^{1}H MR Spectroscopy of Human Bile in the Diagnosis of Chronic Cholestatic Disorders: Primary Sclerosing Cholangitis and Cholangiocarcinoma

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INTRODUCTION: Primary sclerosing cholangitis is an inflammatory and cholestatic liver disease characterized by progressive obliteration of intra- and extrahepatic bile ducts. The etiology of the disease is unknown and the natural history varies from patient to patient. In most cases, the disease progresses slowly towards liver failure. In the end-stages, PSC results in biliary cirrhosis, portal hypertension and is associated with high frequency of cholangiocarcinoma (CC). The common consequence of all forms of cholestatic disorders is the retention of bile acids in the hepatocytes. Bile acids have both protective and damaging effects on hepatocytes. They perform important physiologic functions such as cholesterol homeostasis by their own synthesis from cholesterol, and solubilization of fats and fat soluble vitamins. Elevated levels of bile acids have cytotoxic effects on hepatocytes and cholangiocytes and can bring about apoptosis or necrosis leading to various chronic cholestatic liver diseases such as PSC and CC. In cholestasis, the severity of the liver injury depends not only on increased levels of bile acids but also on the type of bile acid retained in the hepatocytes. The likely candidates are the hydrophobic bile acids such as DCA and CDCA which accumulate in hepatocytes and bring about hepatocellular injury. Convenient and rapid measurement of individual bile acids becomes necessary to identify the bile acid/s responsible for such hepatocellular damage and for the early detection of resulting diseases. MR spectroscopy offers a straightforward method for the rapid identification and quantification of individual conjugated bile acids in human bile [1]. In the present study, we have analyzed bile samples from patients suffering from various hepatobiliary disorders by ^{1}H MR spectroscopy.

MATERIALS AND METHODS: Bile samples were collected from patients (n=22) with cholestatic disorders during ERCP examination, after securing optimal catheter position in the common bile duct (contrast agent iohexol; Omnipaque® 240 mg I/ml). 1D ^{1}H MR spectra, and 2D DQF-COSY, TOCSY spectra were obtained for bile samples on a 360 MHz spectrometer (Bruker Instruments). ^{1}H MR spectra of all the bile samples were obtained with and without homonuclear decoupling of taurine and glycine methylene protons (25-CH_2) to get well resolved signals for amide protons of taurine/glycine conjugated bile acids. By rigorous analyses of all the NMR spectra and comparing with the library of chemical shifts of conjugated bile acids in bile [1], individual conjugated bile acids in human bile were identified.

RESULTS & DISCUSSION: Analysis of human bile using 1D and 2D NMR experiments and with the aid of chemical shifts data from studies on human bile resulted in unambiguous identification of major individual bile acids (GCA, GDCA, GCDCA, TCA, TDCA and TCDCA). All these six conjugated bile acids were seen in bile specimens from subjects with normal bile ducts. They were identified by their characteristic amide NH signals which invariably appeared in the region 7.8-8.1 ppm. It should be noted that the contrast agent did not produce any signal in the amide region of the spectrum. Glycine conjugated bile acids GCA, GDCA, and GCDCA and a taurine conjugated bile acid, TCA were well resolved in proton decoupled ^{1}H MR spectrum of bile, but other taurine conjugated bile acids, TDCA and TCDCA, were not completely resolved due to close resemblance in their chemical shifts. The assignments were further confirmed by 2D DQF-COSY and TOCSY experiments. We have analyzed 22 bile specimens from normal subjects and patients with cholestatic diseases (PSC, n=9; CC, n=7; PSC+CC, n=1; normal bile ducts, n=5). The amide NH protons served as diagnostic marker for the identification of individual conjugated bile acids. We found that in patients with PSC, the amide NH signal due to glycochenodeoxycholic acid (GCDCA) was absent in 8 out of 9 PSC cases, indicating the retention of GCDCA in the hepatocytes. This can be clearly seen in Fig. 1. As a result, GCDCA is not secreted into the bile of patients with PSC. Furthermore, the taurine conjugated bile acids were reduced considerably compared to glycine conjugated bile acids. It has been reported that GCDCA has direct cytotoxic effect on hepatocytes, and its retention in hepatocytes during cholestasis is greater than for other toxic bile acids [2]. Thus, absence of GCDCA in bile may serve as a diagnostic marker for the detection of PSC. In cholangiocarcinoma, the bile composition is greatly altered and the spectral observations revealed significant reductions of bile acids, phosphatidylcholine and cholesterol. As can be seen in Fig. 2, both the normal and the PSC spectra show significant levels of bile acids, which is not the case for CC. Some of the bile spectra of both PSC and CC were also characterized by the absence of phosphatidylcholine signals. These observations substantiate the potential of ^{1}H MRS as a diagnostic tool for the early detection of cholestatic liver diseases.

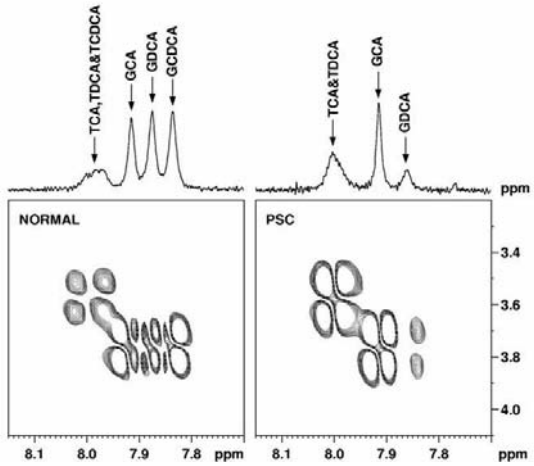

Figure 1: Parts of DQF-COSY spectra of bile showing the amide NH region of the taurine/glycine conjugated bile acids

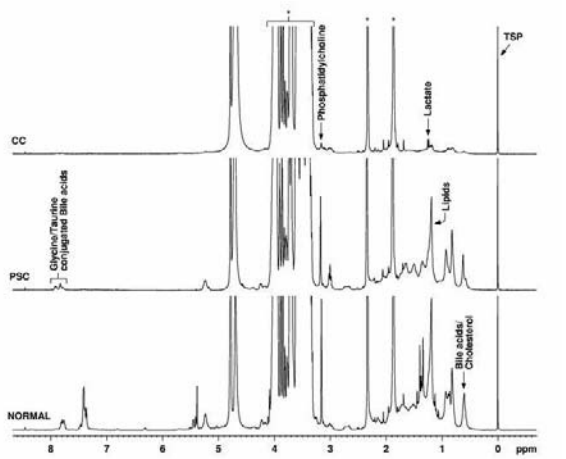

Figure 2: ^{1}H MR spectra of bile from normal, PSC and CC patients (* Contrast agent: Omnipaque)

CONCLUSION: ^{1}H MRS analysis of bile shows that the absence of chenodeoxycholic acid (CDCA) can be a diagnostic marker for the early detection of primary sclerosing cholangitis (PSC). In addition, biliary components were found greatly decreased in cholangiocarcinoma compared to bile from subjects with normal ducts.

REFERENCES

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