# $^1$H MRS of Human Bile in the Differential Diagnosis of Cholangiocarcinoma and Pancreatic Cancer

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**INTRODUCTION:** Cholangiocarcinoma (CC) is a relatively rare but an important malignancy of the biliary tract with an annual incidence of about 3500 in the US, whereas pancreatic cancer is a devastating malignancy with an annual incidence of >35,000 in the US. Both cancers are difficult to diagnose, and are associated with poor prognosis and high mortality. Primary sclerosing cholangitis (PSC) is a predisposing factor for the development of CC. Patients with cholangiocarcinoma present with cholestatic symptoms such as jaundice, pruritus, bilirubinuria, and they also show weight loss and abdominal pain. Diagnosis is usually performed by imaging methods such as ultrasound (US), computed tomography (CT), endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiopancreatography (PTC), magnetic resonance cholangiopancreatography (MRCP) and /or endoscopic ultrasound (EUS). Imaging methods can only detect tumors after the cancer has progressed to a certain extent. Moreover, it is difficult to differentiate CC in patients presenting with PSC. Pancreatic cancer also presents with similar symptoms and is diagnosed by CT, EUS, and ERCP/MRCP. These imaging modalities always fail to differentiate pancreatic cancer in the setting of chronic pancreatitis. Identification of molecular markers will be of value for the detection of these pancreaticobiliary malignancies. To date, there are no specific tumor markers for the detection of both CC and pancreatic cancer. Carbohydrate antigen, CA 19-9 and carcinoembryonic antigen (CEA) are commonly used tumor markers. CA 19-9 has similar sensitivity and specificity for both cancers. Both CA 19-9 and CEA have been measured in bile in benign and malignant pancreaticobiliary diseases, but no consistent differences have been observed [1]. In the present study, we have analyzed the bile samples from CC and pancreatic cancer patients, for the low molecular weight metabolites such as bile salts, phosphatidylcholine, cholesterol, amino acids, and carbohydrates using $^1$H MR spectroscopy.

**MATERIALS AND METHODS:** Bile samples were collected from patients with cholangiocarcinoma (n=12), pancreatic cancer (n=4), and from normal reference (n=10) during an ERCP examination, after securing optimal catheter position in the common bile duct (with/without contrast agent: iohexol, Omnipaque® 240 mg I/ml), about 2-10 ml of bile was aspirated for MRS analysis. 1D $^1$H MR spectra of bile samples were obtained by single-pulse and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences with presaturation, on a 360 MHz spectrometer (Bruker Instruments). 2D $^1$H-$^1$H DQF-COSY and TOCSY experiments were performed for all samples. Constituent bile salts, phosphatidylcholine, glycerophosphocholine, D-glucuronate and other metabolites in bile were identified with the help of these experiments and their presence confirmed by the addition of standard samples.

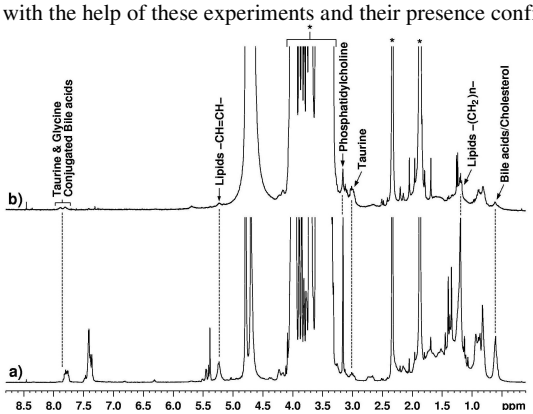


**Figure 1: $^1$H MR spectra of bile from (a) Normal reference and (b) Cholangiocarcinoma patients (*:Contrast agent: Omnipaque).**

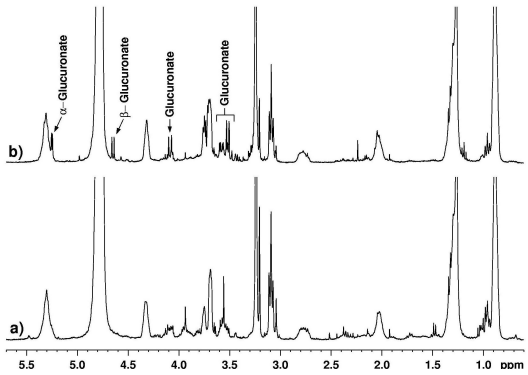


**Figure 2: $^1$H MR spectra of bile from (a) Normal reference and (b) Pancreatic cancer patients obtained by CPMG pulse-sequence.**

**RESULTS & DISCUSSION:** Figure 1 shows typical 1D $^1$H MR spectra of bile samples from (a) normal reference and (b) cholangiocarcinoma. The bile from CC patients is characterized by the reduced levels of major lipid components, bile salts, phosphatidylcholine and cholesterol. The bile contains various glycine- and taurine-conjugated bile acids and they can be identified by looking at their characteristic signals in the region 7.8-8.1 ppm. Furthermore, 2D $^1$H-$^1$H DQF-COSY experiments performed on these samples reveal that phosphatidylcholine is absent in most of the CC patients (n=10). Figure 2 shows typical 1D $^1$H MR spectra bile samples from (a) normal reference and (b) pancreatic cancer obtained by CPMG pulse sequence. The spectral patterns of bile samples from normal reference and pancreatic cancer are well differentiated. The $^1$H MR spectrum of bile from a pancreatic cancer patient is characterized by the presence of D-glucuronate signals (Fig. 2b). This was observed in all the four bile samples from pancreatic cancer patients. The reason for the presence of D-glucuronate in bile of pancreatic cancer patients is unclear. Due to the close structural resemblance of D-glucuronate with D-glucose, and hence very similar chemical shift values, we had initially reported these signals to be due to D-glucose [2]. Later, this biochemical was confirmed to be D-glucuronate after performing other MR experiments (2D DQF-COSY, TOCSY & $^{13}$C MRS) and also by addition of standard compound to the bile. Thus, the presence of D-glucuronate in the bile of pancreatic cancer patients could serve as a diagnostic marker for the detection of pancreatic cancer. Analysis of human bile could be a useful tool for the study of pancreaticobiliary malignancies. Gallbladder cancer (GBC) is another common malignancy of the pancreaticobiliary system which shows similar spectral patterns as that of CC, but the bile samples obtained from GBC patients show elevated levels of amino acids such as leucine, valine, alanine, glycine, phenylalanine, histidine and tyrosine which are not observed in bile obtained from CC patients [3]. Bile samples from GBC patients also show the presence of lactate, acetate, choline, and urea in significant levels. Thus, one may be able to determine the exact type of malignancy in the pancreaticobiliary system by looking at a single spectrum of bile.

**CONCLUSION:** $^1$H MRS of bile could be valuable in differentiating various pancreaticobiliary malignancies based on the biochemical composition. The observations made in the present study will be of great significance in the differential diagnosis of cholangiocarcinoma and pancreatic cancer.

**REFERENCES:**

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