Discrimination of pancreaticohepatobiliary cancer and benign patients in presence and absence of jaundice and cholangitis: 1H and 31P NMR studies of bile

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INTRODUCTION: The increasing rates of mortality of pancreaticohepatobiliary cancer patients warrant the need for early, accurate and non-invasive screening methods. The normally used modalities such as imaging and cytology are often unreliable especially in the detection of tumors at an early stage. The preliminary findings by using ^{1}H and ^{31}P NMR spectroscopy of bile for reduced phosphatidylcholine level in pancreaticobiliary cancer compared to benign patients has kindled a new interest in gastroenterologists for the development of novel diagnostic strategies in cancer management[1]. As reported earlier[1] the discrimination between benign and malignant patients using ^{1}H and ^{31}P NMR spectroscopy of bile is possible only in absence of jaundice and cholngitis. The studies reported herein are aimed at finding out whether the biliary constituents indices provide independent diagnostic indices for the benign and cancer patients in presence of jaundice and cholangitis.

MATERIALS AND METHODS: ^{1}H and ^{31}P NMR spectroscopy were used on bile specimens collected during routine ERCP (Endoscopic retrograde cholangiopancreatography) or PTBD (Percutaneous transhepatic biliary drainage) performed on patients (n=48) being investigated/treated/routine management for undiagnosed extrahepatic biliary obstruction or pancreaticohepatobiliary disease/cancer. Diagnosis of cause of obstruction was based on the basis of history, initial clinical examination, liver function tests and/or ultrasound scanning/imaging modality. Patients were considered as having jaundice if serum bilirubin level >1.0 mg/dL had been documented. Cholangitis was established in patients who had fever >38^{O}C with raised total leucocyte counts (TLC) >11,000 cells/mm^3 (without any other source of infection) and/or bile culture positivity. Patients with intrahepatic biliary obstruction, liver abscess, underlying cirrhosis, or patients in which bile specimens were mixed with blood were excluded from the study. Index bile specimens (~1 mL) were collected during PTBD and ERCP in sterile vials, snap frozen in liquid nitrogen kept in dark till NMR analysis was performed.

Quantitative estimation of phosphatidylcholine (PtC) and inorganic phosphate (Pi) was performed by ^{31}P NMR experiments on neat bile specimens. Other chief biliary components like total bile acids (TBA) and cholesterol (Chol.) were determined by performing ^{1}H NMR experiments on initially lyophilized bile specimens dissolved in DMSO-d_6[2]. Values are reported as median (µM/L) and differences in two groups were determined with Mann Whitney test. Relationships between different variables were determined with Pearson correlation coefficient and Spearman rho correlation coefficient.

RESULTS: The study population (n=48) was divided in 4 groups based on etiology and presence and absence of jaundice and cholangitis**:** The spectrum of etiology of benign patients (n=20) was: choledolithiasis (6), choledocholithiasis (8), pancreatitis (1), benign biliary strictures (4) and cholecystitis (1); and malignant patients (n=28) was: carcinoma of the gall bladder (19), cholangiocarcinoma (7) and periampullary carcinoma (2). Liver function tests were severely deranged in benign as well as malignant patients with jaundice and cholangitis. Based on the presence of jaundice and cholangitis benign and malignant patients were further subdivided each group in two subgroups: (a) Benign patients without jaundice and cholangitis (n=10) [(median bilirubin level 0.4 mg/dL) and cholangitis (median TLC 6800 cells/mm^3)] (b) benign with jaundice and cholangitis (n=10), [median bilirubin level 3.2 mg/dL), and cholangitis 11200 cells/mm^3 (c) malignant without jaundice and cholangitis (n=9)[(median bilirubin level 0.7 and cholangitis (median TLC 8200 cells/mm^3) (d) malignant with jaundice and cholangitis (n=19), [(median bilirubin level 12.4 mg/dL) and cholangitis (median TLC 11250 cells/mm^3)].

^{1}H and ^{31}P NMR spectra of bile (Fig 1-3) suggested that (1) Presence of jaundice significantly suppresses biliary constituents in bile in both benign as well as malignant groups. Benign patients without jaundice and cholangitis vs with jaundice and cholangitis: TBA 74200 vs 15800; Chol. 6800 vs 2200; PtC 10400 vs 1400 ($p<0.05$ for all); Pi 400 vs 600 (p=NS). Malignant patients without jaundice and cholangitis vs with jaundice and cholangitis: TBA 47500 vs ND; Chol. 6700 vs ND; PtC 1100 vs ND ($p<0.001$ for all); Pi 100 vs ND ($p<0.01$). (2) With the increasing level of bilirubin and cholangitis, the biliary constituents (TBA, Chol and PtC) decreased significantly [(with bilirubin level, pearson correlation > -0.60 for all) and with cholangitis (TLC) (spearman rho >-0.49 for all)]; (3) Median concentrations of chief biliary constituents are not different in benign patients with jaundice and cholangitis compared to malignant patients without jaundice and cholangitis (p=NS for all).

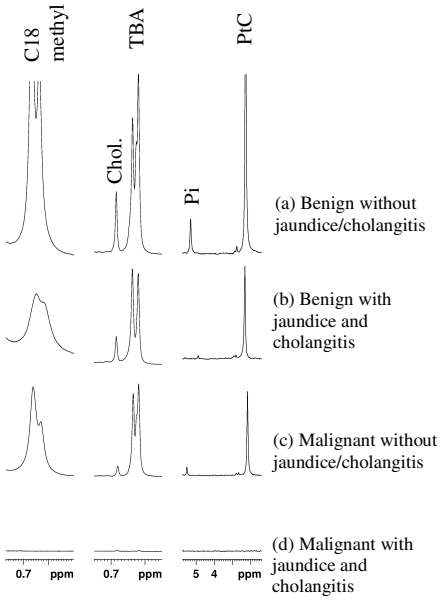

Fig (1) Fig (2) Fig (3)

FIG: Spectra of relevant portion of (1) ^{1}H NMR of neat bile (2) ^{1}H NMR of neat bile in DMSO- d_6 (3) ^{31}P NMR of neat bile

DISCUSSION: Significantly lower indices of Biliary constituents (TBA, Chol., PtC, and Pi) in malignant compared to benign, in the absence of jaundice and cholangitis were observed as reported earlier[1] also. However, jaundice and cholangitis significantly lower biliary constituents in bile in both benign as well as malignant patients probably due to the reduced export of biliary constituents in bile via specific tranporters located on canalicular membrane of hepatocytes to biliary sinusoids and finally to common biliary duct[3]. This hypothesis is supported by previous experimental and human studies demonstrating that function of multi drug resistant P glycoprotein (MDR2) and its human equivalent MDR3: an export pump for phospholipids is down regulated during cholestasis and jaundice[4]. The Biliary constituents in benign with jaundice and cholangitis is the invariant as compared to malignant without jaundice and cholangitis.^{1}H and ^{31}P NMR spectroscopy of bile can discriminate benign and malignant patients on the basis of chief biliary constituents (total bile acids, cholesterol, phospholipids and inorganic phosphate) in the absence of jaundice.

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