Absence of Phosphatidylcholine (PC) in Bile of Cholestatic Patients could be a Potential Risk Factor for Cholangiocarcinoma: A ^{1}H MRS Study

O. B. Ijare[1], T. Bezabeh[1], N. Albiin[2], U. Arnelo[2], B. Lindberg[2], and I. C. Smith[1]

[1]National Research Council Institute for Biodiagnostics, Winnipeg, Manitoba, Canada, [2]Karolinska University Hospital, Karolinska Institutet, Huddinge, Stockholm, Sweden

INTRODUCTION: Cholestasis is an impairment in the transportation of bile from the liver to the intestine. Composition of bile is altered in cholestatic diseases such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and cholangiocarcinoma (CC). Cholangiocarcinoma (CC) is a devastating malignancy, is difficult to diagnose, and is associated with a high mortality. PSC is the commonest known predisposing factor for this cancer. CC rates of 8–40% have been reported in patients with PSC in follow-up studies and explant specimens [1]. PSC is an inflammatory and chronic cholestatic liver disease characterized by progressive obliteration of intra- and extrahepatic bile ducts. It results in biliary obstruction and finally proceeds towards end-stage liver disease. Bile is an aqueous secretion of hepatocytes containing bile salts, cholesterol and phosphatidylcholine (PC) as major lipid components. Bile salts in bile have both protective and harmful effects on the hepatobiliary system. They form vesicles/mixed micelles with phospholipids and cholesterol in the normal physiology, but exercise harmful effects on cholangiocytes in the absence of PC. PC is synthesized in the liver by cystidine diphosphate-choline (CDP-choline) or by the phosphoethanolamine *N*-methyltransferase (PEMT) pathway and is transported to the bile by a specific transport protein, multidrug-resistant protein (MDR3). PC helps bile salts in the formation of mixed micelles with cholesterol. As a result, bile salts and cholesterol solubilize in aqueous bile, and flow to the intestine where they aid in the emulsification of fats/fat-soluble vitamins. In an animal study, MDR2 (a rodent homolog of human MDR3 protein) knock-out mice did not secret PC into the bile [2]. The absence of PC in bile will affect the micellization of bile salts and cholesterol which in turn results in elevated levels of free bile salts in the bile. Free bile salts are hydrophobic in nature and show cytotoxic effects on cholangiocytes due to their detergent properties and may bring about cholangiocellular damage.

MATERIALS AND METHODS: Bile samples were collected from patients with chronic cholestatic diseases (n=47) during an ERCP examination. After securing an optimal catheter position in the common bile duct (contrast agent: iohexol, Omnipaque® 240 mg I/ml), about 2-10 ml of bile was aspirated for MRS analysis. 1D ^{1}H MR spectra, 2D ^{1}H-^{1}H DQF-COSY and TOCSY spectra were obtained for all bile samples on a 360 MHz spectrometer (Bruker Instruments). Constituent bile salts, PC, and glycerophosphocholine (GPC) in bile were identified with the help of 2D DQF-COSY and TOCSY experiments and also by comparison with their chemical shift values reported in the literature.

