

Metabolism of colonic mucosa in patients with ulcerative colitis (UC) and Crohn's disease (CD): An NMR Study

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OBJECTIVE

To study the metabolic derangements in colonic mucosa of patients with ulcerative colitis (UC), Crohn's disease (CD) and controls and to determine the biochemical marker to differentiate UC and CD using in-vitro ¹H-MRS.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of intestine and comprises of UC and CD. Despite using a combination of clinical, endoscopic, histopathological, serological and radiological procedures, diagnosis of UC and CD remains a challenge in 15-20% of patients [1]. The distinction between UC and CD is however; extremely essential as treatment and surgery is different for these two disorders. Metabolomics study using magnetic resonance spectroscopy (MRS) provides information on the early biochemical changes at the molecular level that could signal initiation of the disease processes. There is only one study by Bezabeh et al [2] in the literature on IBD, which documented that proton MRS combined with multivariate method of spectral data analysis can be used to differentiate UC from CD. Earlier we reported a preliminary study on the biochemical profile of colonic mucosa in certain group of IBD patients and controls [3]. In the present investigation we characterized the colonic mucosa of large cohort of IBD patients using in-vitro high-resolution 1D and 2D ¹H-MRS. The metabolic alterations in patients with active and remission stages of UC, CD and controls were investigated to evaluate the biochemistry of diseases processes and to determine a biochemical marker/s, if any, to differentiate between these disorders.

MATERIAL AND METHODS

Twenty six patients with CD and thirty one with UC attending the Gastroenterology clinic of our institute were included in this study. The patients with CD and UC were classified into two sub-groups based on the severity of the disease namely, active (A) or remission (R) type. Of 31 UC patients, 20 had active disease [UC (A)], while 11 had remission [UC (R)]. Of 26 CD patients, 20 had active disease [CD (A)] while 6 were in remission state [CD (R)]. Twenty six subjects undergoing colonoscopic examination for obscure gastrointestinal bleeding and colonic polyps where colon was observed to be normal served as controls. All patients were treated according to standard treatment regimen. An informed consent was taken from patients and controls and the Ethics Committee of our institution approved the study. Ten biopsies were collected from the abnormal (inflamed) regions from patient and normal region from controls undergoing colonoscopy. Water-soluble metabolites were extracted from the tissues following the perchloric acid extraction. Proton NMR spectroscopy was carried out at 400.13 MHz. 1-D spectra with water suppression were acquired using, spectral width = 5000 Hz, data points = 32 K, relaxation delay = 14 s, number of scans = 256. 2-D TOCSY was carried out using mixing time of 75 ms. The concentrations of the metabolites were determined by comparing the integrated intensity of the isolated resonances of the compounds of interest with that of the TSP signal. The statistical analysis was carried out using STATA 9 software. Since the concentration values followed a non-normal (skewed) distribution, non-parametric tests were applied to the data. The median value with the range was taken for statistical analysis. Kruskal Wallis test was applied for comparison of the median values between the groups. For post hoc analysis, Wilcoxon rank test was used for pair wise comparison between the groups.

RESULTS

The concentration of short chain fatty acid (formate) was observed significantly lower in UC (A) patients compared to CD (A) (Fig. 1a). Significantly lower ($p \leq 0.05$) concentration of various amino acids (isoleucine/leucine/valine, glutamic acid + glutamine, alanine); glycolytic product (lactate) (Fig. 1b), intermediate of Krebs's cycle (succinate), membrane metabolites (choline, glycerophosphorylcholine, myoinositol), and formate while significantly higher concentration of glycolytic substrate (glucose) (Fig. 1c) was observed in patients with active state of disease, both UC (A) and CD (A) compared to controls. In the remission state of diseases, concentration of most metabolites was similar to controls except lower concentration of lactate, glycerophosphorylcholine, myoinositol in UC (R) and lactate in CD (R) compared to controls.

DISCUSSION

The gastrointestinal mucosa plays a vital role in digestion and absorption. Present study demonstrated the differences in the metabolic profile of colonic mucosa between patients with UC and CD and also compared to normal colonic mucosa using in-vitro ¹H MRS. Significantly lower concentration of several amino acids, membrane components, glycolytic product, energy stores were observed in the active state of UC and CD compared to controls indicating the decreased protein and carbohydrate metabolism thereby decreased energy status of colonic mucosa in chronic inflammation. Additionally, significantly higher concentration of glucose in IBD supports the reduced rates of glycolytic activity in patients. However, in patients with the remission state, lactate levels were lower both in UC and CD, while glycerophosphorylcholine was lower only in UC compared to controls suggesting that carbohydrate metabolism is also reduced in remission state, however other metabolic pathways are similar to controls. A significant difference in the concentration of formate in the colonic mucosal biopsy was observed between patients with active states of UC and CD, which shows the potential as a possible biomarker for distinguishing UC and CD.

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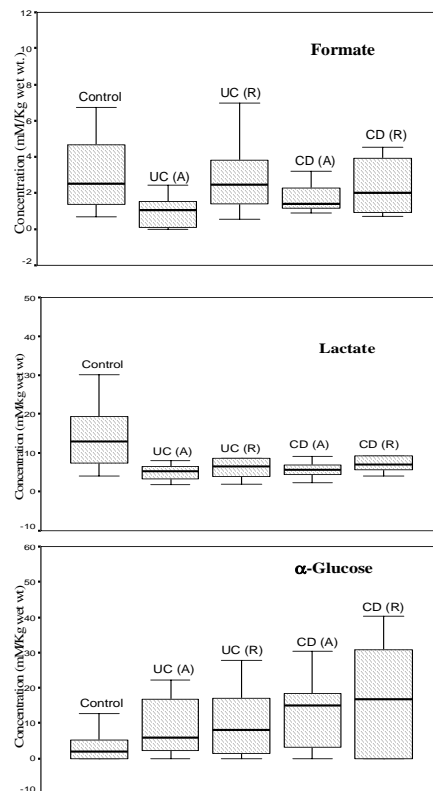


Fig. 1 Concentration of (a) formate, (b) lactate and (c) glucose in UC (A), UC (R), CD (A), CD (R) and controls.