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Serum angiotensin-converting enzyme level as a marker of fibrosis in patients with chronic hepatitis B

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Abstract

Background and aims: Hepatitis B virus (HBV) infection is a public health problem and affects nearly 350 million people worldwide. The present study was conducted in order to investigate the role of circulating angiotensin-converting enzyme (ACE) in the context of renin–angiotensin–aldosterone in newly diagnosed chronic hepatitis B infection. Moreover the association between liver fibrosis and serum ACE levels was also investigated.

Materials and methods: The study was performed on 50 chronic hepatitis B (CHB) patients (24 males, 26 females; median age 39.4 years, range 18–63) and 20 healthy controls. The clinical features of CHB patients including demographics, laboratory and liver biopsy findings were summarized. Serum ACE levels were measured by using commercially available kits.

Results: Serum median ACE levels were 48.4 (14–83) U/L and 26.2 (12–48) U/L for the CHB patients and controls, respectively. Serum ACE levels were significantly higher in patients with CHB compared with the control group ($p<0.001$). Twenty-two patients (44%) had advanced liver fibrosis (Ishak score >2) and 28 patients (56%) had mild liver fibrosis (Ishak score ≤ 2). Mean serum levels of ACE were significantly higher among patients with advanced fibrosis as compared with those without advanced fibrosis (60.3±14.2 U/L *vs*. 39.0±10.5 U/L, $p<0.001$). Receiver operating characteristic (ROC) curve analysis suggested that the optimum ACE level cut-off point for advanced fibrosis was 52.5 U/L (sensitivity: 81.8%, specificity: 82.1%, PPV 78.3%, NPV 85.2%, accuracy 82%, AUC: 0.890).

Conclusions: Our study showed that elevated circulating ACE levels are commonly observed in CHB patients. This finding was more prominent in patients with advanced fibrosis in liver. When evaluating a patient along with other parameters, the inclusion of ACE levels in the evaluation of CHB patients may grant additional prognostic information.

Keywords

Chronic hepatitis B, angiotensin-converting enzyme, renin–angiotensin system, liver fibrosis

Introduction

Hepatitis B virus (HBV) infection is a public health problem and affects nearly 350 million people worldwide. HBV infection occurs with various clinical spectrums ranging from acute and chronic hepatitis to cirrhosis and hepatocellular carcinoma.[1] Chronic HBV infection is a risk factor for hepatocellular carcinoma with an estimated prevalence of less than 1% per year and the risk mainly depends on the stage of the disease, which is appropriately detected with liver histology.[2-4] Although some risk factors including male sex, viral genotype, viral load and the severity of histological findings have been well defined, other factors related to disease progression are still an exciting area of research.

The renin–angiotensin–aldosterone (RAS) axis is a system which involves many essential regulations in the human body for blood pressure, fluid and electrolyte balance.[5] In recent years, the importance of the RAS system in pathogenesis of a number of diseases has been increasingly reported.[6,7] The role of angiotensin (Ang) has also

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shown to be implicated in the pathogenesis of liver disorders in some studies.[8,9] Moreover, angiotensin-converting enzyme (ACE), a vital part of the RAS system, is also cogitated as a governing molecule in systemic and portal circulation in some disorders.[8] The first aim of the study is to investigate the role of circulating ACE in the context of RAS in newly diagnosed chronic hepatitis B (CHB) infection. Moreover the association between liver fibrosis and serum ACE levels was also investigated.

Materials and methods

The study was performed at our tertiary reference centre between August 2008 and January 2010. The study group consisted of 50 newly diagnosed chronic hepatitis B (CHB) patients and 20 healthy controls. Age- and gender-matched healthy controls who initially presented with dyspeptic symptoms were recruited from the gastroenterology clinic. The diagnosis of CHB was established using the following criteria: (1) detectable hepatitis B surface antigen (HBsAg) for ≥6 months, (2) serum HBV DNA $\geq 10^5$ copies/ml.

