Pathophysiology of malabsorption syndrome patients with Small Intestinal bacterial Overgrowth: A 1H NMR spectroscopic study

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SYNOPSIS: In order to study the role of Small Intestinal Bacterial Overgrowth (SIBO), 30 patients with MS and 10 healthy volunteers were examined for biochemical constituents and bacterial growth of jejunal aspirate, using ^{1}H-NMR and microbiological techniques. Bile acids/cholesterol, acetate, lactate, formate and bacterial counts were found higher in MS than in healthy ($p<0.01$ for all). Acetate in MS positively correlated (rho 0.46) with total bacterial count. However, acetate and unconjugated bile acids were higher in MS with SIBO than those without SIBO ($p< 0.01$ for both). Deconjugation of bile salts and bacterial metabolites might be important in pathophysiology of MS.

INTRODUCTION: Malabsorption Syndrome (MS) due to various causes is associated with intestinal stasis that may cause Small Intestinal Bacterial Overgrowth[1-3] (SIBO) ($\geq 10^5$ cfu/mL) in the upper gut fluid. SIBO may cause MS or may result in refractoriness to treatment directed to primary cause of MS. The cause of functional abnormality of small bowel resulting in impairment of absorption of nutrients by SIBO is not clearly understood. We hypothesize, herewith, that bacterial metabolites and deconjugation of bile salts might play role. High resolution ^{1}H NMR spectroscopy is used herein with specific aims to study (a) the differences between the various biochemical constituents in the upper gut aspirate specimens of patients with untreated MS and of healthy controls and (b) the relationship of quantities of these constituents with presence of SIBO and colony counts of bacteria in upper gut as determined using standard microbiological techniques.

MATERIALS AND METHODS: Thirty adult patients with diagnosed and untreated MS using standard criteria as described previously[4] and ten healthy volunteers attending the Luminal Gastroenterology Clinic of a tertiary referral centre were included into the study. Upper gut aspirate was collected in all subjects using a sterilized double-lumen catheter during endoscopy[3]. The specimens were immediately subjected to NMR analysis and microbiological culture, independently. The bacteria were identified using standard method and colonies counted using a serial dilution technique. From the NMR spectra, unambiguous assignment of various metabolites was made using 2-D NMR (DQF-COSY and TOCSY) experiments and spiking experiments using standard chemicals. For the semiquantitative estimation of various metabolites, 500 □L of the untreated upper gut aspirate specimens from each subject was taken in a 5 mm NMR tube. A reusable sealed capillary tube containing 30 □L of 0.375 % Sodium salt of trimethyl silyl propionic acid (TSP) in deuterium oxide was inserted into the NMR tube before recording the spectra. 1D ^{1}H NMR spectra were obtained on a Brukar Avance 400 spectrometer with water suppression by presaturation. Spectral width used was 8000 Hz with time domain data points of 32 K for each sample. Flip angle of the radio frequency pulse was 45^o with a relaxation delay of 5 s to ensure complete recovery of magnetization to equilibrium between the scans. Typically, 128 scans were accumulated for each sample and the resulting data were Fourier transformed after multiplying by exponential window function using a line broadening function of 0.3 Hz and an FT size of 32 K points. Semiquantitative estimation of various metabolites was done based on the signal height normalized to TSP.

RESULTS: Five of 22 (23%) patients with MS in whom upper gut aspirate was cultured for bacteria and none of the 10 healthy subjects had SIBO ($\geq 10^5$ cfu/mL). Median quantity of total bile acids/cholesterol (2 [0 to 12] vs 0.3 [0 to 0.6] mmol/L), lactate (0.7 [0 to 5.2] vs 0 [0 to 0.03] mmol/L), acetate (0.2 [0 to 6.5] vs 0.02 [0 to 0.2] mmol/L) and formate (0.08 [0 to 0.9] vs 0 [0 to 0.05] mmol/L) was higher in patients with MS than in healthy subjects ($p <0.01$ for all, Mann-Whitney U test). In contrast, quantity of amino acid and glucose in upper gut aspirate of patients with MS and healthy subjects was comparable.

Median quantity of acetate (1.3 [0.2 to 6.5] vs 0.1 [0 to 1.4] mmol/L) and unconjugated bile acids (0.5 [0.04 to 0.6] vs 0.01 [0 to 0.3] mmol/L) was higher in upper gut aspirate of MS patients with SIBO than those without SIBO ($p<0.01$ for both, Mann-Whitney U test). Quantity of amino acids, glucose, total bile acid, lactate and formate was however comparable between patients with MS with SIBO and those without SIBO. Quantity of acetate in upper gut aspirate in patients with MS positively correlated with total colony counts of bacteria (Spearman's rho 0.46, two-tailed $p <0.05$).

DISCUSSION: Patients with untreated MS often have SIBO (23%) as compared with healthy subjects. The median quantity of acetate, formate and lactate is significantly higher in MS as compared with controls but only quantity of acetate positively correlated with the total colony count of bacteria in upper gut aspirate of patients with MS. This may mean that the short chain fatty acid (SCFA) such as acetate is produced by bacteria colonizing the small intestine of these patients. Also, patients with MS and SIBO had higher quantity of acetate and unconjugated bile acids than those without SIBO. Deconjugation of bile salts by bacteria has been shown to be important in pathophysiology of MS in patients with SIBO[5]. Our study however showed that metabolites such as acetate produced by bacteria in small bowel might also be important in pathophysiology of MS. SCFA including acetate is known to impair small bowel function[6] and may impair its motility[7]. Further studies are needed to explore how SCFA impair absorptive function of small bowel in these patients.

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