

PROTON NMR SPECTROSCOPIC ANALYSIS OF PUS FOR DIFFERENTIATION BETWEEN AMOEBIC AND PYOGENIC LIVER ABSCESES

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INTRODUCTION:

Liver abscess in human is commonly caused by two types of pathogens, parasites and bacteria. In countries like India, the major cause of liver abscess is protozoan parasite, *Entamoeba histolytica* (EH). Pyogenic liver abscess, which is more common in western countries, is mainly caused by gut derived bacteria such as *E.coli*, *Klebsiella pneumoniae* etc. Methods for diagnosis of liver abscesses are Ultrasonography, Computed Tomography, MRI, Microscopy, Antigen, Culture, PCR and ELISA. For differentiation of Amoebic Liver Abscess (ALA) and Pyogenic Liver Abscesses (PLA) a combination of PCR, ELISA and Culture methods are used. These techniques are time consuming and separate test are required for differentiation between ALA and PLA. Presently there are no techniques available for simultaneous examination of Amoebic Liver Abscess and Pyogenic Liver Abscesses. The utility of ¹H NMR spectroscopic analysis of pus was therefore evaluated for the differentiation of amoebic and pyogenic liver abscess taking PCR and bacterial culture as gold standards.

MATERIALS AND METHODS:

All samples of pus from liver abscesses were snap-frozen in liquid nitrogen immediately after aspiration from patients and then stored in -80°C until NMR experiments were performed. Prior to NMR analysis, samples were thawed and centrifuged to remove pus cells. All samples were highly viscous, and therefore supernatant was diluted to fifty percent with D₂O prior to NMR measurements. ¹H NMR experiments were performed on Bruker BioSpin Avance 400 MHz FT-NMR spectrometer using 5mm Broadband Inverse probe equipped with z-gradient. A single-pulse ¹H NMR and CPMG spectra were recorded with presaturation of water signal for semi-quantitative evaluation of metabolites. For NMR spectral assignment, one and two-dimensional experiments such as COSY, TOCSY etc and spiking experiment using standard compounds, were performed. Known concentration of TSP was used in a capillary for external reference as well as for quantitative estimation. Only fourteen metabolites were chosen for quantitative estimation due to spectral crowding. PCR and culture by standard microbiological method were also performed on all the samples. All NMR raw data were recorded and processed using XWINNMR 3.5.

RESULTS:

One hundred eighteen patients (with mean age 40± 10) were included in this study: 101 with ALA (85.5%), 8 with PLA (5.5%), 9 with mixed infection (7.3%). 85.5% patients with liver abscess were male whereas 14.5% were female. The ¹H NMR spectra of pus were dominated by the presence of lipids and small molecular weight metabolites. A typical CPMG spectrum of amoebic liver abscess (Fig-1A) contains resonances of lactate, isoleucine, leucine, valine, alanine, lysine, threonine, aspartic acid, asparagine, glutamine, methionine, uracil, phenylalanine, tyrosine, histidine, tryptophan, adenine, glucose, ethanol, acetoacetate, arginine, glycine etc. Whereas, ¹H NMR spectrum of pyogenic liver abscess (n=8) showed prominent presence of succinate, acetate and formate (Fig-1B) and absence of asparagine and aspartic acid when compared with ALA. Whether in case of mixed infection (n=9) all succinate, acetate, formate, asparagine and aspartic acid were present (Fig-1C). On the basis of gold standard all subjects were categorized into four groups, viz., PCR-Positive Culture-Negative (ALA), PCR-Negative Culture-Positive (PLA), and PCR-Positive Culture-Positive (MIXED). The χ^2 -test (P=0.52) was applied and no significant difference was found between these two methods. The detailed ¹H NMR analysis of these samples suggested strong resonances from acetate succinate and formate which are products of bacterial fermentation can be treated as a marker for PLA. Whereas in case of ALA, positive predictive value was 92.5%, on the basis of aspartic acid and asparagine. The concentrations of other metabolites showed significant variation from patient to patient which indicative of the length of infection.

