Solid State 13C-NMR Analysis of Human Gall Bladder Stones

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Introduction:
Gall stone disease is suspected to contribute to the pathogenesis of GBC. It is important to identify differences between gall stones from cancer i.e. GBC and benign diseases e.g. CC and XGC so that prophylactic (preventive) cholecystectomy may be offered to those patients who have GBC-like stone to prevent future GBC. Detailed studies of gall stones in native form is highly preferable to account all chemical constituents (both soluble and insoluble) present in GS. In this direction, solid state NMR permits to identify structure of all the chemical constituents in a single experiment without any sample preparation. This makes solid state NMR as an important tool for the study of solids like gall stones in a straight forward and non destructive manner. Though, there is only one report on the solid state ^{13}C-NMR analysis of gall stones based on the morphology of gall stone [2], to the best of our knowledge there is no report on the solid state NMR analysis of the gall stones based on the disease type. In the present study we have undertaken the ^{13}C high resolution solid state NMR analysis of gallstones from patients with CC, XGC and GBC.

Materials & methods:
Gall stones from age-matched patients with CC (n = 12), XGC (n = 5) and GBC (n=10) undergoing cholecystectomy were analyzed. The diagnoses of gall bladder diseases were based on histopathological examination. GS were washed with water, dried and used for the solid state NMR analysis. ^{13}C-CPMAS with TOSS solid state NMR experiments were performed on Bruker Biospin Avance 500 MHz NMR spectrometer using a 4mm NMR Magic Angle Spinning probe. One dimensional ^{13}C-CPMAS spectra were obtained with spinning frequency of 5.0 KHz. The typical parameters used were as follows; spectral width 315 ppm, number of transients 128, data points 2k, acquisition time 0.025sec, line broadening 10 Hz.

Results: Fig 1 represents the solution state natural abundance ^{13}C NMR spectrum (A), solid state ^{13}C-CPMAS spectrum (B) of commercially available cholesterol and part of (145-110 ppm) solid state ^{13}C-CPMAS spectra of gall stones from CC(**C**), GBC (**D**) and XGC(**E**). In the spectra of GS, the C5 peak appears approximately at 144 ppm and C6 shows around 120 ppm. Solution state NMR spectra of cholesterol show single resonance for different ^{13}C atoms due to averaging of chemical shift anisotropy. Whereas in solid state ^{13}C-CPMAS spectra, it shows 4 resonating lines for each carbon atoms corresponding to different non-equivalent orientations. This observation clearly gives information about the presence of 4 different orientation of the cholesterol molecule. ^{13}C-CPMAS spectra of gallstones throw light on the different crystalline orientation in CC, GBC and XGC. The C5 and C6 peaks in the GS in CC (Fig 1[C]) and XGC (Fig 1[E]) shows two peaks corresponding to only two different orientations of the cholesterol molecules whereas in the stones of patients with GBC(Fig 1[D]), it clearly shows four different orientations.
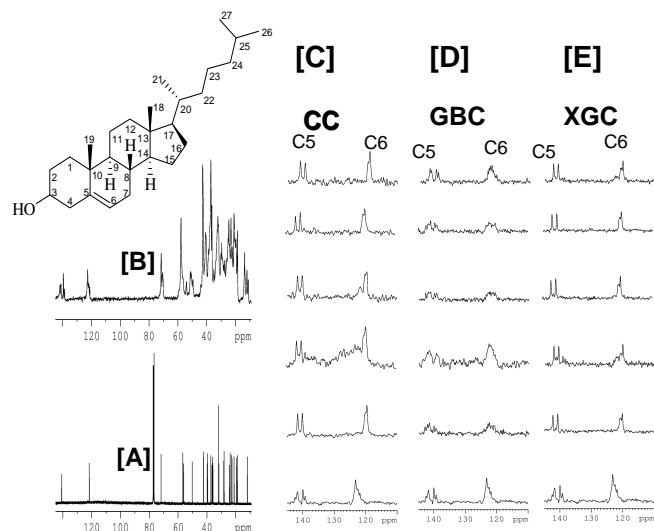

Discussion: Solid state NMR analysis clearly showed the presence of cholesterol as major chemical constituent. ^{13}C-CPMAS spectra of all studied gallstones from CC reveal the presence of mainly two orientations in most of the studied gallstones. Spectra of gallstones from XGC exhibit more similar structure as CC. The resonances at 144 ppm and 120 ppm in GBC are appeared to be broader as compare to the CC and XGC which indicate the presence of cholesterol in amorphous state in GBC. Though, there is no difference in the chemical composition of gallstones from CC, GBC and XGC, there is clear differentiation in the structural variation with respect to orientation of the cholesterol molecule in the gallstones. Our study provides the preliminary observation on the differentiation of gallstones from CC, GBC and XGC. However, more detailed study is required for solid conclusion; this is under way of our project.

Reference:
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Fig1: Solution state ^{13}C-NMR (A) and solid state ^{13}C-CPMAS (B) spectra of cholesterol and ^{13}C-CPMAS spectra of gall stones from CC (C), GBC (D) and XGC (E). Bottom spectra are from the cholesterol which serves as a reference for CC, GBC and XGC.