The status of other hepatitis B markers (HbeAg, Anti-Hbe, Anti-Hbc IgG) was not considered as an inclusion/exclusion criterion. After fulfilling the above criteria, all patients underwent routine liver biopsy before treatment. Liver biopsy specimens that include 11 or more complete portal tracts and are longer than 20–25 mm were thought to be eligible for the pathological assessment. All pathologic specimens were evaluated by an experienced pathologist. The pathological assessments were made using stains including hematoxylin-eosin, Masson's Goldner, Masson's trichrome and reticulin in a blinded fashion. While advanced fibrosis was defined with Ishak staging score >2, mild fibrosis was defined with Ishak staging score ≤ 2.[10,11] AST to platelet ratio index (APRI) was used as a non-invasive test for the assessment of liver fibrosis.[12] APRI test was calculated with using the following formula: APRI = [(AST of the sample/reference AST) × 100] / platelets.

Cirrhotic patients clinically, laboratory and histologically diagnosed were considered ineligible for the study and excluded. Alcohol use (≥20 g/day) and hepatocellular carcinoma were also excluded from the study. For this purpose, all patients in the study were evaluated with abdominal ultrasound, oesophagogastroscopy and serum alpha fetoprotein measurement. Other diseases that can cause chronic hepatitis including autoimmune hepatitis, Wilson's disease and hemochromatosis were deemed not eligible for the study.

Patients with conditions that might affect the serum ACE level, including acute or chronic inflammation, hypertension, diabetes mellitus, renal failure or sarcoidosis, were excluded from the study. Patients receiving drugs such as ACE/ARB inhibitors and any other drugs that might potentially interact with the RAS system were excluded from the study. Serum ACE levels were obtained and paralleled with healthy controls. The study was guided in accordance with the standards of the Helsinki Declaration and written informed consents were attained from each of the patients studied.

Serum ACE activity was measured by observing the alteration in absorbance at 340 nm of the hydrolysis of furylacrylolylphenylalanylglycylglycine (FAPGG) to FAP and GG (Sigma-Aldrich, Poole, UK) on an analyser (Roche MIRA Analyser; Roche Diagnostic Systems, Welwyn Garden City, UK). The ACE activity in the sample was determined by comparing the sample reaction rate with that obtained with the ACE calibrator.

Statistical analyses were performed via using PASW Statistics 17 (SPSS, Chicago, IL, USA). Statistically important changes were evaluated by the chi-square test for categorical variables. The Kolmogorov–Smirnov test tested continuous variables for normality. Normally distributed data are presented as mean and standard deviation (SD). Mann–Whitney *U* test was applied for the independent subgroups. Independent samples *t*-test was used for parametric groups. The differences between CHB groups and controls were compared by the analysis of variance (ANOVA) test. A backward logistic regression analysis, with the Hosmer–Lemeshow goodness-of-fit test, was used to determine the predictors of liver fibrosis. A two-tailed p value 0.05 was considered as statistically significant.

Results

Fifty patients with CHB and 20 control subjects were recruited into the present study. There were 24 males and 26 females in the CHB group and 10 males and 10 females in the control group. The median age of CHB and control patients was 39.4 (18–63) and 40.2 years (24–65), respectively. There was no statistically significant difference between the ages of the study participants. Clinical characteristics and some biochemical features of the study participants are summarized in Table 1. Biochemical values of the cases with CHB and controls are also shown in Table 1.

Serum ACE levels were 48.4 (14–83) and 26.2 (12-48) U/L for the CHB patients and controls, respectively. The ANOVA test showed that serum ACE levels were significantly higher in patients with advanced fibrosis compared with mild fibrosis and controls ($p<0.001$) (Figure 1). Twenty-two patients (44%) had advanced liver fibrosis (Ishak score >2) and 28 patients (56%) had mild liver fibrosis (Ishak score ≤ 2). Mean serum levels of ACE were significantly higher among patients with advanced fibrosis as compared with those without advanced fibrosis (60.3±14.2 U/L *vs*. 39.0±10.5 U/L, $p<0.001$). The characteristic features of these two groups are depicted in Table 2.

