Resemblance of ^{1}H MRS Pattern of Bile to that of Serum may Indicate Alterations in Tight Junctions in Hepatocytes

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INTRODUCTION: Tight junctions (TJs) are associated with hepatocytes and cholangiocytes [1]. Specifically, TJs associated with hepatocytes are concentrated around bile canaliculi, and serve as barrier to keep bile in bile canaliculi away from blood-circulation (sometimes referred to as blood–biliary barrier) [1]. Alterations in TJs have been observed in various chronic cholestatic diseases such as primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), including extrahepatic cholestasis [1]. Such alterations have resulted in an increased paracellular permeability leading to regurgitation of bile from biliary tract. Although bile is isosmotic with blood/plasma, with the secretion of osmotically active solutes such as bile acids into the bile, osmotic gradients are established through hepatocytes and/or via TJs [2]. In cholestatic conditions, any damage to the hepatocytes/TJs may lead to paracellular regurgitation of bile and/or plasma. In this study, we have analyzed bile samples obtained from various chronic cholestatic patients to determine if any of these patients show indications of paracellular regurgitation.

MATERIALS & METHODS: Bile samples were obtained from patients (n=43: PSC = 34; Pancreatic cancer = 5; Cholangiocarcinoma = 3; Gallstones = 1) undergoing endoscopic retrograde cholangiopancreatography (ERCP) examination for various cholestatic conditions. ^{1}H MR spectra of bile samples were obtained using a 360/600 MHz NMR spectrometer (Bruker Biospin, Fällanden). 1D ^{1}H MR spectra were obtained using one-pulse and CPMG sequences with water presaturation; and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) was used as an external chemical shift reference. The following acquisition parameters were used: number of scans = 64, number of points in the time domain = 32 k, spectral width = 7211 Hz, acquisition time = 2.27 s and line broadening for exponential window function = 0.3 Hz. In the CPMG experiments similar parameters were used along with an effective echo-time ($2n\tau$) of 160 (600 MHz)/480 (360 MHz) ms.

RESULTS & DISCUSSION: Figure 1 shows typical ^{1}H MR spectra of bile samples from a PSC patient showing normal spectral pattern (a), and a PSC patient showing serum-like spectral pattern (b). ^{1}H MR spectrum of bile obtained from healthy subjects shows the presence of signals from lipids such as various bile acids conjugated to amino acids – glycine and/or taurine, cholesterol and phospholipids (similar to Fig. 1a). In our earlier studies, we have observed that bile samples from cholestatic patients showed decreased levels of these lipid components [3]. A few patients also showed the absence of either glycine-conjugated bile acid (glycochenodeoxycholic acid) or phosphatidylcholine. We also measured the conjugation ratio of bile acids in bile [i.e. the ratio of glycine-conjugated bile acids (GCBAs) to taurine-conjuagted bile acids (TCBAs)], and observed that this ratio was decreased in cholestatic patients [4]. In the present study, we observed that the ^{1}H MR spectral patterns of bile samples from 2 cholestatic patients (PSC & pancreatic cancer) resembled that of serum (Figure 1b). Moreover, these 2 samples also showed the presence of glucose. We hypothesize that this anomaly could be due to alterations in TJs and regurgitation of blood constituents into the bile.

In normal conditions, TJs act as major anatomical barrier between bile and blood by sealing the lumen of bile canaliculi between adjacent hepatocytes. In an earlier study, Sakisaka et al. have reported that, in patients with PSC and/or extrahepatic cholestasis, TJ-associated protein 7H6 was considerably down-regulated [1]. 7H6 is closely related with paracellular permeability in several organs including liver, and down-regulation of this protein has been correlated to the alterations in TJs. Bile regurgitation takes place in patients with altered TJs due to increased paracellular permeability in hepatocytes [1]. Furthermore, alterations in hepatocyte TJs in PSC has been considered to be a secondary change resulting from impaired bile flow caused by narrowing or obliteration of bile ducts [1]. The serum-like spectral patterns shown in bile samples from 2 patients (PSC and pancreatic cancer) could be due to alterations in the TJs in hepatocytes. Additionally, we have also observed the presence of glucose in both bile samples showing serum-like patterns. Glucose is virtually absent in human bile. The reason for the absence of glucose in bile is unknown, but it is believed that glucose is being removed from the bile by active transport at some site along the biliary tree [5]. The presence of glucose in both bile samples showing serum-like spectral patterns also indicates disruption of the blood-biliary barrier or underlying pathology associated with the biliary tree. Freeze-fraction electron microscopy (FFEM) of biopsied liver tissue specimen is generally performed to evaluate hepatocyte-TJ morphology to identify alteration in the TJs. ^{1}H MRS of bile could be valuable as an indirect measure of detecting TJ abnormality, a possible alternative to FFEM.

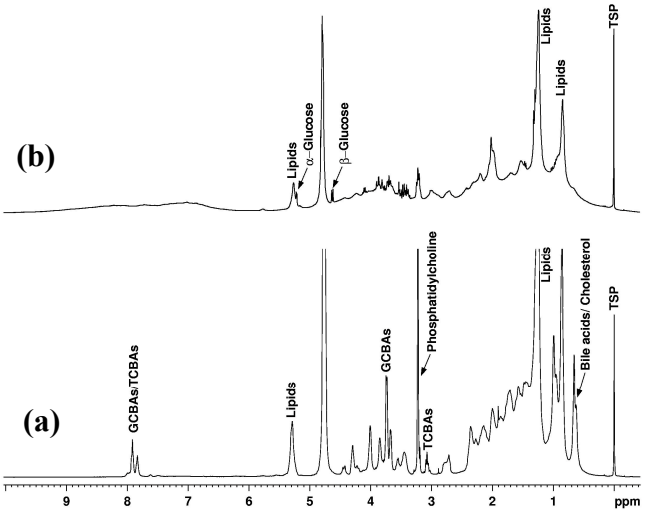

Figure 1: Typical ^{1}H MR spectra (360 MHz) of bile samples from (a) a PSC patient showing normal biliary pattern and (b) a PSC patient showing serum-like spectral pattern.

CONCLUSION: Serum-like spectral pattern of bile samples in cholestatic patients may indicate underlying pathology associated with the alterations in TJs in hepatocytes. Such pathological condition may be detected by ^{1}H MRS of bile.

REFERENCES: 1. Sakisaka S, Kawaguchi T, Taniguchi E et al. *Hepatology* 2001;**33**:1460-1468.
2. Boyer JL. *Hepatology* 1983;**3**:614-617.
3. Albiin N, Smith ICP, Arnelo U. *Acta Radiol* 2008;**49**:855-862.
4. Ijare OB, Bezabeh T, Albiin N et al. *J Pharm Biomed Anal* 2010;**53**:667-673.
5. Guzelian P, Boyer JL. *J Clin Invest* 1974;**53**:526-535.