Proton NMR-based Metabolomic Profiling in Pulmonary Tuberculosis Patients

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Introduction: Infection with *Mycobacterium tuberculosis* (Mtb) is associated with changes in cellular metabolism. Classically Mtb infection induces inflammation leading to granuloma formation. The tubercle bacilli are able to survive within the granuloma for extended time periods, therefore it is important to understand the metabolic relationship between the pathogen and the host. In this regard, metabonomics study of body fluids such as blood serum may aid in the identification of unique metabolite(s)/patterns of metabolites that would be useful for diagnosis and treatment of patients. Thus the objective of the present study were: (a) to determine and compare the concentration of metabolites in the serum taken from untreated pulmonary tuberculosis patients (PTB); household healthy contacts free of clinical tuberculosis (HHC); healthy controls (HC); and successfully treated pulmonary tuberculosis patients (TPTB) using in-vitro ^{1}H NMR spectroscopy, and (b) to analyze the metabolome profile using multivariate analysis to identify the biomarker(s) which would help in diagnosis and treatment of patients.

Patients and Methods: Whole blood samples was collected from 45 individuals (untreated PTB=15, HHC =13, HC =14 and TPTB =13). Patients and TPTB were registered in the out patient department of the National Institute of Tuberculosis and Respiratory Diseases, New Delhi. An informed consent was taken and the Institute Ethics Committee approved the study. All subjects were treated according to standard treatment regimen. Blood samples were collected in morning pre-prandial and centrifuged (2000 g, 10 min at 4° C) and serum was separated and stored at -70° C until NMR spectroscopic analysis**.** Formate (0.05 mM) was added as an internal standard to serve both as chemical shift reference and concentration standard for proton NMR spectroscopy. One dimensional (1D) and two dimensional (2D) total correlation spectroscopy (TOCSY) NMR experiments were carried out at 700 MHz (Agilent, U.S.A.).^{1}H NMR spectra of serum samples was carried out using 1D CPMG with water pre-saturation. Typical parameters for 1D NMR were: spectral width 9000 Hz with 32 K data points; echo time =15 ms; number of scans=64; and relaxation delay=70 s. The following parameters were used for 2D NMR experiments: data points 2 K in F2 dimension with a spectral width 9000 Hz and relaxation delay of 2 s. A mixing time of 80 ms was used for TOCSY. Comparisons of metabolites in all the four groups of subjects were carried out using one-way analysis of variance (ANOVA) followed by Bonferroni test. Probability values of 5% were considered significant ($p<0.05$). Partial least squares-discriminant analysis (PLS-DA) was performed to explore the biochemical dissimilarities among PTB, HHC, HC and TPTB using Unscrambler 10.2 (CAMO Software, Oslo, Norway).

Results & Discussion: In all 41 metabolites were assigned unambiguously using 1D and 2D NMR spectroscopy. PLS-DA score plots showed clear differences between PTB & HHC, PTB & HC and PTB & TPTB (see Fig.1). Our results revealed lower concentration of citrate and higher concentration of phosphocreatine in sera of PTB patients compared to HHC. The concentrations of 13 metabolites were significantly different in sera of PTB patients compared to HC. Of the 13 metabolites, 12 metabolites namely glycine, methionine, betaine, creatine, phosphocreatine, creatinine, succinate, acetate, choline, methylguanidine, valine and trimethylamine were elevated in PTB patients compared to HC. However the concentration of citrate remained low in PTB patients compared to HC. Data further showed higher level of sarcosine, phosphocreatine and trimethylamine in PTB patients compared to TPTB where as the concentration of citrate remained low in untreated PTB patients .The metabolite which was found to be significantly reduced in PTB patients compared to all other groups was citrate, an intermediate of the tricarboxylic acid cycle.

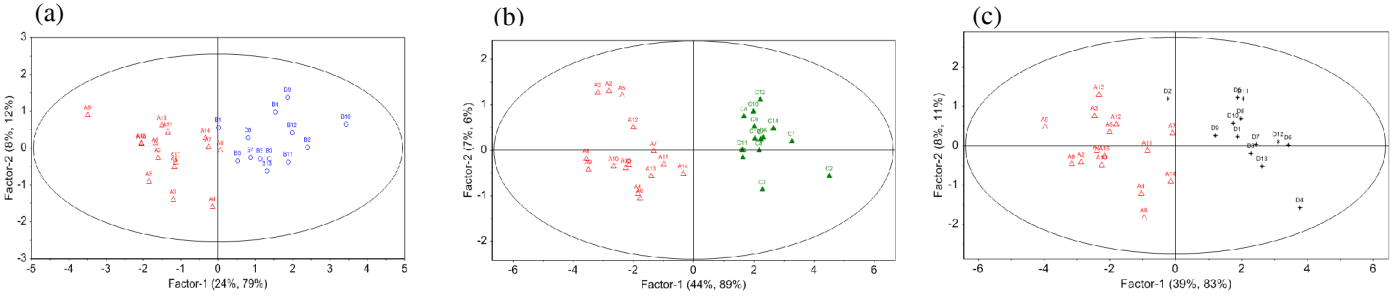

Figure 1: PLS-DA score plots derived from the ^{1}H NMR spectra of serum samples obtained from (a) pulmonary tuberculosis patients & household healthy contacts (b) pulmonary tuberculosis patients & healthy controls and (c) pulmonary tuberculosis patients & treated pulmonary tuberculosis patients. △- Pulmonary tuberculosis patients (PTB), ○ - Household healthy contacts (HHC), ▲ - Healthy controls (HC), + - Treated pulmonary tuberculosis patients (TPTB).

Conclusion: Our study revealed that the metabolic profile of sera of PTB patients is distinct from the HHC, HC and TPTB and implying that NMR based metabonomics may provide an insight to understand the biochemical alteration(s) that occur in PTB patients.