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Oxidative stress markers, secondary bile acids and sulfated bile acids classify the clinical liver injury type: Promising diagnostic biomarkers for cholestasis

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ABSTRACT

Clinicians sometimes encounter difficulty in choosing a therapeutic strategy due to the uncertainty regarding the type of liver injury. In particular, cholestasis is difficult to diagnose by conventional markers at an early stage of disease. The aim of this study was to identify promising biomarkers for distinguishing the symptom-based types of liver injury (e.g. hepatocellular injury, cholestasis), which was derived from a rigorously statistical perspective. The associations between diagnostic biomarkers (e.g. bile acid components, oxidative stress markers and liver fibrosis markers) and the liver injury types were assessed by a multiple logistic regression analysis using 304 blood samples from patients with liver disease. As a result, reductions in the lithocholic acid (LCA) and deoxycholic acid (DCA) levels, and elevation of the serum sulfated bile acid (SSBA), liver fibrosis marker IV collagen (type IV collagen), hyaluronic acid (HA) and reactive oxygen species (ROS) levels were all significantly associated with cholestasis. On the other hand, elevations in the LCA and type IV collagen levels, and a reduction in the ursodeoxy cholic acid (UDCA) level, were significantly associated with hepatocellular injury. The receiver operating characteristic (ROC) analyses showed that the largest area under the ROC curve (AUC) was found for ROS, followed by DCA, HA, LCA, SSBA and type IV collagen in the cholestatic-type cases. These results indicated that ROS, the secondary bile acid levels such as DCA and LCA, and SSBA are promising biomarkers for cholestasis and for classifying the type of liver injuries. This comprehensive approach will allow for an accurate diagnosis, which will facilitate the selection of an appropriate therapy at the onset of disease.

1. Introduction

During the clinical diagnosis of liver injury, clinicians must make an early assessment of the extent of progression from cholestasis in the bile canaliculi to severe hepatocellular injury, and subsequently make an appropriate decision with regard to the therapeutic strategy [1,2]. Liver injuries are generally classified into two symptom-based types; cholestasis and hepatocellular injury, as well as a mixed type (with both kinds of injuries) [1]. Cholestatic liver injury is characterized by the impairment or cessation of bile flow, resulting in an accumulation of bilirubin, cholesterol and its metabolites. Hepatocellular injury refers to a process involving the impairment of primarily the hepatocytes, and can result in hepatocyte necrosis [3,4].

Hepatocellular injury generally results in elevations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), with little or no elevation of alkaline phosphatase (ALP), whereas increases in ALP and γ-glutamyl transpeptidase (γ-GTP) are seen in cases with cholestasis. Clinically, the ALP and ALT levels have been used as the criteria for diagnosing cholestasis, hepatocyte injury or mixed type cases [3–5]. Although the typing of liver injuries based on the symptoms or etiology is crucial for selecting the treatment,

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there are limitations to diagnosing a patient based solely on the biochemistry findings [1,2,6].

Regardless of the etiology of the liver disease, the clinical condition changes time-dependently with the progression or recovery of illness, e.g., hepatocellular injury with a high level of serum transaminase activity may initially appear in patients with acute viral hepatitis B, and then the patient may later develop cholestasis, or vice versa. The diagnosis of the current clinical condition is therefore required to select the most appropriate therapeutic strategy at the onset of disease, and frequent assessments of the clinical condition are needed to ensure that appropriate treatments are administered based on the changes in the patient's condition. However, physicians usually diagnose patients using a combination of biochemical examinations and symptom assessment during their treatment [1]. Therefore, obtaining a precise and early diagnosis using one or more biomarkers would help to more clearly classify the liver injury type [6], especially to specify cholestasis, and to select the optimum treatment [1,2].

In addition to the basic biochemistry parameters, the individual bile acid components have been investigated as potentially sensitive markers for the clinical diagnosis of hepatic diseases [7–12]. Bile acids are synthesized and conjugated by hepatic microsomal, mitochondrial and lysosomal enzymes [13]. In humans, cholic acid (CA) and chenodeoxycholic acid (CDCA) are the major primary bile acids synthesized via the classical pathway. In the intestine, primary bile acids are deconjugated and 7α-dehydroxylated by anaerobic microflora to secondary bile acids, mainly deoxycholic acid (DCA) and lithocholic acid (LCA). Physiologically, these bile acids are conjugated with taurine or glycine and excreted into bile. The sulfation of bile acid maintains bile acid homeostasis under pathological conditions. The formation of serum sulfated bile acid (SSBA) increases in patients with cholestatic diseases, and urinary sulfated bile acid (USBA) quantification is used to monitor patients with biliary atresia, because it reflects the degree of cholestasis in both adults and newborns [9,14,15]. Therefore, the measurement of changes in serum bile acids might indicate the presence, and possibly the nature, of liver and biliary diseases.

