NMR study of gallstones from benign and malignant gallbladders

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SYNOPSIS: Gallstones from chronic cholecystitis (CC) (n=23; benign), xanthogranulomatous cholecystitis (XGC) (n=11; benign), and gallbladder cancer (GBC) (n=11; malignant) were studied using ^{1}H NMR to explore aetio-pathophysiology of gallbladder cancer. Significantly less cholesterol and high calcium were observed in GBC compared to CC ($p<0.0001$; $p<0.002$, respectively) or XGC ($p<0.0001$; $p<0.03$, respectively). Magnesium was high in GBC compared to CC ($p<0.002$). Striking differences in the gallstone composition of the malignant and benign patients that can be obtained rapidly from single pulse NMR methods may provide new insights into the aetio-pathophysiology of gallbladder cancer.

INTRODUCTION: Patients with chronic cholecystitis (CC) are associated with gallstones (GS). 1-3% of cholecystectomies done for GS disease turn out to have gallbladder cancer (GBC). About 60-90 % of GBCs are associated with GS. Xanthogranulomatous cholecystitis (XGC) is a special variant of CC associated with GS. This frequent association of GS with GBC remains undisputed. However, the mechanisms which explain the possible aetiological role of GS constituents in causing GBC are not clearly understood. Detailed studies to understand the differences in the composition of stones in benign and malignant disease need to be done to understand the aetio-pathophysiology of GBC. In the present pilot study we have evaluated the biochemical composition of GS in patients suffering from CC, XGC and GBC.

MATERIALS AND METHODS: Gallstones from patients with CC (n=23), XGC (n=11)) and GBC (n=11) of age matched patients undergoing cholecystectomy (laproscopic or open) were analyzed. The diagnosis of gall bladder diseases was based on histopathological examination. GS were washed with water, dried and used for analysis of cholesterol, calcium and magnesium using ^{1}H NMR spectroscopy. For cholesterol analysis 5 mg of finely powdered GS was dissolved in 500 µl of deuterated chloroform and ^{1}H spectra were recorded using single pulse sequence. From the resultant spectra, the quantity of cholesterol was estimated using the integrals of the cholesterol signals at 3.54/5.37 ppm (Figure) relative to the reference signal from trimethylsilylpropionic acid sodium salt (TSP) dissolved in deuterium oxide (taken in a co-axial capillary). For the analyses of calcium and magnesium, 5 mg each of GS were dissolved in 500 µl of double distilled water at pH=4 and the spectra were recorded after increasing the pH in presence of excess of EDTA. Intense water signal was suppressed by presaturation. The quantities of calcium and magnesium were simultaneously determined from the integrals of their EDTA-complex signals at 2.54 and 2.69 ppm (Figure), respectively, relative to TSP taken in a coaxial capillary. All NMR experiments were performed on a Bruker Biospin Avance 400 MHz spectrometer using 5 mm broadband inverse probe. Typical parameters used were: spectral width: 8000 Hz; time domain points: 32K; relaxation delay: 3s; pulse angle: 45°, number of scans: 64; spectrum size: 32 K and line broadening: 0.3 Hz. The quantities of cholesterol, calcium and magnesium in the gallstones of CC, XGC and GBC were statistically analyzed using two-tailed t-test using SPSS 12 software (SPSS Chicago, IL, USA). P values of less than 0.05 were considered significant.

RESULTS: The concentrations of cholesterol, calcium and magnesium determined using ^{1}H NMR for a total of 45 gallstones from three categories of patients are shown in the Table.

Table: Comparison of gallstone constituents of CC, XGC and GBC obtained using ^{1}H NMR.

Chemical constituent*	Chronic cholecystitis (CC)	Xanthogranulom-atous cholecystits (XGC)	Gallbladder cancer (GBC)	P-value
Calcium	0.35 (0.04 - 4.12)	0.27 (0.07 - 4.5)	1.62 (0.44 - 30.22)	< 0.002 (CC vs GBC) < 0.03 (XGC vs GBC) ns (CC vs XGC
Magnesium	0.02 (0.004 - 0.098)	0.02 (0.01 - 0.70)	0.19 (0.08 - 0.82)	< 0.002 (CC vs GBC) ns (XGC vs GBC) ns (CC vs XGC
Cholesterol	686.6 (221.8 - 857.8)	595.09 (431.9 - 795.8)	338.5 (293.4 - 395.2)	< 0.0001 (CC vs GBC) < 0.0001 (XGC vs GBC) ns (CC vs XGC)

*The values are mg/g; median (range); ns: not significant

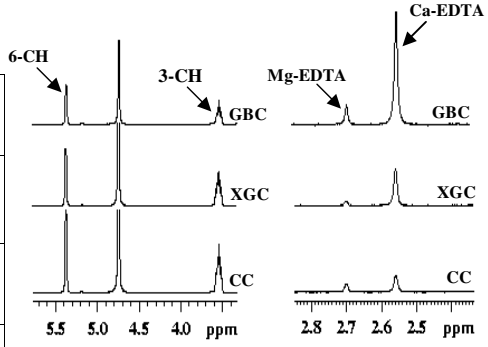

Figure: Typical ^{1}H NMR spectra indicating different quantities of cholesterol (left); calcium and magnesium (right) in the three categories of gallstones (all spectra plotted with identical vertical scale).

DISCUSSION: Presence of gallstones is a well established risk factor for gallbladder cancer. Studies have postulated the risk of gallbladder cancer to be associated with the size and number of the gallstones in the gallbladder.[1-2] However, there are no exhaustive studies on the relationship between composition of gallbladder stones and the risk of gallbladder cancer although it has been reported that no patient with gallbladder cancer had either black or brown pigment gallstones.[2] Our study on the chemical composition of gallstones arising from different pathological conditions reveals that calcium was significantly higher in stones in GBC as compared to CC and XGC. Magnesium was significantly higher in GBC as compared to CC. Gallbladder cancer is shown to be associated with cholesterol gallstones. Reduced cholesterol in GBC when compared to CC or XGC may be due to its dissolution and replacement with calcium and magnesium[3]. Marked changes in the chemical composition of gallstones of GBC with reference to CC or XGC may be useful for the study of aetio-pathophysiology of GBC and it may be used as an early indicator of cancer. Our preliminary results on bile are also in line with these studies. Further experiments on a large number of specimens would through more light on the findings.

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