

Metabonomics of Gastrointestinal Mucosa in Celiac Disease using in-vitro proton NMR Spectroscopy

Naranamangalam R Jagannathan¹, Uma R Sharma¹, Sujeet Mewar¹, and Govind K Makharia²

¹Department of NMR & MRI Facility, All India Institute of Medical Sciences, New Delhi, Delhi, India, ²Department of Gastroenterology & Human Nutrition, All India Institute of Medical Sciences, New Delhi, Delhi, India

Introduction: Celiac disease is the common chronic autoimmune disorder caused by dietary protein gluten present in wheat, barley and rye in genetically susceptible individuals (HLA-DQ2 or -DQ8 haplotype). The intolerance to gluten in celiac disease leads to atrophy of intestinal villi through an inflammatory cascade, which results in multisystem manifestations such as chronic diarrhoea, malabsorption, short stature, anemia, infertility, and metabolic bone disease [1]. In a community based study, the prevalence of celiac disease is 1.04% [2]. The diagnosis of celiac disease is challenging due to wide variation in clinical manifestations. Histological features are suggestive of the diagnosis, they however are not specific. Recently the utility of ¹H magnetic resonance spectroscopy (MRS) combined with multivariate analysis of urine and plasma in differentiation of celiac disease patients from controls was reported [3]. In this direction, a systemic study of metabolic profile of intestinal mucosa may provide a biomarker for assessment of villous abnormality and diagnosis in these patients. Thus the objectives of the present study are: (a) to investigate the metabolic profile of intestinal mucosal biopsies of patients with celiac disease and controls using in-vitro nuclear magnetic resonance (NMR) spectroscopy, and (b) to determine the biomarker/s for differentiation of celiac disease from controls and management of patients with celiac disease.

Patients and Methods: Twenty three patients with celiac disease (mean age 25.6±11.2 yrs) were recruited for this study. Twelve subjects (mean age 35.4±11.0) undergoing endoscopic examination for dyspepsia and GERD served as controls. An informed consent was taken and the Institute Ethics Committee approved the study. All subjects treated according to standard treatment regimen. The diagnosis of celiac disease was made on the basis of European Society of Pediatric Gastroenterology Hepatology and Nutrition. Approximately, 10 mucosal biopsies (58±15 mg) were obtained from the intestinal mucosa and perchloric acid extracts were prepared and the lyophilized powder obtained was dissolved in 0.6 ml of D₂O solvent. Sodium trimethyl silyl- (2,2,3,3-H₄) propionate (TSP) was added as a standard for chemical shift and quantification of concentrations of metabolites. One-dimensional and two-dimensional total correlation spectroscopy (TOCSY) NMR experiments were carried out at 700 MHz (Agilent, U.S.A.) Comparison of metabolites in celiac patients and controls were carried out using Mann Whitney test using SPSS 11.5 and partial least squares-discriminant analysis (PLS-DA) was performed to explore biochemical dissimilarities between patients with celiac disease and controls using Metaboanalyst 2.0 (The Metabonomics Innovation Centre (TMIC)).

Results & Discussion: Celiac patients showed higher concentration of aspartate (Asp), leucine (Leu), succinate (Succ) and fumarate (Fum) in comparison with controls (see Fig.1 and Table 1). PLSDA clearly differentiated patients with celiac disease from controls (Fig.2). To the best of our knowledge, this is the first study that presented comprehensive biochemical characterization of intestinal mucosa of patients with celiac disease. Our data revealed significantly higher concentration of Leu, and Asp in patients with celiac disease compared to controls. Amino acids are the major fuel for the small intestinal mucosa [4] and higher levels of amino acids in mucosa may be suggestive of lower activity of transaminases leading to accumulation of amino acids. Asp plays an important role in the urea cycle along with amino acids like ornithine, glutamate and citrulline. Accumulation of Asp in intestinal mucosa may thus lead to deficiency of Asp for urea cycle in liver and in turn may affect the liver. Liver abnormalities have been reported in patients with celiac disease [5]. Our results also revealed significantly higher concentration of Succ and Fum in the mucosa of patients with celiac disease suggesting abnormality in Krebs's cycle and leading to energy deficiency in celiac disease which may affect mucosal integrity. The present study provides clinically relevant biochemical signatures of intestinal mucosa of celiac disease patients and suggests the utility of NMR spectroscopy in determining the alternative biomarker/s for assessing the small bowel injury.

References: (1) Fasano A et al., *Arch. Int. Med.*, 2003; 163: 268; (2) Makharia GK et al., *J Gastroenterol Hepatol.* 2011; 26: 894; (3) Bertini I et al., *J Proteome Res.* 2009; 8: 170; (4) Guoyao Wu, *J. Nutr.* 1998; 128: 1249-1252; (5) Alberto Rubio-Tapia and Joseph A. Murray, *Hepatology*, 2007; 46: 5.

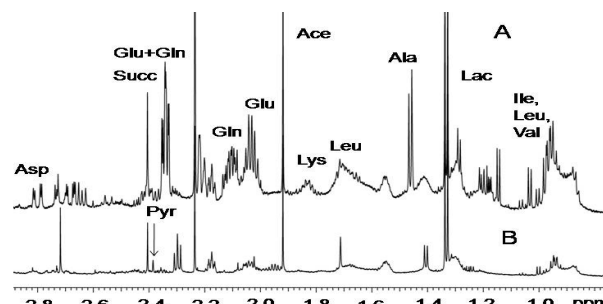


Fig 1: ¹H MR spectrum of celiac disease patient (A) and control (B).

Table 1: Concentration (mM/Kg wet weight) of metabolites in celiac patients and controls.		
Metabolites	Control (n=12) Median (Range)	Celiac (n=23) Median (Range)
Leu	1.20(0.18 - 5.45)	2.17(0.26 - 2.45)
Succ	0.19(0.01 - 2.81)	1.21(0.01-0.74)
Asp	0.85(0.01 - 4.82)	1.23(0.01-167)
Fum	0.08(0.01-3.72)	0.26(0.01-0.88)

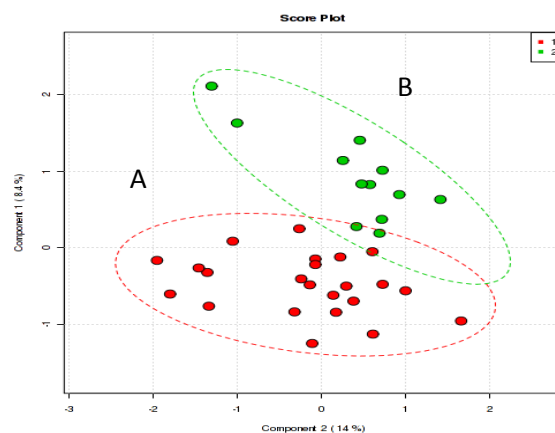


Fig 2: PLS-DA score plot showing the two first components (PCs) for (A) celiac disease and (B) controls.