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Reduced cortisol in the absence of bacterial infection in patients with hepatitis B virus cirrhosis

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ABSTRACT. In liver cirrhosis with bacterial infection, hepatoadrenal syndrome has been described recently as a progressive impairment in the adrenocortical reserve, with deficient production or action of glucocorticoids resulting in adrenal insufficiency. The aim of this study was to explore the characteristics of cortisol in hepatitis B virus (HBV) cirrhosis patients in the absence of bacterial infection. Fasting peripheral venous blood samples were collected from 107 patients with HBV cirrhosis in the absence of bacterial infection and 18 patients with chronic hepatitis B (CHB) infection at 7 a.m. in the morning. The carbohydrate, cortisol-binding globulin, routine chemistry, liver function, and hepatitis B indicators were tested, and free cortisol was calculated. Cortisol (COR) levels were 18.72 ± 6.60 μg/dL in the CHB group and 14.20 ± 7.55 μg/dL in the HBV cirrhosis group (P = 0.002). COR levels were 15.11 ± 5.56, 14.88 ± 6.96, and 12.68 ± 8.36 μg/dL in Child-Pugh class A, B, and C cirrhotic patients, respectively (P = 0.006). Adrenocorticotropic hormone levels were 35.42 ± 24.49, 26.57 ± 15.72, and 19.65 ± 10.72 pg/mL in Child-Pugh class A, B, and C cirrhotic patients, respectively (P = 0.000). Patients with HBV

cirrhosis had significantly lower serum COR levels compared with those of CHB patients, even if they are in the absence of bacterial infection. COR levels negatively correlated with Child-Pugh scores. The hypothalamic-pituitary-adrenal axis might be damaged in patients with HBV cirrhosis.

Key words: Hepatitis B; Cirrhosis; Cortisol; Unbacterial infection; Patient; Liver

INTRODUCTION

The hepatitis B virus causes one of the most common chronic infectious liver diseases, affecting 350-400 million individuals worldwide (McMahon, 2010). In China, a hepatitis B epidemiological investigation in 2009 reported a hepatitis B virus surface antigen (HBsAg) carrier rate of 7.18% in the general population aged 1-59 years (Liang et al., 2009). Bacterial infection is a common complication and one of the most important causes of death in patients with cirrhosis (Lazzarotto et al., 2013). Child-Pugh class C cirrhotic patients are more prone to refractory shock (33%) than patients with Child-Pugh class A or B cirrhosis (8%), indicating that refractory shock gradually increases along with the severity of cirrhosis (Fernández et al., 2006). Relative adrenal insufficiency has recently been described in 62-80% of heterogeneous groups of patients with liver disease (Arabi et al., 2010). Adrenal insufficiency can affect the liver function, increase the mortality, and seriously influence the prognosis (O'Beirne et al., 2007; Aravinthan et al., 2009; Galbois et al., 2010; Fede et al., 2011). Our study explored whether cortisol (COR) levels are reduced in patients with HBV cirrhosis and what factors correlate with COR level.

A cross-sectional study was employed in our research. We determined the Child-Pugh class of HBV cirrhosis and serum level of COR, free cortisol (FC), cortisol-binding globulin (CBG), and adrenocorticotropic hormone (ACTH) in patients with chronic hepatitis B (CHB) and HBV cirrhosis. We also assessed the characteristics of COR in patients with HBV cirrhosis

MATERIAL AND METHODS

Subjects

A total of 107 patients with HBV cirrhosis, including 11 patients with Child-Pugh class A cirrhosis, 35 patients with Child-Pugh class B cirrhosis, and 61 patients Child-Pugh class C cirrhosis, aged 18-68 (51.29 ± 13.23) years, were selected from Beijing Youan Hospital affiliated to Capital Medical University between September 2012 and March 2013; another 18 CHB patients aged 30-58 (40.39 ± 8.49) years were also recruited for the study. This study was conducted in accordance with the Declaration of Helsinki and with approval of the Ethics Committee of Capital Medical University. Written informed consent was obtained from all participants.

Inclusion criteria

Inclusion criteria were derived from "Management of hepatitis B: summary of a clinical research workshop" (Hoofnagle et al., 2007).

Exclusion criteria

Exclusion criteria were as follows: 1) other viral infections, fatty liver, or autoimmune disease; 2) malignant tumor, trauma, cardiovascular disease, adrenocortical disease, or thyroid disease; 3) use within 6 months of a medication known to affect cortical hormone levels; and 4) infection, defined as a systemic inflammatory response syndrome with simultaneous positive bacterial or fungal culture (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference, 1992).