DISCUSSION:

The results of ¹H NMR spectroscopy of liver pus specimens provide substantial differences in metabolic profile of PLA, ALA and mixed infection. Culture method is highly sensitive but some technical problems often associated with collecting, handling and processing of liver pus along with drug treatment prior to aspiration, may affect the sensitivity. PCR has lower detection limit but is applicable only for ALA. ELISA test has good sensitivity but it gives false positive results in case of amoebic dysentery. The major advantage of NMR spectroscopy is that it is less time consuming, and easy sample preparation as compared to other techniques for simultaneous examination of both the infections (ALA & PLA). Metabolic profiling by NMR spectroscopy may provide a better platform to study the pathophysiology of different types of liver infection. We believe that NMR spectroscopic analysis of pus may be useful in conjunction with other routine diagnostic methods (culture and PCR) for better discrimination between pyogenic with amoebic liver abscesses which will help in better management and early prognosis of disease.

REFERENCES: 1- S.Iodhi et al. *Tropical Medicine and International Health*, Vol. 9 No. 6; 718-723: 2004.

2- Conter et al. *Surg Gynecol Obstet*, Vol. 162 No. 2; 114-120: 1986.

3- Ashish Gupta et al. *NMR in Biomed*, Vol. 19; 1055-1061: 2006

4- Lindon et al. *Concepts Magn Reson*, Vol. 12, No. 5; 289-320: 2000

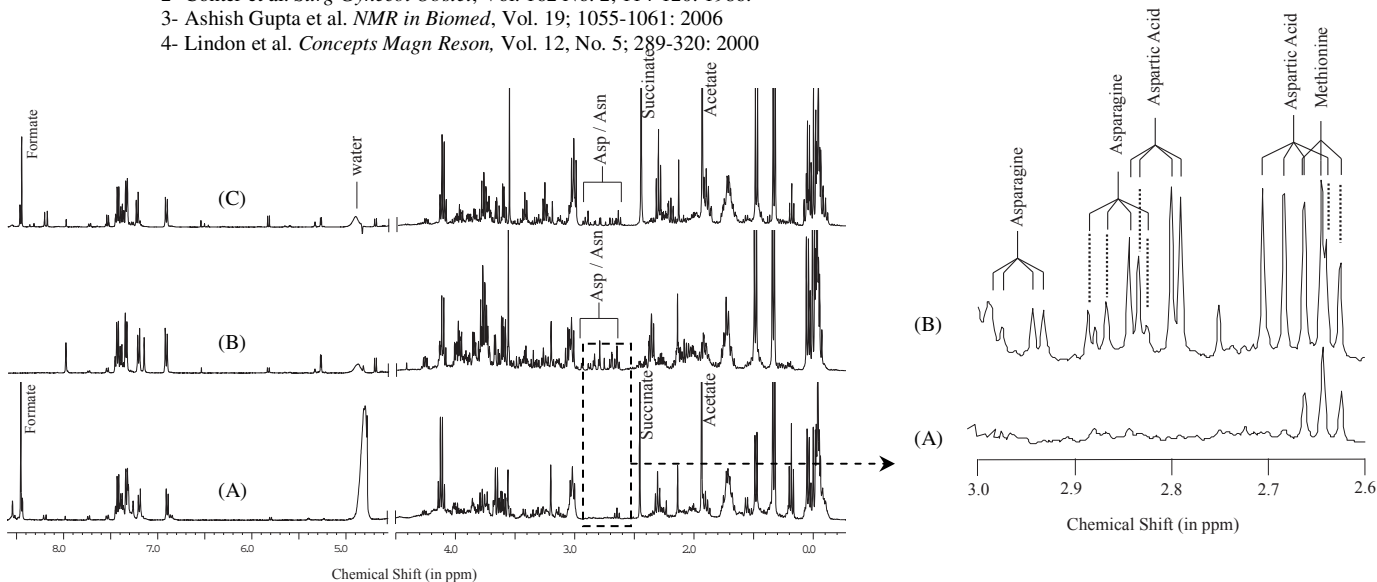


Figure 1: Typical ¹H NMR spectrum of liver abscess from patients with (A) Pyogenic liver abscess (B) Amoebic liver abscess and (C) liver abscess due to mixed infection, showing the difference in the metabolites in three groups.