A non-invasive and early diagnosis of primary scelerosing cholangitis using MR spectroscopy

Sanaz Mohajeri[1,2], Tedros Bezabeh[1], Scott B. King[1], Omkar B. Ijare[1], M.A. Thomas[3], Gerald Minuk[2], Jeremy Lipschitz[2], Allan B. Micflikier[2], Iain Kirkpatrick[2], Lawrence Ryner[1], and Ian C.P. Smith[1]

[1]National Research Council, Institute for Biodiagnostics, Winnipeg, Manitoba, Canada, [2]University of Manitoba, Winnipeg, Manitoba, Canada, [3]University of California, Los Angeles

INTRODUCTION: Cholestasis results from failure to clear bile from the liver into the duodenum (1). It eventually results in intracellular accumulation of bile acids and consequently hepatocellular injury (2). Primary sclerosing cholangitis (PSC) is a chronic progressive cholestatic liver disease with ongoing inflammation, obliteration, and fibrosis of both intrahepatic and extrahepatic bile ducts (3). There is no effective medical therapy for PSC other than Orthotopic Liver Transplantation (OLT), which is not effective in the presence of established Cholangiocarcinoma (CC) (4). In-vitro studies in our lab have shown the potential role of Phosphatidylcholine (PC) and glycine/taurine conjugated bile acids in the pathophysiology of cholestatic liver diseases (5). One theory in this regard is the hydrolysis of PC to glycerophosphocholine (GPC) due to a reflux of pancreatic juice or the presence of ROS which can result in higher levels of free bile acids and subsequent inflammation. Therefore, this phenomenon can be an important predictor of liver damage and cancer development (5). In the current study, we have tried to compare the bile composition between normals and PSC patients in-vivo in order to determine the metabolites with possible roles in disease mechanism.

MATERIALS AND METHODS: *In vivo* 1-D ^{1}H MRS experiments were performed on gallbladder bile in 8 healthy volunteers and 7 PSC patients using a Siemens 3T Magnetom Trio clinical scanner and our home made receive array coil based on a protocol approved by local research ethics boards. The following parameters were used for PRESS sequence: voxel size = $12 \times 12 \times 12$ mm^3., TE = 30 ms, TR = 2000 ms, bandwidth = 2000 Hz and NS = 256. Respiratory gated sequences decreased motion artifacts, and spatial saturation bands reduced contamination from fat and/or the liver. After integration of peak areas, the ratio of phosphatidylcholine/unsuppressed water was calculated and compared between normals and PSC patients using Student's t-test. In addition, the PRESS sequence was edited to 2-D L-COSY [6], and the following parameters were used for the 2D experiments: NS =12, time increment (Δt1) = 0.8 ms, measurements = 50. Both 1-D and 2-D spectra were analyzed using the 2007 FELIX software (FELIX NMR Inc., San Diego, California).

RESULTS & DISCUSSION: In this study, we compared 1-D spectra of gallbladder bile in-vivo from healthy volunteers and PSC patients. After integration of peak areas (PC and glycine/taurine conjugated bile acids), the areas were normalized using the signal of unsuppressed water and compared between healthy volunteers and PSC patients using the Student's t-test. There was a statistically significant difference in the PC region, 3.22 ppm (P=0.009) between normals (mean ± S.D., $4.80E^{-03} \pm 1.83E^{-03}$) and PSC patients (mean ± S.D., $2.09E^{-03} \pm 1.60E^{-03}$). Furthermore, there was also a statistically significant difference (p = 0.004) between normals (mean ± S.D., $1.30E^{-03} \pm 2.30E^{-04}$) and PSC patients (mean ± S.D., $6.32E^{-04} \pm 4.0E^{-04}$) in the glycine conjugated bile acids region (8.0 ppm). Considering the protective role of PC against bile acids, the decrease in the levels of this metabolite may have a role in the inflammation. Therefore, determining the levels of PC may be useful in predicting the extent of hepatobiliary damage and cancer risk (5). In the 2-D L-COSY spectra, cross peaks observed from phosphatidylcholine-glyceryl signals (5.34ppm/4.2 ppm; 4.4 ppm/4.3 ppm), lactate (4.1ppm/1.32ppm), and glycine/taurine conjugated bile acids (8.0 ppm/3.7 ppm) could also be helpful in differentiating PSC patients from normals.

Figure 1. *In vivo* 1-D and 2-D ^{1}H MRS spectra acquired from human gallbladder bile (top: normal (7), bottom: PSC patient) showing spectral differences between normals and PSC patients.

CONCLUSION: There were statistically significant differences in the normalized PC and glycine conjugated bile acids peak areas (1-D spectra) between normals and PSC patients in this preliminary study. However, a larger sample size is required to investigate these interesting results further. 2-D experiments will also be helpful in evaluating differences in glycine/taurine conjugated bile acids, phosphatidylcholine-glyceryl, and lactate (8) regions between normals and PSC patients which can have additional predictive value in inflammation and cancer risk assessment.

REFERENCES:
1. Vleggaar FP, Van Ooteghem NA, Van Buuren HR, Van Berge Henegouwen GP. *Scand J Gastroenterol Suppl*. 2000 ;(232):86-92.
2. Spivey JR, Bronk SF, Gores GJ. *J. Clin. Invest.* 1993; 92: 17–24.
3. Lee YM, Kaplan MM; *Practice Guideline Committee of the ACG. American College of Gastroenterology. Am. J. Gastroenterol.* 2002 Mar; 97(3):528-34.
4. LaRusso NF, Shneider BL, Black D, Gores GJ, James SP, Doo E, Hoofnagle JH. *Hepatology*. 2006 Sep; 44(3):746-64.
5. Ijare OB, Bezabeh T, Albiin N et al. *NMR Biomed* 2009; 22:471-479.
6. Thomas MA, Yue K, Binesh N et al. Magn. Reson. Med. 2001; 46: 58-67
7. Mohajeri S., Bezabeh T., King S., et.al. Proceedings of ISMRM, 2011.
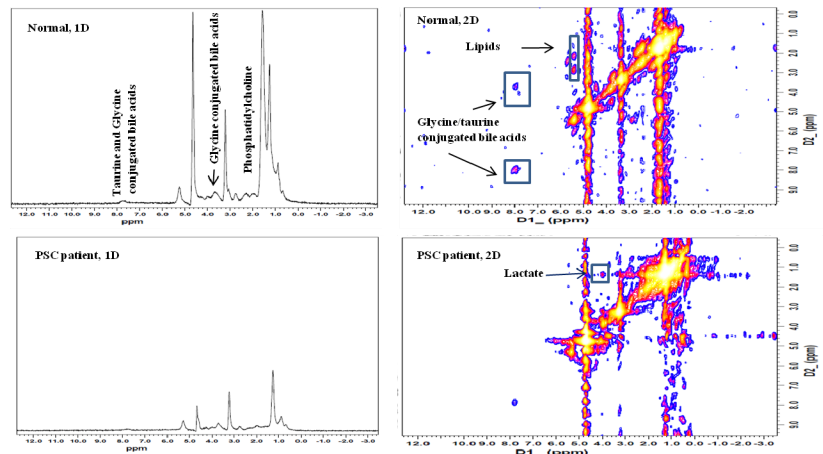8. Nishijima T, Nishina M, Fujiwara K. *Jpn J Clin Oncol.* 1997 Feb; 27(1):13-7.