Test items

After admission to the hospital, each patient's volume of ascites was determined using abdominal ultrasound. At 7 a.m. on the following morning, fasting peripheral blood samples were collected, and total bilirubin (TBIL), albumin (ALB), prothrombin time (PT), blood ammonia (BA), HBsAg, hepatitis B virus E antigen (HBeAg), hepatitis B virus E antibody (HBeAb), and HBV DNA levels were determined in our clinical laboratory. An additional 4 mL of blood was collected in a biochemical tube containing coagulant and separation gel, followed by centrifugation at 2500 r/min for 10 min at 4°C; the supernatant was then transferred to an Eppendorf PCR tube and stored at -80°C. After the frozen serum was thawed at room temperature and agitated, COR, CBG, and ACTH levels were measured.

Detection methods

COR and ACTH levels were measured using enzyme-amplified chemiluminescence with a kit from Diagnostic Products Corporation (Immulite 2000, Siemens Company, USA). CBG level was determined using radioactive immunoassay (RIA) with an RIA kit from Biosource (Belgium).

An automatic biochemical analyzer was employed to detect TBIL, ALB, and BA in peripheral blood; an automated blood coagulation analyzer was utilized to test PT and partial prothrombin activity (PTA).

HBV serologic markers were identified by enzyme-linked immunosorbent assay (ELISA), and HBV DNA level was measured by reverse transcription-polymerase chain reaction (RT-PCR), with 100 copies/mL as the lowest limit of quantification. The test Kit for HBV DNA was provided by Roche Diagnostics GmbH (Germany).

Formula

Coolens formula was used to calculate FC concentration (Coolens et al., 1987): FC concentration (μM) = (Z2 + 0.122C)1/2 - Z, Z = 0.0167 + 0.182 (T-C).

Statistical analysis

Data analysis was performed with the SPSS 11.5 software (IBM Corporation, USA). Normally distributed measurement data are reported as means ± standard deviation. Comparison of mean values among 3 groups used one-way analysis of variance, and comparison between 2 groups utilized independent samples *t*-test. Pearson's correlation analysis was performed. $P < 0.05$ was considered to be statistically significant.

RESULTS

Demographic and background characteristics

A total of 107 patients (93 males and 32 females), including 11 patients with Child-Pugh class A cirrhosis, 35 patients with Child-Pugh class B cirrhosis, and 61 patients with Child-Pugh class C cirrhosis, aged 29-68, were selected for the research group; another 18 patients with CHB were recruited for the control group. All patients were observed to be free of bacterial infections (Table 1).

Table 1. Demographic and virological indicators of the subjects.

	CHB (18 cases)	Child-Pugh classification of cirrhosis			P value
		Class A (11 cases)	Class B (35 cases)	Class C (61 cases)	
Male/female	10/8	8/3	26/9	49/12	0.879
Age (years)	40.4±8.5	48.3 ± 10.3	50.5 ± 13.5	55.8 ± 13.5	0.000$^{\Delta}$
Family history (Yes/No)	8/10	7/4	8/27	25/36	0.796
Antiviral Therapy (Yes/No)	11/7	10/11	8/27	25/36	0.879
HBeAg (+/-)	5/13	4/7	11/24	23/38	0.780
LogHBV DNA	3.5±2.4	2.7 ± 2.2	2.8 ± 2.1	2.8 ± 2.2	0.004

*$P < 0.05$, significant; $^{\Delta}P < 0.01$, highly significant.

Level of COR, FC, CBG, and ACTH

COR, FC, CBG and ACTH levels were significantly lower in the HBV cirrhosis group than in the CHB group ($P < 0.01$, Table 2).

Table 2. Comparison of COR, FC, CBG and ACTH level between HBV-cirrhosis and CHB groups.

	CHB (18 cases)	HBV-cirrhosis (107 cases)	*t* value	P value
COR (μg/dL)	18.72 ± 6.60	14.20 ± 7.55	-3.16	0.002$^{\Delta}$
CBG (μg/mL)	54.84 ± 16.53	31.89 ± 14.18	-7.22	0.000$^{\Delta}$
FC (μM) (100X)	9.44 ± 0.02	9.41 ± 0.37	-4.57	0.000$^{\Delta}$
ACTH (pg/mL)	31.19 ± 11.28	25.92 ± 17.77	-6.79	0.000$^{\Delta}$

As FC had small order of magnitude, it was multiplied by 100 times in Table 2 in order to describe easily. Similarly hereinafter $^{\Delta}P < 0.01$, highly significant.

The levels of COR, CBG, and ACTH elevated with the increase of Child-Pugh class, but FC levels did not distinctly change (Table 3).

Table 3. COR, FC, CBG, and ACTH levels in patients with HBV-cirrhosis.

	Child-Pugh classification of cirrhosis			P value
	Class A (11 cases)	Class B (35 cases)	Class C (61 cases)	
COR (μg/dL)	15.11 ± 5.56	14.88 ± 6.96	12.68 ± 8.36	0.006$^{\Delta}$
CBG (μg/mL)	45.63 ± 13.01	30.92 ± 12.73	26.50 ± 12.62	0.001$^{\Delta}$
FC (μM, 100X)	9.415 ± 0.045	9.409 ± 0.376	9.416 ± 0.319	0.700
ACTH (pg/mL)	35.42 ± 24.49	26.57 ± 15.72	19.65 ± 10.72	0.000$^{\Delta}$

$^{\Delta}P < 0.01$, highly significant.

