METABOLIC SIGNATURES IN HISTOPATHOLOGICALLY PROVEN GALLBLADDER CARCINOMA TISSUES BY HRMAS NMR SPECTROSCOPY

S. K. Bharti[1], R. Roy[1], A. Behari[2], V. K. Kapoor[2], and C. L. Khetrapal[1]

[1]CBMR, Centre of Biomedical Magneetic Resonance, Lucknow, Uttar Pradesh, India, [2]Dept. of Surgical Gastroenterology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

INTRODUCTION:

Gall bladder cancer (GBC) represents the fifth most common malignancy of the gastro-intestinal tract world wide. The exact etiology of GBC is unknown, but several risk factors have been identified, including cholelithiasis, family history and increased stone size. Discrimination between the benign Chronic Cholecystitis (CC), Xanthogranulomatous Cholecystitis (XGC) and GBC has importance in management of patients care. In this study ^{1}H HRMAS NMR based metabolomics approach has been applied for discrimination of (CC), (XGC) and GBC tissues types and to see the metabolic variations among them.

MATERIALS AND METHODS:

Gallbladder tissue specimens(n=83, CC=51, XGC=11, GBC=21) were collected from patients undergoing laparoscopic cholecystectomy/open cholecystectomy at the tertiary care super specialty hospital in India and stored at -80°C until NMR spectra were recorded. A part of the specimen was sent to the department of pathology as a routine histopathology test and a part for NMR analysis. ^{1}H NMR spectra of all gallbladder tissue specimens were recorded on 800 MHz (Bruker BioSpin, Avance-III) NMR spectrometer equipped with triple resonance HRMAS probe and a spinning rate of 8 KHz. Spectral width of 16 ppm, relaxation delay 4.0 sec, 2.55 sec aq time, No. of scan 128 and 4 dummy scans. CPMG spectra were recorded with a total echo time of 20 ms. All NMR spectra were recorded at 8°C in order to restrict metabolic activities of the tissue specimens.

The CPMG spectra obtained from gallbladder tissues were subjected to multivariate analysis. The CPMG spectra were reduced to 421 (0.5 to 4.7 ppm) discrete chemical shift regions to produce a series of sequentially integrated regions of 0.01 ppm width. OSC-filtered PLS-DA was performed using The Unscrambler X 10.0.1 (Camo Software, USA). For self validation of the model, 50% of the data were randomly selected and predicted on the basis of that model.

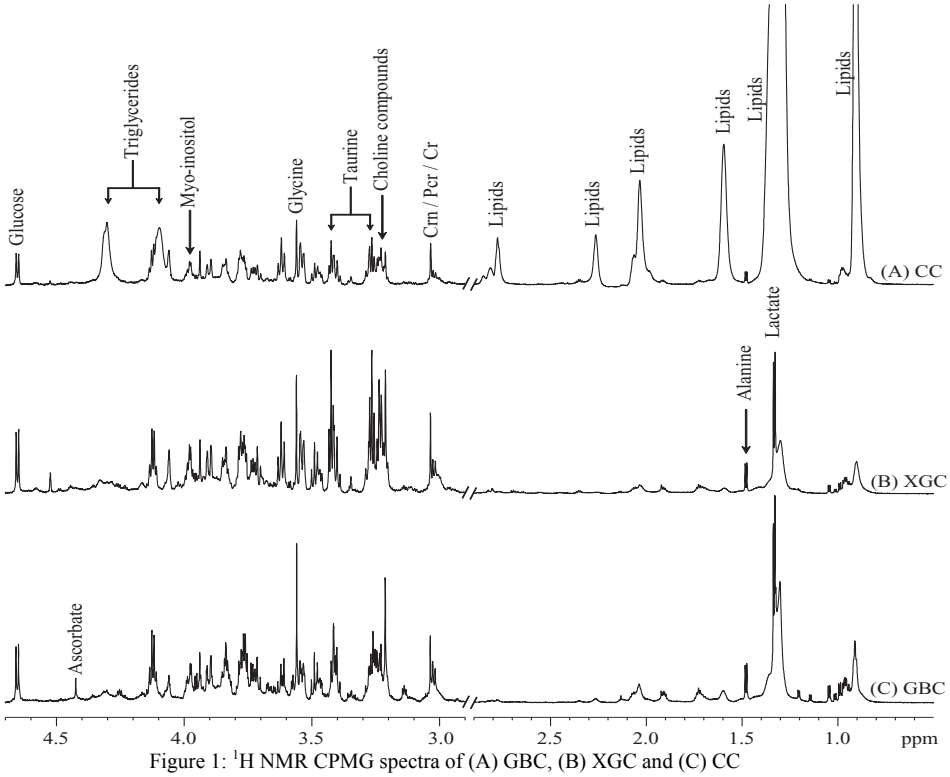

Figure 1: ^{1}H NMR CPMG spectra of (A) GBC, (B) XGC and (C) CC

RESULTS AND DISCUSSION:

Using ^{1}H HRMAS NMR analysis, more than 45 endogenous metabolites were assigned that includes lipids, amino acids, organic acids, choline containing compound, creatine, glucose etc. The NMR metabolic profile of gallbladder tissues was found to be dissimilar from the other previously reported malignant and nonmalignant tissues like brain, breast, oral, kidney and prostate tumor tissues. Proton NMR spectra of CC, XGC and GBC are shown in figure 1. OSC-filtered PLS-DA score plot obtained from analysis of NMR spectra is shown in figure 2. Regression plot PLS components 1 and 2 are also shown in figures 2B and C. Clustering between the groups was observed due to lactate, myo-inositol, creatine, choline containing compounds, glycine, ascorbate and lipid components. The levels of lactate, choline compounds and glycine were high in XGC, lower in GBC and lowest in CC. whereas the creatine, myo-inositol and lipid components were higher in CC groups and low in XGC. Even the variations within the groups were also governed by the concentration of choline, taurine, myoinositol and creatine resonances. The validation of the 50% of the data provided correct classification demonstrating the robustness of the model.

CONCLUSION:

The HRMAS spectroscopic analysis clearly demonstrates metabolic finger printing of CC, XGC and GBC tissues. The HRMAS studies may also be extended to Fine Needle Aspiration Cytology (FNAC) samples obtained from patients prior to surgery for additional metabolic information.

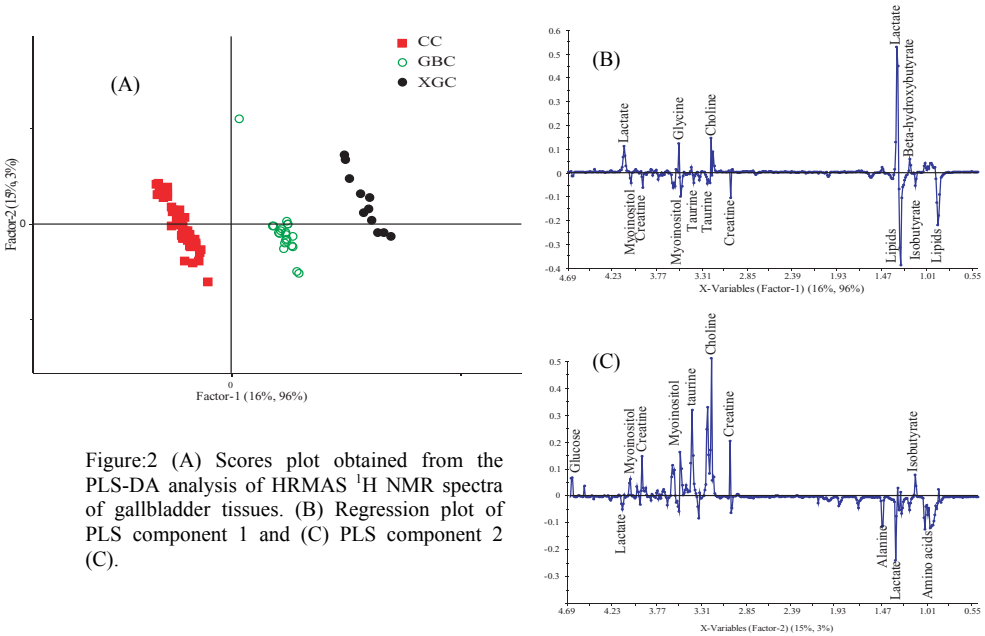

Figure:2 (A) Scores plot obtained from the PLS-DA analysis of HRMAS ^{1}H NMR spectra of gallbladder tissues. (B) Regression plot of PLS component 1 and (C) PLS component 2 (C).

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