A serum metabolomic analysis for diagnosis and biomarker discovery of primary biliary cirrhosis and autoimmune hepatitis

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BACKGROUND: Because of the diversity of the clinical and laboratory manifestations, the diagnosis of autoimmune liver disease (AILD) remains a challenge in clinical practice. The value of metabolomics has been studied in the diagnosis of many diseases. The present study aimed to determine whether the metabolic profiles, based on ultraperformance liquid chromatography-mass spectrometry (UPLC-MS), differed between autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC), to identify specific metabolomic markers, and to establish a model for the diagnosis of AIH and PBC.

METHODS: Serum samples were collected from 20 patients with PBC, 19 patients with AIH, and 25 healthy individuals. UPLC-MS data of the samples were analyzed using principal component analysis, partial least squares discrimination analysis and orthogonal partial least squares discrimination analysis.

RESULTS: The partial least squares discrimination analysis model (R^2Y=0.991, Q^2=0.943) was established between the AIH and PBC groups and exhibited both sensitivity and specificity of 100%. Five groups of biomarkers were identified, including bile acids, free fatty acids, phosphatidylcholines, lysolecithins and sphingomyelin. Bile acids significantly increased in the AIH and PBC groups compared with the healthy control group. The other biomarkers decreased in the AIH and PBC groups compared with those in the healthy control group. In addition, the biomarkers were downregulated in the AIH group compared with the PBC group.

CONCLUSIONS: The biomarkers identified revealed the pathophysiological changes in AILD and helped to discriminate between AIH and PBC. The predictability of this method suggests its potential application in the diagnosis of AILD.

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KEY WORDS: autoimmune liver disease; biomarkers; metabolomics; autoimmune hepatitis; primary biliary cirrhosis; overlap syndrome

Introduction

Autoimmune liver disease (AILD) is characterized by self-perpetuating inflammation of the liver with indeterminated causes.[1] AILD includes autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), primary sclerosing cholangitis, and overlap syndrome (OS). AIH and PBC are two types of AILD with a higher incidence than PSC and OS.[2] The diagnosis of AILD is challenging due to diverse clinical and laboratory manifestations.[3, 4] The diagnosis of AILD requires a combination of clinical features, abnormal liver biochemical patterns, auto-antibodies and abnormal liver histology.[4, 5] However, liver biochemical patterns and auto-antibody levels are not pathogenic or required for disease.[6] Furthermore, atypical liver histology confounds the diagnosis.[7, 8] Differentiating between AIH and PBC is difficult because of non-specific behaviors and the overlap of diseases, but the differentiation is important because the treatment regimens for AIH and PBC are different. Although PBC is currently treated with ursodeoxy-

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cholic acid,[9, 10] AIH is mainly treated with prednisolone/prednisone with or without azathioprine.[11]

In previous studies[12, 13] on the diagnosis of AILD, proteomic analyses were performed to identify many auto-antibodies. However, auto-antibodies are insufficient to diagnose AILD.[6, 14, 15] Antimitochondrial antibodies are present in >90% of PBC patients,[16-18] and no antibody is highly specific in patients with AIH. Many scoring systems have been designed for the diagnosis of AILD. Three criteria for the diagnosis of AIH have been proposed by the International Autoimmune Hepatitis Group.[19-21] Compared with the revised criteria in 1999, which were primarily designed for research purposes, the simplified scoring system has enhanced clinical applicability and practicability.[22] The revised original scoring system performs well in patients with few or atypical features of AIH, whereas the simplified system is better at excluding the diagnosis in diseases with concurrent immune manifestations.[23] However, the specificity of the revised original scoring system is not satisfactory, whereas the specificity of the simplified system is low. Furthermore, these scoring systems list liver biopsy results as a criterion. Although being a generally safe procedure, liver biopsy carries a risk of complications.[24] Therefore, developing new methods to detect AILD accurately is important.

With the development of high-throughput methods, "omic" studies have become important to elucidate biological processes. As the products of cellular adjustment processes, metabolites levels are regarded as the ultimate readouts for genetic or environmental changes in biological systems.[25, 26] Metabolomic methods have been used for clinical diagnosis and drug discovery. Using these methods, researchers have discovered many specific biomarkers and successfully diagnosed several diseases.[27-29] Previous studies used metabolomic methods for the diagnosis of biliary tract cancer, renal cell carcinoma, and diabetes mellitus.[30-32] These studies suggested that the accuracy and predictability of these methods can augment current diagnostic approaches. However, no study has characterized AILD using metabolomic methods.

Nuclear magnetic resonance spectroscopy and gas or liquid chromatography coupled to mass spectrometry (GC-MS and LC-MS, respectively) are common tools for metabolomic studies.[33, 34] In contrast to nuclear magnetic resonance and GC-MS, LC-MS has higher resolution and sensitivity and is considered to be a powerful tool to obtain rapidly and effectively multiparametric metabolite profiles from biological fluids.[35] In this study, we evaluated the feasibility of a metabolomic method to diagnose AILD. Our method was based on ultraperformance liquid chromatography (UPLC)-MS; we evaluated two common AILDs, AIH and PBC. This study aimed to determine whether the metabolic profiles were different between AIH and PBC, to identify specific metabolomic markers, and to establish a model for the diagnosis of AIH and PBC.

Methods

Study population

We selected 19 patients with AIH and 20 with PBC who were hospitalized at the First Affiliated Hospital of Zhejiang University School of Medicine (Hangzhou, China) from March 2009 to December 2010. The study protocol was approved by the Ethics Committee of the hospital and written informed consent was provided by all enrolled patients. The patients with AIH were diagnosed according to the revised criteria proposed by the International Autoimmune Hepatitis Group in 1999. Their scores confirmed definitely the diagnosis of AIH.[20] Patients with PBC were diagnosed according to the entry criteria for clinical trials of PBC based on the recommendations of the American Association for the Study of Liver Diseases.[36] Patients taking medication or supplements, or those with gallstones diseases or other factors that cause cholestatic abnormal liver profiles were excluded. In both groups, patients with primary sclerosing cholangitis, OS (such as PBC and AIH or AIH and primary sclerosing cholangitis), hepatitis virus infection, HIV co-infection, hepatocellular carcinoma, or diabetes were excluded. Twenty-five healthy persons who visited our hospital for physical check-ups were recruited as healthy controls. These individuals exhibited normal liver functions and had no evidence of disease. No statistically significant differences in age and gender were found among the PBC, AIH and control groups (Table 1, $P>0.05$).

Sample collection

At admission, before breakfast and initiation of drug treatment, blood was taken from 39 patients and 25 healthy individuals and centrifuged at 3600×g for 6 minutes at 4 ℃. Blood serum was transferred into tubes and stored at -80 ℃ until analysis.

Chemicals

Acetonitrile, isopropyl alcohol, formic acid, methanol, leucine enkephalin (HPLC grade), bile acid, sphingomyelin, lysophosphatidylcholine and fat acid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Distilled water was filtered through a Milli-Q system (Millipore, Bedford, MA, USA).

UPLC-MS analysis

The serum samples were thawed at 4 ℃. Eight qual-