DISCUSSION

The hepatitis B virus causes one of the most common chronic infectious liver diseases, affecting 350-400 million individuals worldwide (McMahon, 2010). A prospective study of 684 patients with CHB identified the estimated annual incidence of cirrhosis in CHB patients to be 2.1% (Papatheodoridis et al., 2008). Bacterial infection is a common complication and one of the most important causes of death in patients with cirrhosis (Lazzarotto et al., 2013). The fatality rate of cirrhotic patients with septic shock is 60-100% (Tsai et al., 2006; Thierry et al., 2008). In 2006, Fernández et al. (2006) studied 75 cirrhotic patients with septic shock and discovered that Child-Pugh class C cirrhotic patients were more prone to refractory shock (33%) than patients with Child-Pugh class A or B cirrhosis (8%), indicating that the incidence of refractory shock gradually increases with the severity of cirrhosis.

More than 70% of COR in the blood is bound by CBG, and 20% is bound by ALB; thus, free COR, which plays an important biological role, composes less than 10% of total blood COR (Qureshi et al., 2007). CBG is insufficiently synthesized in patients with cirrhosis and end-stage liver disease, which might influence the biological role of COR. Basal COR level is affected by multiple factors and is unstable in patients; thus, it is not reliable to evaluate COR in patients with insufficient CBG synthesis due to cirrhosis or patients under stress conditions such as infection and shock.

Our research aimed to determine serum levels of COR, FC, CBG, and ACTH in patients with chronic hepatitis B and HBV cirrhosis. Previous research did not combine Child-Pugh classification with study of patients with HBV cirrhosis, whereas in our research, we explored COR level and its related factors in patients with different Child-Pugh classes of HBV cirrhosis.

Our research results revealed that COR and FC levels were significantly lower in the HBV cirrhosis group than in the CHB group, and COR level and Child-Pugh score of HBV cirrhosis were negatively correlated, which has rarely been reported. The Child-Pugh scoring system uses 5 indicators (ALB, ascites, TBIL, PT, and encephalopathy) to assess the severity of cirrhosis. Basal COR level is affected by multiple factors and is not reliable for evaluating patients under stress conditions such as infection and shock. Therefore, the biological activity of COR in patients is better reflected by the FC level.

Fede et al. (2011) studied 101 non-infected cirrhotic patients with stable hemodynamic status and demonstrated that the correlation coefficient between FC and basal COR was 0.94 because of the existence of CBG, whereas the correlation coefficient between FC and peak COR was 0.90. Similarly, in our research, the correlation coefficient between FC and COR was 0.907. CBG level decreased along with the decrease in Child-Pugh class, indicating insufficient synthesis of CBG in cirrhotic patients. During the acute stage of disease, CBG concentration and bound COR level decline rapidly, resulting in an increase in the FC level (Perogamvros et al., 2011). Our research revealed that the FC level in patients with HBV cirrhosis was markedly lower than that in CHB patients, but no significant difference was observed within the HBV cirrhosis group, suggesting that CBG might have affected the changes in FC and COR. The lower FC level in the HBV cirrhosis group suggests that FC might be a sensitive indicator for the diagnosis of HBV cirrhosis.

Under physiological conditions, the secretion of COR follows a circadian rhythm and is controlled by the hypothalamic-pituitary-adrenal (HPA) axis. Furthermore, COR affects the HPA axis through negative feedback, reducing COR secretion to maintain a dynamic equilibrium among the 3 glands (Xiong and Zhang, 2013). When COR level decreases, an internally

reactive increase of ACTH should occur to promote the secretion of COR by adrenal cells.

In 2008, Wiest et al. (2008) studied the HPA axis and discovered that COR level decreased in some patients, but the decrease was not sufficient to induce an obvious ACTH change. The researchers concluded that COR feedback regulation was damaged under certain physical conditions, such as trauma, severe infection, and shock, i.e., COR reduction did not correspond to ACTH increase. Our research showed a positive correlation between ACTH and COR levels, with a correlation coefficient of 0.256 ($P < 0.05$). The COR level progressively decreased along with an increase in the Child-Pugh score; thus, ACTH failed to effectively stimulate the COR level. We believe that the impaired HPA axis in the patients with HBV cirrhosis induced the insufficient plasma COR level. However, prospective studies and ACTH stimulation tests are required to confirm our findings.

CONCLUSIONS

Patients with HBV cirrhosis had significantly lower plasma COR levels compared with those in CHB patients, and COR level negatively correlated with Child-Pugh score. The HPA axis might be damaged in patients with HBV cirrhosis.

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