Receiver operating characteristic (ROC) curve analysis suggested that the optimum ACE level cut-off point for advanced fibrosis was 52.5 U/L (sensitivity: 81.8%, specificity: 82.1%, PPV 78.3%, NPV 85.2%, accuracy 82%, AUC: 0.890) (Figure 2). In the meantime, APRI test cut-off point for advanced fibrosis was 0.46 (sensitivity: 63.6 %,

Table 1. Demographic features and laboratory values of the patients and controls

	Chronic hepatitis B patients (n = 50)	Control group (n = 20)	p
Mean age (years)	39.4±11.9 (18–63)	40.2±10.8 (24–65)	NS
Sex (M/F)	24/26	10/10	NS
Smoking status (+/-)	18/32	6/14	NS
BMI (kg/m^2)	21.3±5.7	22.6±4.8	NS
Blood pressure (BP)			
Systolic BP (mmHg)	117±16	122±9	NS
Diastolic BP (mmHg)	76±12	81±11	NS
Staging (0–6)	2 (0-5)		
Grading (0–18)	7.0±2.9 (0-14)		
INR	1.01±0.11	0.95±0.11	NS
Leucocyte (/mm^3 × 10^3)	6.07±1.43	6.17±1.25	NS
Platelet count (/mm^3 × 10^3)	192±42	215±56	NS
ALT (*N*: 0-40 U/L)	99 (17–368)	20.5 (14–31)	<0.001
AST (*N*: 0-40 U/L)	45.5 (20–314)	22.5 (10–40)	<0.001
ALP (*N*: n40-130 U/L)	75 (26–214)	60 (49–80)	0.002
GGT (*N*: 8-61 U/L)	27 (10–205)	25 (17–36)	NS
T.Bil (*N* <1.2 mg/dl)	0.83±0.29	0.85±0.25	NS
HBV-DNA (copy/ml)	7224 × 10^3 (2.6-2000000 × 10^3)		
HbeAg (+)/Anti-Hbe (+)	17/33		
AFP (ng/ml)	2.55 (0.77–8.9)		
ACE (U/L)	48.4±16.2	26.2±10.3	<0.001

Data are presented as median (range) or mean±SD. NS: non-significant: INR: international normalized ratio; ACE: angiotensin-converting enzyme; AFP: alpha fetoprotein; T.Bil: total bilirubin.

specificity: 60.7%, PPV 66%, NPV 68%, accuracy 62%, AUC: 0.61). Logistic regression analysis was performed to determine factors possibly associated with liver fibrosis. No correlation could be established between liver fibrosis and age, sex, alanine aminotransferase (ALT) and albumin level. Independent predictive factors for liver fibrosis in chronic hepatitis B patients were calculated as serum ACE level (odds ratio, OR = 1.21, 95% confidence interval, CI: 1.08–1.36), HBeAg, HBV-DNA and international normalized ratio (INR) (Table 3).

Discussion

In the present study, circulating ACE levels were significantly higher in patients with newly diagnosed CHB in comparison with the control group. Also patients with more severe histological findings had higher serum ACE levels as compared with those with lesser degrees of fibrosis.

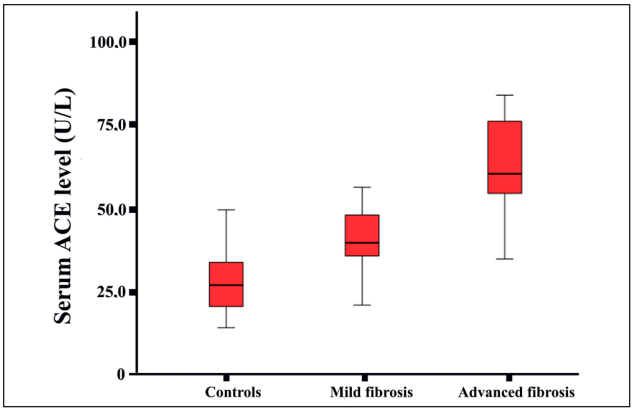

Figure 1. Serum angiotensin-converting enzyme (ACE) levels in patients with chronic hepatitis B (CHB) (mild and advanced liver fibrosis) and controls.

