Differentiation of Cervical Tubercular with non-reactive Lymphadenitis tissues by Proton HR-MAS NMR spectroscopy

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Introduction: Cervical lymphadenitis is the most common extra pulmonary manifestation of tuberculosis (TB)[1]. The incidence of mycobacterial lymphadenitis has increased in parallel with the incidence of TB, causing 2 million deaths worldwide every year. Cervical lymphadenitis has been observed in nearly 35 per cent of extrapulmonary cases of TB. If it remains untreated, it may further lead to morbidity and mortality. It remains a therapeutic challenge as it mimics other pathological processes such as more frequent non-reactive hyperplasia, other infections (viral, bacterial or fungal), neoplasms (lymphoma, sarcoma or metastatic carcinoma), sarcodiosis, toxoplasmosis etc. Diagnosis of tubercular Cervical lymphadenitis often requires combination of fine needle aspirated cytology (FNAC), polymerase chain reaction (PCR), staining of acid-fast bacilli and histopathological examination (HPE) using incisional biopsy. Among these, HPE can be considered as a gold standard technique. Therefore, objective of the present study is the evaluation of proton HR-MAS NMR spectroscopy as an efficient alternative diagnostic method on incisional biopsied human (n= 109) tissue specimens obtained from cervical lymphadenitis in order to understand the metabolic differentiation of tubercular and non-reactive lymphadenitis in conjunction with HPE of the same tissue specimens.

Materials and Methods: Incisional biopsy tissue specimens (n=109) were obtained from 109 patients (45 males and 64 females) who were enrolled with a written consent to participate in the study that was approved by the ethics committee. The stored tissues at -80°C were thawed and washed with D_2O prior to NMR analysis, to remove blood and other impurities. The tissues were then dissected and inner-core (25-30 mg of wet-weight) of each tissue was taken for HR-MAS NMR experiments. Typically, the sample was packed into a 4 mm ZrO_2 rotor of 50 µl capacity; a volume of 20µl of D_2O having 0.03% TSP was used as a chemical shift reference. The sample-rotor-setup was then transferred into the HR-MAS NMR probe for NMR analysis on Bruker Avance III 800 MHz NMR spectrometer. Proton NMR spectra with water suppression were acquired using one-dimensional NOESY, CPMG spectra at 8.0°C. The same tissue specimens were then fixed in formalin for further HPE. A representative class of CPMG proton MR-spectra (0.5 – 4.25 ppm) obtained from cervical lymph node tuberculous and non-reactive lymphadenitis tissue specimens along with the histopathology of tubercular tissue is shown in Figure 1(a). It shows tubercular granuloma surrounded by epitheloid cells. The NMR data from 109 patients (109 tissue specimens) were subjected to the spectral binning (0.005ppm) of the full region (0.0ppm – 10.0ppm, removing the water region) for multivariate PCA followed by PLS-DA analysis using the software 'The Unscrambler X' Software package (Version 10.0.3, Camo ASA, Norway) using full cross validation and were then compared with the histopathological examination. Since the loading plots were found to be dominated by lipid signal, therefore PCA was performed on the NMR binned (0.005ppm) spectral region of 1.85ppm – 4.70ppm.

