis, exhibits tissue-specific expression. Renin is mainly produced by the juxtaglomerular cells of the kidney, where it is stored in granules and released into circulation (Laragh, 1992). Circulatory renin has a well-established role in blood pressure and volume homeostasis. On the other hand, renin is minimally expressed, or not expressed, in the liver (Fukamizu et al., 1994).

Table 1. The Correlation Between Renin Levels and the Biochemical Data in Patients with Hepatocellular Carcinoma

Renin	Pearson Correlation (R)	Significance
Prothrombin	- 0.460	0.003
Total Protein	- 0.125	0.369
Serum Albumin	- 0.391	0.003
Total Bilirubin	0.144	0.291
Direct Bilirubin	0.119	0.598
α-fetoprotein	0.254	0.041
Tumor size	- 0.008	0.954

Biochemical and histologic evidence has been established for the existence of a tissue-based RAS (local RAS) within a variety of tissues such as blood vessels, liver, kidney, spleen (Zhang et al., 2003).

In the present study, the renin level was elevated significantly in the cirrhotic patients. Plasma rennin activity was found to be increased in chronic liver disease patients (El-Raziky et al., 2005) including liver cirrhosis (Arroyo et al., 1981). Increased local RAS activity in the hepatic and splenic vessels is due to cirrhotic portal hypertension, and the synthesis of local Ang II increases, which contract the hepatic sinusoid, stimulate hyperplasia of hepatic satellite cells (HSC) and proliferation of vascular smooth muscle cells (VSMC), and also interfere with the met abolism of other vasoactive substances. All these enhance the degree of cirrhosis and portal hypertension (Zhang et al., 2003).

In the current study, the renin level was elevated significantly in HCC versus the control group and the cirrhotic patients group. No significant difference was found between the HCC patients group versus the cirrhotic patients group. Furthermore, in HCC patients, it was found that the renin levels is negatively correlated with serum albumin and prothrombin time and positively correlated with the levels of α-fetoprotein. HCC is a hypervascular tumor, and blood support comes from new branch vessels of hepatic artery (Sun & Tang, 2004). Experimental and clinical data indicate that in human HCC tumor progression is associated with angiogenesis and that an increase in microvascular density (MVD) is associated with a poor prognosis (Sun et al., 1999; Sun et al., 2006). The renin-angiotensin system is frequently activated in patients with chronic liver diseases including HCC. Perindopril, an angiotensin converting enzyme inhibitor, inhibits angiogenesis by reducing vascular endothelial growth factor (VEGF) production (Wu, 2008).

In conclusion, the renin levels are altered apparently in the patients with liver cirrhosis and HCC and suitable medical intervention should be placed for management of such alteration. Moreover, further studies are needed to explorer its prognostic significance.

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