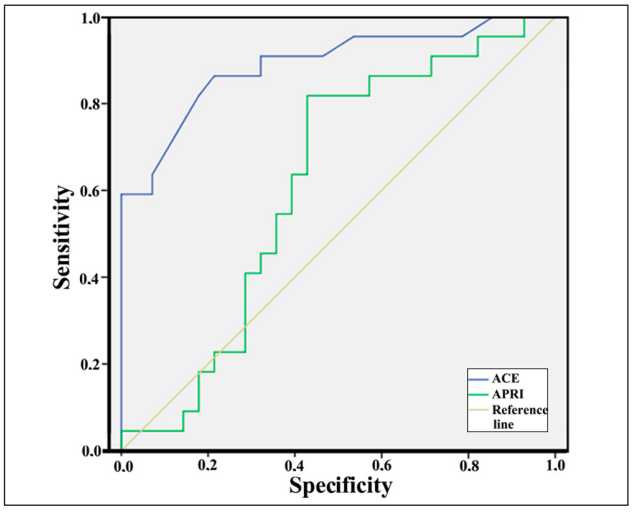

Figure 2. Receiver operating characteristic (ROC) curves of angiotensin-converting enzyme (ACE) and AST to platelet ratio index (APRI) for differentiating advanced fibrosis from mild fibrosis.

Table 2. Biochemical values of the patients according to their fibrosis levels

	Advanced fibrosis (n = 22)	Mild fibrosis (n=28)	p
Mean age (years)	40.6±13.4 (21–63)	38.4±10.7 (18–56)	NS
Sex (M/F)	14/8	10/18	NS
HBV-DNA (copy/ml)	43974 ×10^3(96–2000000 ×10^3)	3450 ×10^3(2.6–1000000 ×10^3)	NS
ACE (U/L)	60.3±14.2	39.0±10.5	<0.001
ALT (N: 0–40 U/L)	67.5 (20–303)	66 (17–368)	NS
AST (N: 0–40 U/L)	48 (20–269)	43.5 (20–314)	NS
INR	1.0 (0.8–1.2)	1.0 (0.7–1.3)	NS
Total bilirubin (mg/dl)	0.82±0.30	0.84±0.30	NS

NS: non-significant; ACE: angiotensin-converting enzyme; INR: international normalized ratio.

Table 3. Multivariate logistic regression analysis for liver fibrosis in chronic hepatitis B patients

	OR	CI (95%)	p
Age	0.94	0.85–1.04	NS
Sex	2.38	0.19–30.6	NS
ALT	1.0	0.99–1.01	NS
Albumin	0.41	0.06–2.7	NS
ACE	1.21	1.08–1.36	0.001
INR	0	0–3.4	NS
HbeAg	0.245	0.03–1.91	NS
HBV-DNA	1	1–1	NS

NS: non-significant; OR: odds ratio; CI (95%): 95% confidence interval; ACE: angiotensin-converting enzyme; INR: international normalized ratio.

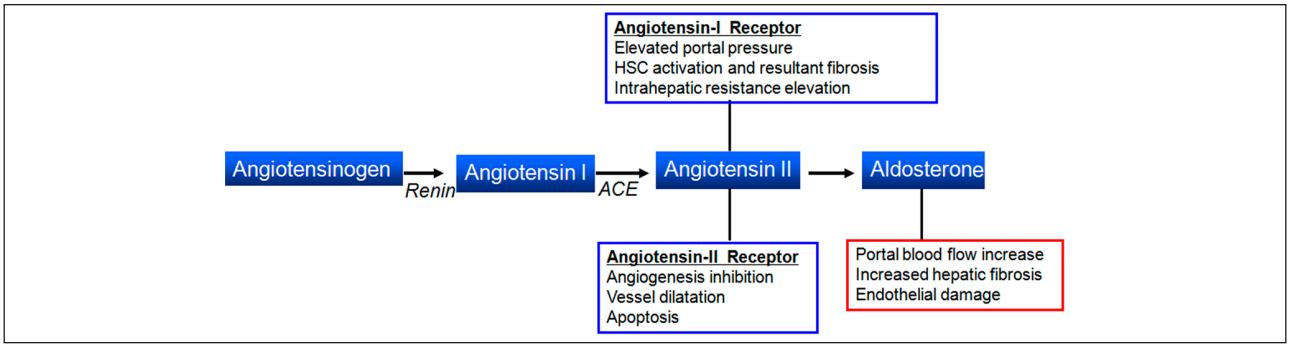

Figure 3. The potential role of the renin–angiotensin–aldosterone (RAS) system in the pathogenesis of liver diseases.

