# Increasing indices of bile constituents following decompression therapy are indicators of restorative function of hepatocytes: 1H and 31P NMR studies

**P. Tripathi[1], L. Bala[1], G. Choudhuri[2], and C. L. Khetrapal[1]**

[1]CBMR, Centre of Biomedical Magnetic Resonance, Lucknow, Uttar Pradesh, India, [2]Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

**INTRODUCTION:** Among various causes of extrahepatic biliary obstruction secondary to malignancy, the common ones are tumor of the gall bladder and common bile duct, carcinoma of head of pancreas and malignant stricture of bile duct. Jaundice and cholangitis are common complications of majority of malignant biliary obstructed patients and are often considered as risk factors predicting advance disease and early mortality (1, 2). Surgery in jaundiced patients with a tumor is associated with a higher risk of postoperative complications compared with surgery in non jaundiced patients. Hence, prevention and palliation of debilitating symptoms is critically important for management of such patients. With the advancements in biliary catheterization techniques, Percutaneous Transhepatic Biliary Drainage (PTBD) and stenting has evolved to be a safe, effective and established technique of biliary decompression and became popular as a preoperative, risk reducing measure for treatment/palliation of symptoms. Although long-term survival is poor in patients with malignant biliary obstruction especially those with unresectable tumors, drainage has been shown to improve prognosis and quality of life (3). It has been reported earlier that reduced concentration indices of biliary constituents are indicator of jaundice and cholangitis (4). However, serial alterations in the biliary constituents in response to the decompression therapy in patients with malignant biliary obstruction and infection, has not yet been reported. Such studies have been reported herein. Specific aims addressed are (a) Indices of biliary constituents at presentation (day 0; at the time of PTBD); (b) serial changes in indices of biliary constituents following drainage; (c) extent of restoration of biliary constituents indices in patients with/without cholangitis.

**MATERIALS AND METHODS:** $^{1}$H and $^{31}$P NMR spectroscopy were used on bile specimens collected from the patients (n=19) undergoing PTBD as a routine management for malignant extrahepatic biliary obstruction. Diagnosis of cause of obstruction was based on the basis of detailed history, initial clinical examination, liver function tests, ultrasonography and/or imaging modality (computed tomography/MRI). Serial bile specimens were collected aseptically on day 0 (at presentation while performing PTBD), thereafter, on day 1, midweek and at the end of 1 week. 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 >38ºC 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, extrahepatic obstruction with benign cause, liver abscess, underlying cirrhosis, or patients in which bile specimens while sampling were mixed with blood while performing PTBD, patients in whom serial bile sampling could not be followed till the end of 1 week or patients who did not have cholangitis at presentation but developed it later because of any methodological flaws (complications related to stent placement principally sepsis) following PTBD were excluded from the study. For NMR analysis, about 1 mL of bile specimens were collected, snap frozen in liquid nitrogen, kept in dark and stored in -80$^0$C, till NMR experiments were performed. Quantitative estimation of phosphodiesters (PDE) 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-$d_6$.

**RESULTS**: Nineteen patients median age 54.5 yrs [range (36-76) yrs; male 7] with extrahepatic malignant biliary obstruction with/without infection were included in the study**.** The etiology of patients was as follows: carcinoma of the gall bladder (10), cholangiocarcinoma (7), malignant biliary stricture (2). Liver function tests clearly indicate a significant progressive drop in bilirubin level, TLC and a trend of liver function tests returning towards normal by the end of 1 week. Based on the presence and absence of cholangitis the patients were further divided in two subgroups: **Patients without cholangitis (n=8):** Eight patients were established as without cholangitis, however, these patients had clinically proven extrahepatic malignant biliary obstruction and clinical symptoms of jaundice (bilirubin level > 1.0 mg/dL). **Patients with cholangitis (n=11):** Eleven patients were established as having cholangitis before inserting catheter via PTBD on day 0. All the patients had leucocytosis (TLC > 11,000 cells/mm$^3$). Fever exceeded 38.5ºC in nine patients (81.8%). Out of nine, bile culture was positive for bacteria in eight patients (88.9%) and negative in one. Two had mild fever with negative bile culture. These two patients who had a negative bile culture had also purulent bile and were on antibiotic treatment prior to entry. All eleven patients had clinical evidence of jaundice (bilirubin level >1.0 mg/dL) with proven malignant biliary obstruction.

