Discrimination of malignant ascites from cirrhosis associated ascites in a multivariate analysis model: 1H NMR analysis of ascitic fluid

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SYNOPSIS: ^{1}H NMR quantitative analysis of Ascitic Fluid (AF) specimens in ascites associated with cirrhosis (n=47) and malignant ascites (n=7) were carried out on 14 metabolites viz. 3-hydroxybutyrate (BHBT), lactate, alanine, acetate, acetone, acetoacetate, pyruvate, glutamine, citrate, glucose, histidine, tyrosine, phenylalanine and formate. Multivariate Discriminant Function Analysis (DFA) was performed to find out the important biochemicals to discriminate two groups. The best discrimination was achieved when BHBT, lactate and tyrosine were considered together as marker biochemicals in the model ($P<0.0001$). The proposed model could classify 98.1% cases correctly between two groups with the sensitivity of 97.9% and specificity of 100%.

INTRODUCTION: Ascites, the accumulation of pathological fluid in abdominal cavity, manifests clinical problems and is an important risk factor of morbidity and mortality[1]. Among the various causes, ascites associated with cirrhosis is predominant (>84%), while malignant ascites (MA) are relatively low (<10%)[2]. The differentiation between malignant and benign ascites remains serious clinical problem for further diagnostic and therapeutic point of view. Analysis of ascitic fluid (AF) is most effective way for the differential diagnosis and evaluation of different causative etiologies. Although cytology is considered the "gold standard" in terms of diagnostic specificity (~98%), its diagnostic sensitivity is only 40-60%[3]. Therefore a wide variety of biochemical markers have been evaluated in recent years, but complete differentiation has not been achieved[4-5]. Thus a detailed metabolic profile of AF is desired. This has prompted us to explore quantitative high resolution ^{1}H NMR spectroscopy on AF specimens with specific aims to study (a) the differences between the various biochemical constituents in the AF specimens of patients with MA and cirrhosis (b) and to apply multivariate Discriminant Function Analysis (DFA) to find out important biochemicals in the differentiation process.

MATERIALS AND METHODS: In a prospective study, 100 consecutive patients with ascites who underwent routine abdominal *paracentesis* in the Gastroenterology ward of a tertiary referral centre of northern India, participated in the study. An imaging study was also performed to assess cause of ascites formation. The patients with clinically unstable condition and sepsis were excluded from the study and only patients with cirrhosis as confirmed by a histologic and/or clinical diagnosis and patients with a histologic diagnosis of malignancy (independent of ascitic fluid cytology) were included in this study. For ^{1}H NMR analysis the sterile AF specimens collected in a fasting state before commencing treatment were immediately centrifuged at 5000 rpm for 10 min at 0^0C. The supernatants were stored at -70^0C until analysis. ^{1}H NMR measurements were made on a Brukar Avance 400 MHz spectrometer using Carr- Purcell-Mieboom (CPMG) sequence with water suppression by presaturation. The parameters used were: Spectral width, 8000 Hz; data points, 32 K; Flip angle, 90^o; relaxation delay, 10 s; CPMG pulse train 277 ms; Scans, 128; line broadening function, 0.3 Hz. For the quantitative estimation of various metabolites, measured amount of trimethyl silyl propionic acid (TSP) in deuterium oxide was used in capillary. The data were subsequently log transformed to adjust for non-homogeneity of variance before subjecting DFA with the step–wise backward selection procedure (using the minimization of Wilk's lamda) to describe important biochemicals for differentiation.

RESULTS: After positive diagnosis was reached based on above criteria, a total no. of 62 patients were included in the study upon positive inclusion criteria: 47 cases had a non malignant ascites (NMA) due to cirrhosis [median age 43 (range 27-79) years; 40 males]; 7 cases with malignant ascites (MA) [median age 50 (range 35-65) years; 4 males] with positive ascitic fluid cytology; 8 cases [median age 58 (range 49-70) years; 7 males] ungrouped had negative ascitic fluid cytology and were highly suspicious of malignant related ascitis.

In a univariate analysis, mean quantities of BHBT, lactate, acetone, were significantly higher in AF specimens of MA compared to NMA ($p<0.05$ for all) while quantities of glutamine, glucose, tyrosine, and phenylalanine significantly low in AF specimens of MA compared to NMA ($p<0.05$ for all). However, mean quantities of alanine, acetate, acetoacetate, pyruvate, citrate, histidine and formate were comparable. This is shown in Fig.1. The DFA model showed that 3 biochemicals; BHBT, lactate and tyrosine, when considered together have the maximum discriminating power between 2 groups ($P<0.0001$) to correctly classify the 98.1% of the original grouped cases with a sensitivity of 100% (7/7 MA cases) and specificity of 97.9% (46/47 NMA cases). The DFA scores can be calculated by the following equation:

DFA score = -1.020*ln (tyrosine) + 0.316*ln (BHBT) + 0.642*ln (lactate) with canonical correlation= 0.761 (ln = natural log)

Functions at group centeroids are NMA = - 0.444 while for MA = 2.982

When 8 suspicious malignant related ascites cases with negative cytology were subjected to this model, 3 cases were predicted to MA group.

DISCUSSION: We report an association of 3 AF biochemicals: BHBT, lactate and tyrosine giving the maximum discriminating power between two groups, after trying with several DFA models. This suggests that BHBT, lactate and tyrosine serve as the marker signals in multivariate analysis. Significant quantity of lactate in MA patients is an indicative of high glycolytic activity of cancerous cells in AF specimens. They experience a hypoxic environment, have smaller number of mitochondria consequently anaerobic glycolysis is the major source of ATP production. In such a situation glucose consumption increases several times to meet energy demands. This is coupled with the catabolism of fatty acids. This is evident with significantly low glucose level and high ketone bodies (chiefly BHBT) level in MA patients. Acetoacetate forms the intermediate product.

An increased amount of aromatic amino acids tyrosine and phenylalanine in cirrhotic patients is an indicative of increased release of these amino acids from muscle and reduced capacity of further degradation by failing liver[6] thereby accumulation in plasma and eventually in AF. Though the present study has a limitation of small sample size, the efficiency of the above model in predicting MA cases with 100% specificity is encouraging. Further, the model predicts 3 cases as MA out of 8 cases in malignancy suspected group having negative cytology.

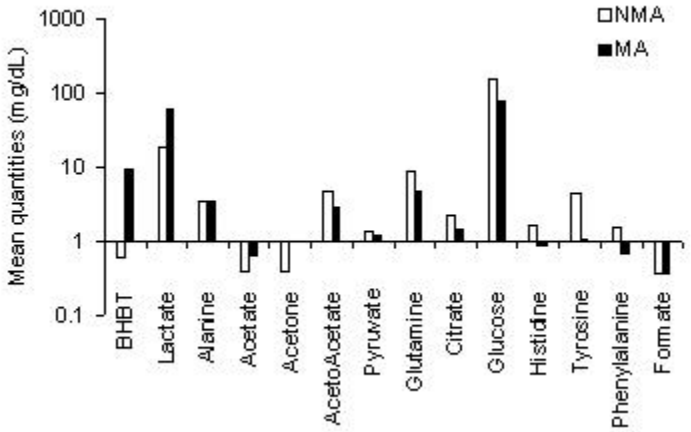

Fig.1 Comparison of various biochemicals in ascitic fluid between two groups

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