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Serum sphingomyelin has potential to reflect hepatic injury in chronic hepatitis B virus infection

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SUMMARY

Objective: To explore the relation between serum sphingolipids and hepatic injury in chronic HBV infection.
Methods: A cohort of participants including 48 healthy persons, 103 chronic HBV-infected patients containing chronic hepatitis B (CHB) and HBV-related cirrhosis were included. High performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was performed to detect serum sphingolipids. The serological indicators were detected and quantified. The valid liver biopsy specimens were acquired from twenty five CHB.
Results: Twenty four serum sphingolipids were detected. There were eighteen sphingolipids showing significant differences between the healthy control and chronic HBV infection groups. In patients with chronic HBV infection, fourteen sphingolipids differed significantly between CHB and HBV-related cirrhosis. Among sphingolipids with a significant difference in both HBV infection *vs* healthy control and CHB *vs* cirrhosis, seven sphingolipids were independently related to the presence of cirrhosis. SM(d18:1/24:0), a sphingomyelin (SM) compound, was found to have a negative correlation with model for end-stage liver disease (MELD) score. Additionally, SM(d18:1/24:0) was demonstrated to have a correlation with inflammation grades by liver biopsy in CHB patients.
Conclusions: Serum sphingolipids have close relation with hepatic injury in chronic HBV infection, especially that SM(d18:1/24:0) might be a potential serum biomarker.

1. Introduction

Chronic liver disease caused by persistent hepatitis B virus (HBV) infection is a potentially life-threatening disease affecting the world population. Chronic hepatitis B (CHB) is thought to be responsible for approximately 30% of cirrhosis, which has become the 14th most common cause of death worldwide.[1,2] Chronic HBV infection is characterized by a functionally inefficient immune system that fails to eliminate HBV from the liver but maintains continuous hepatocellular injury thought to be responsible for disease progression.[3] Meanwhile, hepatic fibrosis which can occur at the advanced stage of CHB is primarily driven by the development of necroinflammation in response to hepatic injury.[4] It has been suggested that the termination of chronic HBV infection by available antiviral therapies has been associated with reduced occurrence of cirrhosis.[5] Thus, the prevention of hepatic injury will benefit for the outcome of CHB.

Sphingolipids constitute structural components of biological membranes, and regulate the signal processes including proliferation, differentiation and apoptosis.[6] It has been proved that sphingolipids are enriched in lipid rafts that provide distribution platform for signal molecules.[7–9] And pathogens are able to exploit lipid rafts to get into their target host cells.[10] The in vivo animal

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study showed that HBV replication could be inhibited through the repression of host sphingolipid biosynthesis.[11] Additionally, the clinical trial demonstrated that glycosphingolipid alpha-galactosylceramide could be used as a monotherapy for CHB by the regulation of antiviral immune responses.[12] We previously found that glycosphingolipid and sphingomyelin were closely related to hepatic necroinflammation and steatosis in chronic hepatitis C patients, respectively.[13,14] Recently we also found that serum dihydroceramide may be a useful prognostic indicator for the early prediction of HBV-related acute-on-chronic liver failure.[15] According to the previous finding, we speculated that the one or subclass of sphingolipids might play different roles in different etiologies and progressing stage of liver disease, and certain sphingolipids might be involved in the hepatic injury caused by chronic HBV infection.

Thus, in order to explore the role of sphingolipids in chronic HBV infection, we included a cohort of patients containing CHB and HBV-related cirrhosis. With the help of the metabolomics technologies and available liver biopsy, we analyzed the profile of serum sphingolipids and the relationship between sphingolipids and hepatic injury in chronic HBV infection.

2. Materials and methods

2.1. Patients

A cohort of 151 participants including 48 healthy controls, 103 chronic HBV infection patients from Beijing YouAn Hospital (Capital Medical University, Beijing, China) were included from 2009 to 2013 in the present study. The chronic HBV infection patients included 52 CHB and 51 HBV-related cirrhosis. Chronic HBV infection and cirrhosis diagnosis were made in accordance with established criteria.[16] Chronic HBV infection was established by the positive for HBsAg or HBV DNA using polymerase chain reaction assays for more than 6 months before enrollment. Patients with serum bilirubin $\geq$ 10 mg/dl and prothrombin activity (PTA) $<$40% were excluded. Additionally, patients with presence of other causes of liver disease or hepatocellular carcinoma were also excluded. All healthy controls were negative for HBsAg and showed normal liver function.

