'Mycolates and Phenolic glycolipids as biomarker for tubercular ascites' : A Proton Magnetic Resonance spectroscopic approach

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Introduction:

Tuberculosis continues to be a major problem, being responsible for 7-10 million new cases and 6% of death world-wide. It can involve any part of the body. Abdominal tuberculosis is the sixth most frequent site to be involved after lungs. Ascites is one of the pathological presentation of abdominal tuberculosis. The diagnosis of abdominal tuberculosis is difficult because of its protean manifestation. There is no definitive test, radiological or serum based for abdominal tuberculosis. However barium meal follow through, histopathology, PCR, Serum adenosine deaminase all may help in reaching the diagnosis Therefore, it is pertinent at this stage to review the role of newer diagnostic modalities in abdominal tuberculosis that would supplement the traditional methods for better management of disease. We herein report a preliminary NMR spectroscopic studies on ascitic fluid with different disease etiology and to identify finger print metabolic biomarker associated with tuberculous ascites.

Materials and Methods:

Ascitic fluid obtained from 15 patients who were enrolled with a written consent to participate in the study that was approved by the ethics committee. For ^{1}H NMR analysis, the ascitic fluid specimens were centrifuged at 3000 *g* for 10 min at 4°C. The supernatants were stored at -80°C until analysis. The NMR experiments were performed on Bruker Avance III 800 MHz NMR spectrometer equipped with 5 mm triple resonance TCI cryoprobe. Proton NMR spectra with water suppression were acquired using one-dimensional NOESY and CPMG spectra at 25.0 °C. The samples (n=15) were subjected to PCR analysis and eight samples were confirmed to be positive for tuberculosis. The spectrum of all these patients were compared with non-tubercular PCR negative patients, and other non-tuberculous ascitic diseases, by unsupervised statistical method of principal component analysis (PCA).

Results:

One of the striking features observed in ascitic fluid of tuberculosis patients is the presence of mycolates in the region 0.1 to 0.2 ppm, as well as signal arising due to aromatic protons between 7.5 to 8.2 ppm was indicative of presence of phenolic glycolipid. Moreover, a doublet at 1.2 ppm and multiplet at 4.2 ppm corroborates the presence of 1,2-propandiol in the Ascitic fluid of tuberculosis patients. The common metabolites observed in both the types of ascitic fluid, were glucose, lactate, branched chain amino acid, tyrosine, phenylalanine, N-acetyl glycoprotein, acetoacetate, creatine, choline, phosphocholine, taurine and Glycerophosphocholine.
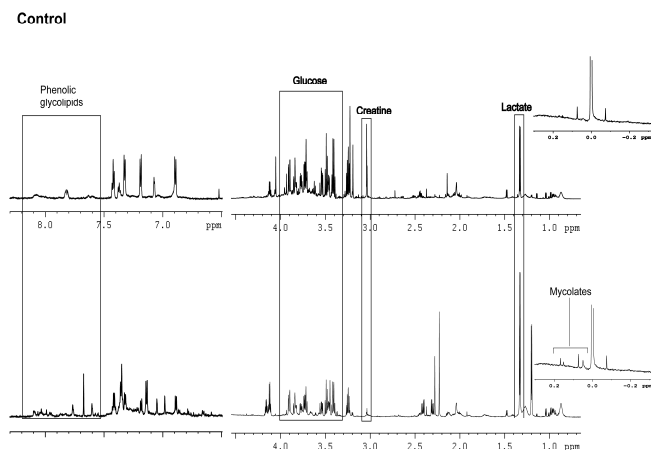

Figure 1: The ^{1}H NMR spectra obtained from tubercular (n=8) and non-tubercular (control; n=7) ascitic fluids. The resonances indicated are significantly important with respect to principal component analysis.
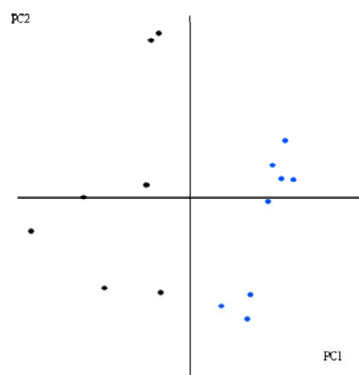

Figure 2: The PCA score plots obtained from ^{1}H NMR spectra of tubercular (n=8) and non-tubercular (control; n=7) ascitic fluids. * Black dots represent spectra obtained non-tubercular while blue dots represent tubercular ascetic fluid. The score plot is indicative of group separation between the controls and tuberculosis sample.

Discussion:

The metabolic profile of ascitic fluid may play a very important role for the diagnosis of abdominal tuberculosis. The presence of mycolates and phenolic glycolipid prove to be an straight forward differentiating factor for the abdominal tuberculosis. Since mycolates and phenolic glycolipid are integral cell wall components of *M. tuberculosis*, they can be used as finger print biomarker for the presence of tubercular bacilli. Mycobacterium species constantly shed phenolic glycolipid within the intracellular milieu of the host during infection. The phenolic glycolipid observed in discrete form in the present study may represent cell wall of mycobacteria and hence one could identify these chemical entities in the ascitic fluid. As *Mycobacterium tuberculosis* contains long chain fatty acid (C_{60}) such as mycolates, its presence in ascitic fluid may be due to these bacterial lipids, more over breakdown of these mycolates may result in libration of free gaseous form of cyclopropane and hence the signal assigned by ^{1}H NMR spectra showed combination of mycolates and cyclopropane. The study has examined the speculation that the presence of mycolates, phenolic glycolipid may help to identify, tubercular ascites more reliably and could thus differentiate them other diseases producing ascites.

Conclusion:

PCR positive ascitic fluid when subjected to ^{1}H NMR spectroscopic analysis showed distinct presence of mycolate and phenolic glycolipid in the spectra compared to non PCR positive ascitic fluid and other non tubercular ascitic diseases. Thus it can be safely concluded that the metabolite mycolate and phenolic glycolipid may act as fingerprint biomarker in the diagnosis of abdominal tuberculosis. Thus providing help in management of tubercular patients. It was a pilot study and the sample size was small but the power of study can be increased by accruing more patients. To the best of our knowledge and belief this study is first of its kind and opens a new investigative area in the management of abdominal tuberculosis.