With respect to the individual bile acid components, several studies have attempted to apply quantification of these levels to the clinical diagnosis of hepatic disease, but the numbers of patients included in these studies were too small to permit any general conclusions to be drawn, and the measurement methods were complicated, with limited sensitivity and specificity [7,9,16–19]. Although the bioanalytical methods used to measure the bile acid levels is one of the key factors allowing a correct assessment of liver injury, highly sensitive methods have only recently been developed [20–25]. In this study, the primary and secondary bile acids and their conjugates were determined in human serum using a simple, sensitive and highly selective LC-MS/MS method.

The aim of this study was to identify definitive biomarkers for distinguishing liver injury types. To this end, we investigated the relationships between various types of liver disease and the levels of serum biomarkers, such as bile acid components, SSBA, reactive oxygen species (ROS), 8-hydroxy-2′-deoxyguanosine (8-OHdG), hyaluronic acid (HA) and type IV collagen, in addition to the standard serum biochemistry parameters, in patients with liver injuries.

2. Materials and methods

2.1. Study design

Patients with liver disease (n = 154) and healthy volunteers (n = 46) were recruited from Jikei University Hospital, Tokyo, Japan from March 2011 to September 2012. All subjects agreed to participate in the prospective study and provided written informed consent for blood collection. The study protocol conformed to the ethical principles of the Declaration of Helsinki and was approved by the local research ethics committees of the Jikei University School of Medicine and Daiichi Sankyo Co., Ltd. The study design is shown in Fig. 1.

2.2. Data collection

Within the 311 blood samples from 154 patients, 7 blood samples from 4 patients were excluded due to the missing data about the biomarkers, therefore, the total of 304 blood samples from 150 patients were used for analysis (Fig. 1). At enrollment, a 5–10 mL blood sample was collected from each participant once to three times on different days using disposable needles and vacuum syringes. A total 304 and 46 blood samples from patients and healthy volunteers, respectively, were separated by centrifugation and stored at −80 °C until subsequent serological and biochemical tests. The assays of the levels of biochemical parameters were performed using commercial kits, i.e., ALT, AST, ALP, γ-GTP, total bile acids (TBA), albumin (ALB) and the prothrombin time (PT) were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan), and total bilirubin (T.BIL) and direct bilirubin (D.BIL) were

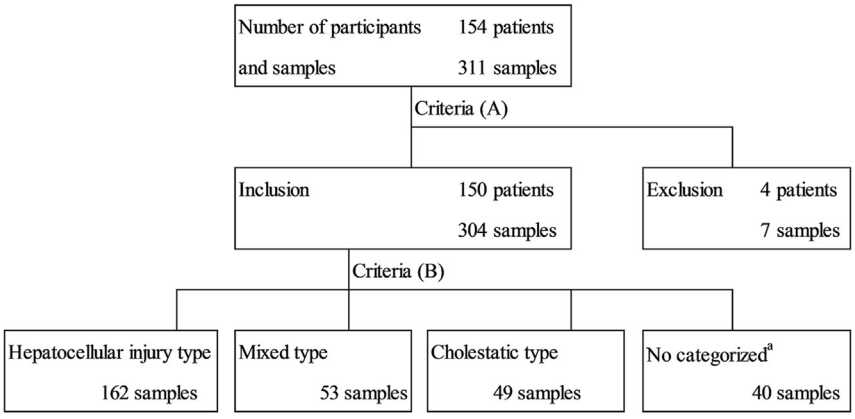

Fig. 1. The study flow diagram and background of the participants and samples. Criteria (A): The samples with the data for all markers used were included in the statistical analysis. Criteria (B): The types of liver injury were diagnosed by three physicians and were classified based on the assessment of at least two of the three physicians. [a] The type of liver injury was diagnosed as neither hepatocellular injury nor cholestatic type by two of three physicians.