The RAS system is divided into two groups: classical RAS system and alternative RAS system. While classical system includes ACE, Ang II, Ang receptor type 1 (AT1); the alternative RAS system has some different elements, which encompass Mas receptor, ACE2, Ang(1-7). Both classical and alternative RAS systems play a significant role in the liver diseases' pathogenesis.[13]

According to the current literature, the RAS system is connected with at least three major pathogenetic mechanisms in liver with respect to liver disease pathogenesis, which include increased portal pressure due to vasospastic microocclusion, hepatic stellate cell proliferation and inflammation (Figure 3). Our findings are consistent with the previous studies, which demonstrate the role of ACE in liver in both human and animal studies.[14,15] We verified the importance of the RAS system in portal hypertension due to hepatoportal sclerosis and we reported that the circulating ACE level might have a role in creating vasospasm in microcirculation due to circulating ACE in hepatoportal sclerosis related portal hypertension in our previous study.[8] Corey et al.[16] studied the effect of Ang-blocking agents on liver fibrosis in patients with hepatitis C and concluded that less fibrosis was related to the protective role of ACE inhibitors in the patients. Moreover, a review performed by Koh

et al.[17] stressed some potential benefits of the inhibition of the RAS system in liver injury and fibrosis. In this review, authors stated that the impeding of the RAS system incites liver repair and prevents cancer development. Moreover, Bataller et al. revealed some intriguing implications regarding the role of the RAS system and liver disease relation.[18,19] In that study, the authors found that Ang II stimulates vessel shrinkage and works as a proliferative factor for hepatic stellate cells via AT1 receptors. These findings extrapolated to the clinical practice for the development of some potential drugs which affect the activated hepatic stellate cells (HSCs) causing the blockage of Ang II in intrahepatic circulation. Cirrhosis is the end result of all chronic liver disease. For that reason, targeting the HSCs is a promising alternative for the prevention of the progression of liver fibrosis.

CHB is another important cause of cirrhosis in common clinical practice. While some patients with CHB might present with advanced liver disease, other patients are found to have only mild or minimal fibrosis at the time of diagnosis. Effective antiviral treatments are recommended for all chronic hepatitis patients according to biopsy findings today even in cirrhotic patients.[1] Today, viral suppression is achieved with the available effective antiviral treatments with minimal side effects. In routine clinical practice, we commonly decide the treatment using liver biopsy findings, viral load and liver function tests.

Liver biopsy is the gold standard to detect damage in the liver caused by HBV. However, sampling errors are the weakest point of the procedure and even in advanced liver fibrosis the pathologist could mistakenly evaluate samples as normal due to sampling error. On the other hand, some patients with CHB may present as mild cirrhosis or early cirrhosis at the time of admission with all normal laboratory findings. Performing liver biopsy in such patients also does not reflect disease activity if the biopsy has a sampling error. In this respect predicting liver fibrosis and cirrhosis by application of simple models consisting of routine laboratory data are crucial. As a novel index, APRI test was developed to estimate the degree of liver fibrosis in treatment naïve chronic hepatitis C patients.[12] Wai et al.[12] demonstrated that the area under curve (AUC) of APRI for prediction of significant fibrosis and cirrhosis was 0.87 and 0.93 respectively. In the present study overall accuracy of APRI for estimating advanced fibrosis was 0.61 with sensitivity, specificity, NPV and PPV of 63.6%, 60.7%, 68% and 66% respectively. The low sensitivity and specificity regarding APRI values found in this study validates a need for different non-invasive methods for estimating liver fibrosis. From this perspective serum ACE levels as an indirect measure of liver fibrosis may open new diagnostic and therapeutic avenues. Demonstration of elevated circulating ACE levels in chronic hepatitis B at the time of diagnosis may be an early sign of advanced liver fibrosis. Moreover, along with other parameters, the inclusion of ACE levels in the evaluation of CHB patients may grant additional prognostic information.

In conclusion, when taking account of the importance of treatment individualization in CHB, elevated circulating ACE levels may be a reflection of impending cirrhosis in newly diagnosed patients. In this way, more aggressive and intensive management options could be considered. Although large randomized controlled trials are needed to change the clinical practice in CHB, our cross sectional small sample size study will broaden our horizons in the context of well-known mechanisms for the RAS system in chronic liver diseases.

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Conflict of interest

There is no conflict of interest to declare.

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