From $^{1}$H NMR spectra it can be seen that on day 0 the concentration of various biliary constituents; total bile acids, cholesterol and PDE in neat index bile are low in almost all nineteen bile specimens (Fig.1 and 2). $^{1}$H NMR spectra of day 1 bile obtained after 24 hrs after PTBD procedure shows that biliary constituents started appearing in almost all nineteen bile specimens in significant concentration (P<0.01). In midweek bile biliary constituents were more in comparison to day 1 bile. Bile by the end of 1 week shows significantly high concentration indices of biliary constituents with respect to day 0 (P<0.001).

Restoration of bile acids and cholesterol is more pronounced in general compared to PDE in both the groups. In patients with cholangitis restoration of TBA, Chol and PDE is more significant in Day 1 (P<0.01) (Fig.1) compared to Day 0, as compared to the patients without cholangitis (P<0.05) (Fig.2).

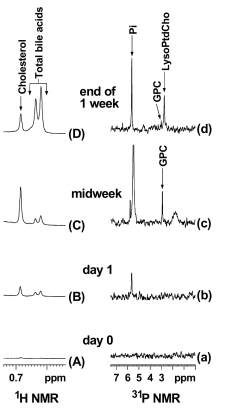


Fig.1 Spectra of relevant portion of $^{1}$H NMR of neat bile in DMSO-$d_6$ (A, B, C and D) and $^{31}$P NMR of neat bile (a, b, c and d) in a typical patient without cholangitis obtained serially following drainage.

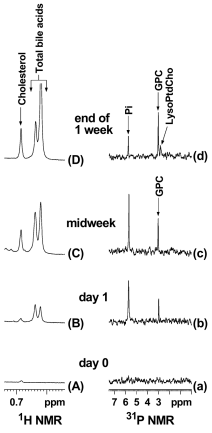


Fig.2 Spectra of relevant portion of $^{1}$H NMR of neat bile in DMSO-$d_6$ (A, B, C and D) and $^{31}$P NMR of neat bile (a, b, c and d) in a typical patient with cholangitis obtained serially following drainage.

**DISCUSSION:** The observation of undetectable to significantly lower indices of biliary constituents in index bile (day 0) in all cases can be explained as follows: bile is synthesized in liver and concentration of biliary constituents are maintained via a complex network of signals that regulate synthesis, expression and function of specific transporters located at the canalicular side of hepatocyte that determine their export from biliary sinusoids to canaliculus (5). Bile is normally secreted at a pressure of about 15-25 cm $H_2O$. Extrahepatic malignancy renders persistent mechanical obstruction to bile ducts and impedence to normal flow of bile and as such causes an increase in the back pressure on the liver. Pressure rise to about 35 cm $H_2O$ results in suppression of bile flow and therefore jaundice. Experimental studies in cholestatic models have indicated that infection of the bile above the obstruction leads to cholangitis and further suppresses bile flow via down regulation of expression of transporters in canalicular side (6).

Our studies further report significant restoration of biliary constituents following drainage. This is in conformity with earlier report indicating upregulation of expression levels of the multidrug resistance-associated proteins of the canalicular bilirubin conjugate export pump (MRP2) and the canalicular bile salt export pump (BSEP) in the liver following decompression (7). The recovery in biliary constituents following decompression was more pronounced in patients with cholangitis compared to those without cholangitis. This indicates decompression therapy may have important implication in patients with cholangitis.

**REFERENCES:** (1) Hawkins WG et al, Ann Surg Oncol. 2004; **11**: 310-315. (2) Boey JH et al, Ann Surg. 1980; 191: 264-270. (3) Abraham NS et al, Gastrointest Endosc. 2002; **56**: 835-841. (4) Bala L et al, NMR Biomed. 2008 (Epub; 7oct 2008). (5) Geier A et al, Biochim Biophys Acta 2007; **1773**: 283-308. (6) Trauner M et al, Gastroenterology 1997; **113**: 255-264. (7) Shoda J et al, Am J Gastroenterol. 2001; **96**: 3368-3378.