Magnetic Resonance Spectroscopic Evidence for Pancreaticobiliary Reflux in Hepatobiliary Malignancies

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INTRODUCTION: Regurgitation of pancreatic juice (PJ) into the biliary tract and the subsequent mixing with bile [also known as Pancreaticobiliary (PB) reflux], is frequently observed in patients with pancreaticobiliary maljunction (PBM). PBM is a congenital anomaly characterized by an uncommon union of biliary and pancreatic ducts outside the duodenal wall, forming a long common channel [1]. In patients with PBM, the common channel can be so long that the action of sphincter of Oddi does not functionally affect the junction, resulting in mutual regurgitation of bile and PJ [2]. Presence of PBM has been considered to be a predisposing factor for the carcinogenesis of bile duct and gallbladder (GB), as well as pancreatic disorders such as acute pancreatitis [1,2]. PB reflux has also been observed in some patients without PBM [1]. These patients either had relatively long common channel or normal pancreaticobiliary junction. Previous *ex vivo* studies tested the suitability of MRS for the detection of PB reflux through the analysis of bile [3]. In this study, we analyzed bile samples from patients with cholangiocarcinoma (CC) and GB cancer (GBC) to determine if any of these patients show PB reflux.

MATERIALS & METHODS: Bile samples from patients (n = 50: Primary sclerosing cholangitis (PSC) = 34; CC = 3; GBC = 7; Pancreatic cancer = 5; Gallstones = 1) undergoing endoscopic retrograde cholangiopancreatography (ERCP), cholecystectomy or liver transplantation were collected, and stored at -80°C until analysis. ^{1}H MR spectra were obtained using a 360/600/800 MHz NMR spectrometer (Bruker Biospin, Fällanden). 1D ^{1}H MR spectra were obtained using one-pulse and CPMG sequences with presaturation of water resonance. An external standard of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) was used as a chemical shift reference. The following acquisition parameters were used: number of scans = 64, recycle delay = 5 s, number of points in the time domain = 32 k, spectral width = 12.0 ppm, 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 160/480 ms. The clinical diagnoses were made by the use of US, CT, ERCP, and/or MRI. All malignancies were verified by histopathology.

RESULTS & DISCUSSION: Figure 1 shows typical ^{1}H MR spectra of bile samples from patients with gallstones (control), GBC and CC. Spectra from patients with GBC and CC clearly show the presence of signals due to aromatic amino acids tyrosine, phenylalanine, histidine and tryptophan. Moreover, CPMG experiment revealed the presence of other amino acids – valine, leucine, isoleucine, threonine, alanine, lysine, glutamate, glutamine, and glycine – in the aliphatic region. Amino acids are generally absent in human bile under normal physiology. From a previous *in vitro* study in our laboratory, we have confirmed that one of the reasons for the presence of amino acids in bile is due to the PB reflux [3]. In the present study, 5/7 patients (71.4%) with GBC and 1/3 (33.3%) CC patients showed PB-reflux. Twelve out of 34 PSC patients (~35%) also showed this phenomenon, while it was absent in the pancreatic cancer (n = 5) patients.

In the earlier *in vitro* study, a mixture of bile and pancreatic juice (1:1, v/v) incubated for 48 hrs showed a gradual decrease in the levels of phosphatidylcholine (PC), which was attributed to its hydrolysis [3]. In this study, we confirmed the hydrolysis product to be lysophosphatidylcholine (lysoPC), suggesting the presence of phospholipase-A_2 as an active phospholipase in PJ samples mixed with bile [4]. Since PC is an important component of bile protecting hepatocytes and biliary epithelium from harmful effects of bile acids, its absence from bile could be cytotoxic to both hepatocytes and cholangiocytes. An *in vitro* study on model bile containing lysoPC also showed significant inhibition of the proliferation of human GB epithelial cells [5], indicating detrimental effects of lysoPC on epithelium. In patients with cholestatic/inflammatory diseases such as PSC/cholecystitis, the absence of PC in bile could be a potential risk factor for the progression towards malignant transformations [6]. In this study, we have observed the absence/hydrolysis of PC in all of the 18 bile samples that were mixed with PJ inside the body; and notably in 5/7 GBC and 1/3 CC patients. Twelve of the 34 PSC patients with PB-reflux also showed the absence of PC in the bile. These patients could be at increased risk for developing CC in the long run. All these observations support the hypothesis that reflux of PJ into the biliary tract or GB could be a potential risk factor for the development of cholestatic conditions and the ultimate progression towards carcinogenesis of bile ducts/GB, as observed in patients with PBM [1, 2].

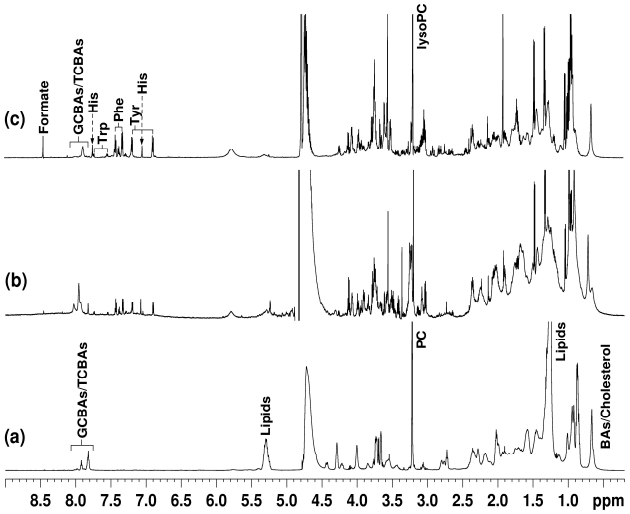

Figure 1: Typical ^{1}H MR spectra of bile samples from (a) control subject, (b) gallbladder cancer (GBC) and (c) cholangiocarcinoma (CC); the latter two showing the presence of aromatic amino acids – Tyr, His, Phe, and Trp – indicative of PB reflux (BAs: Bile acids; GCBAs: Glycine-conjugated bile acids; TCBAs: Taurine-conjugated bile acids; PC: Phosphatidylcholine).

CONCLUSION: The present study supports the hypothesis that PB reflux is a potential risk factor for hepatobiliary malignancies. This study could be extended to *in vivo* applications, which may lead to non-invasive diagnosis of PB reflux using MRS of GB bile. Such an approach would be valuable in the non-invasive diagnosis of hepatobiliary malignancies.

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