RESULTS & DISCUSSION: Human bile samples obtained from patients with PSC (n=20), CC (n=12), CC+PSC (n=4), and normal reference patients (n=11) were analyzed using 1D and 2D NMR experiments. ^{1}H MRS patterns of all patient-groups were compared. Spectra were analyzed for major lipid components such as bile salts, cholesterol and phosphatidylcholine (PC). Figure 1 shows parts of ^{1}H-^{1}H DQF-COSY spectra of the bile samples from various patient-groups: (a) Normal reference, (b) PSC, and (c) CC. Spectral patterns of (CC+PSC) were almost similar to CC. The ^{1}H MR spectrum of normal bile shows characteristic signals for PC due to 2-CH (at ~5.34 ppm) and 1-CH_2 (at H_a:4.43 & H_b: 4.23 ppm) of the PC-glycerol backbone. We could observe the presence of these signals in the normal reference patient spectrum shown in Figure 1a. If we compare the DQF-COSY spectra of bile samples obtained from CC and PSC patients with that of a normal reference patient, it is clearly observed that both the CC and PSC patients showed an absence of PC-glycerol signals and hence PC in their bile (Figure 1b&c). Out of 16 CC/(CC+PSC) patients, 13 showed absence of PC; and also out of 20 PSC patients, 13 showed an absence of PC. Of the 11 normal reference patients, PC was present in 9 of them. The two patients whose bile samples did not show the presence of PC had elevated levels of plasma bilirubin, which is an indication of underlying cholestasis. The absence of PC in bile, in CC and/or PSC patients may be due to its hydrolysis in the presence of inflammatory mediators or to a possible defect in the PC-transport protein, MDR3 [2].

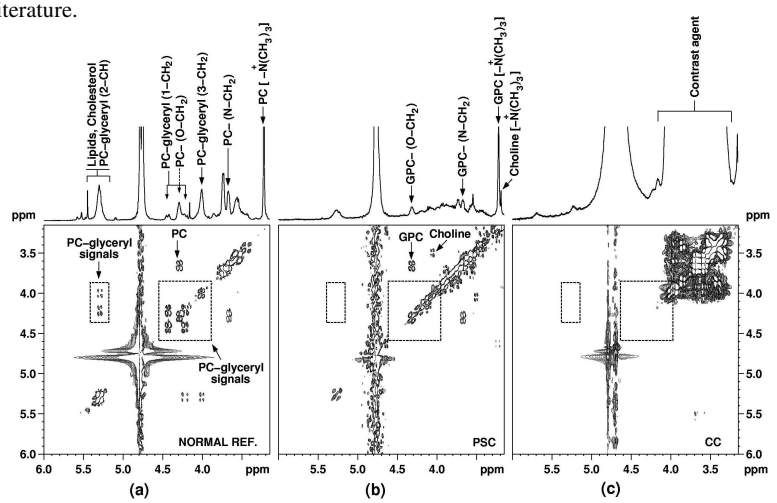

Figure 1: Parts of ^{1}H-^{1}H DQF-COSY spectra of typical bile samples from (a) Normal reference (b) PSC and (c) CC patients showing the presence/absence of phosphatidylcholine (PC) signals. PC was observed only in the normal reference patient (Most of the bile samples analyzed contained contrast agent, but we used bile without contrast agent from normal reference & PSC patients for clarity in the peak labeling).

We have observed the absence of PC in most of the CC (10/12) and CC+PSC (3/4) patients. PC is an important component of bile, helping in the micellization of bile salts and cholesterol, and protecting cholangiocytes from the harmful effects of the free bile salts. PSC has been considered as a potential risk factor for the development of CC. In the present study, we have observed the absence of PC in 13/20 (65%) bile samples obtained from PSC patients. In patients with PSC, the bile devoid of PC will be rich in free bile salts and the cholangiocytes will be constantly exposed to the toxic bile salts, which may lead to proliferation of cholangiocytes via the activation of epidermal growth factor receptor (EGFR) [3]. The absence of PC in chronic cholestatic diseases such as PSC could be treated as a potential risk factor for the development of CC.

CONCLUSION: ^{1}H MRS analysis of bile shows the absence of phosphatidylcholine (PC) in chronic cholestatic diseases such as PSC, which could be a potential risk factor for the development of cholangiocarcinoma (CC) in this patient cohort.

REFERENCES:

1. Khan SA,Thomas HC, Davidson BR et al. *Lancet* 2005; **366**, 1303-1314.
2. Pratt DS. *Curr Opin Gastroenterol* 2005; **21**: 270-274.
3. Wernburg NW, Yoon JH, Higuchi H et al. *Am J Physiol Gastrointest Liver Physiol* 2003; **285:**G31-G36.