Each subject signed the informed consent at the beginning of the study. The study protocol was conducted in accordance with the provisions of the Declaration of Helsinki and approved by the Institutional Review Board of Beijing YouAn Hospital, Capital Medical University (Beijing, China).

2.2. HPLC-MS/MS

Fasted blood samples were prospectively collected in 16 $\times$ 100 mm $\times$ 10 mL BD Vacutainer® glass serum tubes (Becton Dickinson, Franklin Lakes, NJ) in the morning of the second day after the subjects were admitted to the hospital. The tubes were incubated at room temperature for 20 min to allow the blood to clot, and then centrifuged at 750 $\times$ g for 15 min to obtain the serum. Serum samples were stored at -80 °C immediately after collection.

The high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) was performed using an Agilent 6410B Triple Quad mass spectrometer (Agilent Technologies Inc., Santa Clara, CA) comprising a triple quadrupole MS analyzer equipped with an electrospray ionization interface and an Agilent 1200 RRLC system (HPLC-MS/MS). The HPLC-MS/MS methodology used in this study was described in our previous report.[15] Sphingolipidomic assays were performed at the Institute of Materia Medica, Peking Union Medical College (Beijing, China).

2.3. Liver Biopsy

Liver biopsies were obtained from CHB patients under ultrasound guidance. Finally, the valid liver biopsy specimens were acquired from twenty five CHB patients. Specimens were fixed in formalin and embedded in paraffin. Specimens longer than 1.5 cm, containing at least six complete portal areas, were reviewed by two senior pathologists who were unaware of the patients' clinical data. The Scheuer scoring system was used to assess hepatic inflammatory activity.[17]

2.4. Statistical analysis

Data were expressed as mean $\pm$ standard deviation (SD). In univariate analysis, depending on the data distribution, differential significance of continuous variables between the two groups was analyzed by the independent-samples t test or Mann–Whitney test. Moving forward, the (LR) multivariate logistic regression analysis was performed, and the P values of entry and removal were respectively set to 0.05 and 0.1. The diagnostic value of the indicator was evaluated by the area under the receiver operating characteristic (ROC) curve analysis. Categorical variables were analyzed by Pearson χ^2 test. Correlation analysis was performed by Spearman's rank correlation or Pearson correlation. Statistical analysis was performed using SPSS version 19.0 (Chicago, IL, USA), while a two-sided P value $<$0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the participants

The clinical characteristics of participants are summarized in Table 1. The patients with chronic HBV infection with a mean age of 44.94 years, showed elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. The mean serum HBV DNA load of these patients was high (3.0E+07 cps/mL). Between the CHB and cirrhosis groups, the sex proportion resulted in no significant difference between the two patient groups (P = 0.083), and ALT, total bilirubin (TBIL), albumin, and HBV DNA load showed statistical differences ($P < 0.05$). The available liver biopsies were acquired from twenty five CHB patients. Inflammation grade 1 and 2 were found in most of these patients (80.0%, 20/25). For HBV-related cirrhosis, the proportion of compensated cirrhosis accounted for 21.6% (11/51).

3.2. The alteration of serum sphingolipids' level

Based on our quantitative lipidomic platform, twenty four serum sphingolipids were detected by HPLC-MS/MS. In order to explore the role of sphingolipids in chronic HBV infection, we analyzed the alteration of sphingolipids between healthy and chronic HBV infection. There were eighteen sphingolipids which showed significant differences (Fig. 1). With the progress of chronic HBV infection, fourteen sphingolipids differed significantly between CHB and HBV-related cirrhosis (Fig. 2).

After comparing these sphingolipids, 13 sphingolipids which differed between healthy and chronic HBV infection, also presented with significant differences between CHB and HBV-related cirrhosis. Fig. 3 depicts these 13 species in the metabolic pathway of sphingolipids. Of these sphingolipids, there were 6 ceramides (Cer), 2 hexosylceramide (HexCer), 3 sphingomyelins (SM), one ceramide-1-phosphate and one dihydroceramide (dhCer).