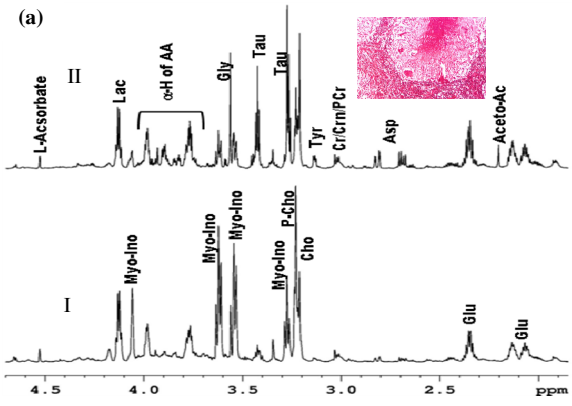
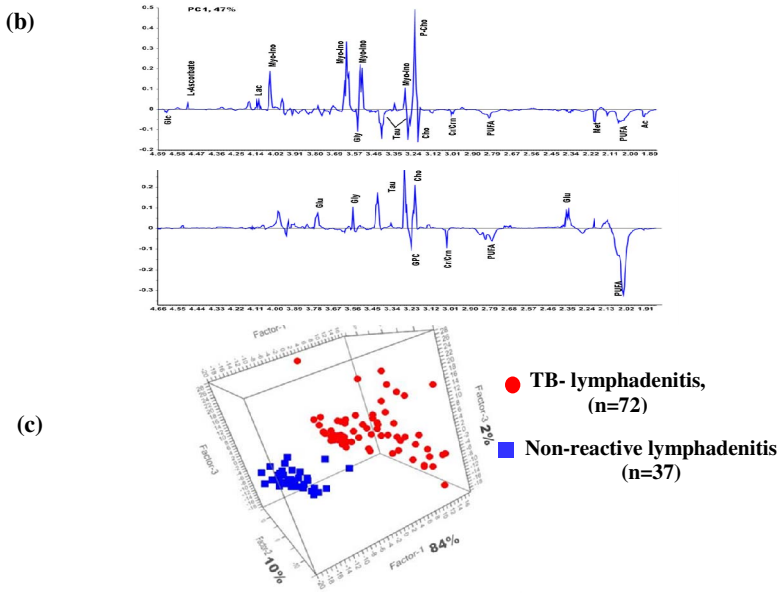


Figure 1:(a) A typical ^{1}H CPMG NMR spectra of non-reactive (I) and tubercular Cervical lymphadenitis (II) tissue of the binned region and the corresponding histopathology of tubercular tissue (b) PCA loading plots of tissue biopsies obtained from NMR spectra indicating the variations of resonances among tubercular and non-tubercular tissues and (c) PLS-DA 3D scattered plot clearly distinguishes tubercular and non-tubercular groups.



Results and Discussion: The PCA loading plots indicated that tubercular tissues had higher levels of acetate (Ac), taurine (Tau), glycine (Gly), amino acids (leucine, lysine and isoleucine), Cr/Crn, glucose (Glc) and PUFA which are positively correlated, and lower levels of myo-inositol (Myo-Ino), lactate (Lac), ascorbic acid and phosphocholine (P-Cho) representing altered metabolic processes (lipidogenesis, protein synthesis, volume regulation and controlled cell proliferation) during the progression of tubercular infection (Fig. 1 b). Taking these metabolic patterns into considerations from the PC1 loading plot, two groups were defined and then supervised three component PLS-DA model was generated using full validation of all the samples (Fig.1c). The same tissue specimens were subjected to HPE. The PLS-DA model of NMR data had correctly classified for tubercular lymphadenitis (n=72) and non-reactive lymphadenitis (n=37) in its respective histological categories with >98% of correct classification (including two border-line tissue specimens). The overall representation of metabolic shift in this prospective ^{1}H HR-MAS NMR study seems to be in concordance with metabolic alterations of tubercular lymphadenopathy, which is explained by the results of multivariate statistical analyses. One of the striking features is the low concentration of P-Cho in tubercular tissues which can be utilized in differentiation of lymphoma associated lymphadenitis. As P-Cho is a marker for cell proliferation.

Conclusions: We believe that HR-MAS NMR spectroscopy may be used as an efficient and alternative diagnostic method in order to start anti-tubercular treatment in patient suffering from cervical TB lymphadenitis. However larger sample size is required for such endeavor in order to categorized different stages of tubercular lymphadenitis and other associated pathologies. The above mentioned biomarkers may further be explored using *in vivo* single-voxel MR spectroscopy non-invasively for differentiating TB lymphadenitis.

References:

[1] S.Manolidis et al. *Otolaryngol Head Neck Surg.* **109**, 427-33 (1993).