Alteration in the Conjugation Pattern of Bile acids in Human Bile during Cholestasis: A ^{1}H MRS Study

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INTRODUCTION: Conjugation of bile acids with the amino acids glycine and taurine is an important phenomenon in bile formation in the liver. It facilitates normal bile flow and protects the liver and bile ducts from the harmful effects of unconjugated bile acids. In healthy humans, the ratio of glycine- to taurine-conjugated bile acids is generally 3:1 [1]. This ratio, however, is altered in cholestatic diseases and determining the conjugation pattern could be valuable in the diagnosis of hepatopancreaticobiliary diseases. In this study, we have analyzed bile samples from patients with various cholestatic diseases and determined the ratio of taurine- to glycine-conjugates.

MATERIALS AND METHODS: Bile samples were obtained from patients (n=17) undergoing endoscopic retrograde cholangiopancreatography (ERCP) examination/surgery for various cholestatic conditions. Medical diagnosis is based on the levels of plasma-bilirubin (P-bilirubin) (Non-cholestatic: ≤ 26 μM; Cholestatic: > 26 μM). 1D ^{1}H MR spectra with/without ^{1}H-decoupling were obtained for all bile samples on a 360 MHz spectrometer (Bruker Instruments). The glycine-conjugated bile acids (GCBAs) and taurine-conjugated bile acids (TCBAs) were quantified from the peak areas of their characteristic methylene (CH_2) signals resonating at 3.73 and 3.07 ppm respectively. Since the GCBAs signal partially overlaps with another biliary lipid signal, its peak area was obtained by deconvolution (XWINNMR software), using 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) as an external standard. The same technique was used with TCBAs.

RESULTS & DISCUSSION: Conjugation of bile acids with amino acids- glycine and/or taurine is the final step in the bile acid synthetic pathway, and this reaction is mediated by two cytoplasmic enzymes – bile acid CoA ligase and bile acid-CoA:amino acid N-acetyltransferase [1]. Most of the bile acid pool in bile is conjugated to glycine and/or taurine, generally, in the ratio 3:1. Figure 1 depicts the ^{1}H MR spectra of human bile from (a) non-cholestatic and (b) cholestatic patients showing relative levels of GCBAs and TCBAs in both patients. Figure 1(a') & (b') show the amide proton (NH) signals of individual glycine- and taurine-conjugated bile acids in the respective bile samples (a) and (b) [glycochenodeoxycholic acid (GCDCA); glycodeoxycholic acid (GDCA); glycocholic acid (GCA); taurochenodeoxycholic acid (TCDCA); taurodeoxycholic acid (TDCA); taurocholic acid (TCA)]. From the ^{1}H MR spectrum of cholestatic bile [Fig. 1(b & b')], it is obvious that TCBAs are highly elevated whereas GCBAs concentrations are reduced compared to those of non-cholestatic bile [Fig.1 (a & a')]. We quantified the levels of GCBAs and TCBAs in bile samples and determined their ratios. The median value of the GCBAs:TCBAs was found to be 2.23. Six out of 17 patients showed elevated levels of TCBAs, whereas 2 patients showed elevated levels of TCBAs along with reduced levels of GCBAs (see Table 1 for details). Two non-cholestatic patients (Bile # 2 & 4, with normal levels of P-bilirubin) showed elevated levels of TCBAs and decreased GCBAs:TCBAs ratio (< median), whereas a cholestatic patient (Bile # 16, with elevated P-bilirubin) showed TCBAs levels and GCBAs:TCBAs ratio comparable to those of non-cholestatic patients (> median). A detailed medical diagnosis and closer follow-up may be needed in such patients. The mean ± SD values of this ratio were found to be 2.88 ± 1.47 and 1.47 ± 0.83 for the non-cholestatic and cholestatic samples respectively. Using a Student's t-test, the difference between the two mean values was found to be statistically significant ($p = 0.01$). This alteration in the conjugation pattern of bile acids could be attributed to the defect in the bile acids synthetic pathway [1].

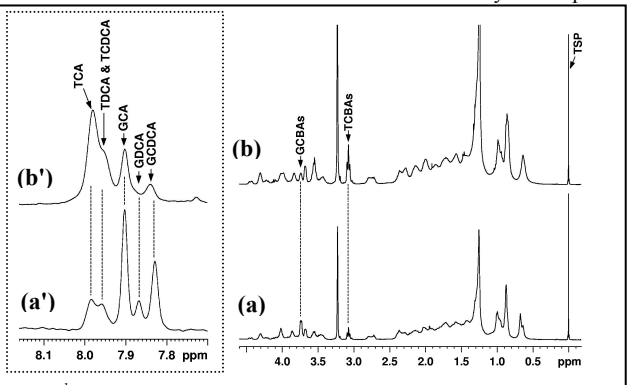

Figure 1: ^{1}H MR spectra of human bile from (a) non-cholestatic (b) cholestatic subjects showing elevated levels of taurine-conjugated bile acids (TCBAs) and reduced levels of glycine-conjugated bile acids (GCBAs). Inset: The amide (NH) proton regions of the respective bile spectra (a) & (b), showing individual taurine- and glycine-conjugated bile acids. Due to similar chemical shifts of TDCA and TCDCA, they have not been resolved at the magnetic field strength used in this study (360 MHz).

Table 1. Levels of GCBAs, TCBAs in bile samples collected from patients with various hepatopancreaticobiliary diseases along with their ratio.

Bile #	Medical Diagnosis	GCBAs (mM)	TCBAs (mM)	GCBAs:TCBAs
1	Non-cholestatic	27.98	8.33	3.35
2	**Non-cholestatic**	**11.71**	**11.8**	**0.99**
3	Non-cholestatic	19.01	5.96	3.19
4	**Non-cholestatic**	**37.41**	**25.19**	**1.49**
5	Non-cholestatic	42.73	13.64	3.13
6	Non-cholestatic	18.26	5.69	3.2
7	Non-cholestatic	10.05	2.82	3.56
8	Non-cholestatic	15.54	3.06	5.08
9	Non-cholestatic	28.36	12.69	2.23
10	Non-cholestatic	6.08	2.39	2.54
11	Cholestatic	8.99	4.56	1.97
12	Cholestatic	16.35	8.03	2.04
13	Cholestatic	19.72	18.36	1.07
14	Cholestatic	16.75	9.05	1.85
15	Cholestatic	7.95	19.39	0.41
16	**Cholestatic**	**15.54**	**6.27**	**2.48**
17	Cholestatic	2.04	4.68	0.44

In our earlier studies, we have observed reduced levels of major biliary lipids (bile acids, phospholipids and cholesterol) in patients with cholangiocarcinoma [2] and the absence of GCDCA in some patients with cholestatic diseases such as PSC [3]. In furthering our studies on the identification of biomarkers for the detection of hepatopancreaticobiliary diseases using MRS, in the current work we have observed elevated levels of TCBAs in patients with various hepatopancreaticobiliary diseases and also observed reduced levels of GCBAs in two of these patients. Both these changes contribute towards reducing the GCBAs:TCBAs ratio. These observations are indicators of underlying cholestatic disease and may have potential diagnostic value.

CONCLUSION: Conjugation of bile acids with glycine/taurine has an important role in bile formation which minimizes the harmful effects of bile acids on the hepatocytes and biliary epithelium. Alteration in the conjugation pattern is suggestive of underlying cholestatic condition which can be easily detected by ^{1}H MRS.

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