Metabonomics of Celiac Disease by in-vitro Proton NMR Spectroscopy of Blood Plasma

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Introduction: Celiac Disease (CeD) is an autoimmune enteropathy caused by ingestion of gluten and related prolamines present in cereals like wheat, rye, and barley in genetically predisposed individuals[1]. The diagnosis of CeD is challenging due to multisystem clinical manifestations. Since, the pathology of CeD is a consequence of abnormal metabolism, metabonomics of the intestinal mucosa and body fluids like blood plasma may provide an insight into the biochemistry of CeD. Our previous study using intestinal mucosa of CeD patients showed enhanced levels of several metabolites like succinate, aspartate and leucine compared to controls indicating the abnormalities in several metabolic processes in CeD[1]. Multivariate analysis combined with nuclear magnetic resonance (NMR) spectroscopy of serum and urine has also been reported for differentiation of patients with CeD from controls[2]. However, no data on absolute concentration of metabolites in blood plasma of CeD patients has been reported which may aid in identification of a suitable biomarker for diagnosis. Thus the objectives of the present study are (a) to determine the concentration of metabolites in blood plasma of patients with CeD and compare them with patients with gastro oesophagus reflex disease (GERD) and dyspepsia who served as diseased controls (DC) and healthy controls (HC) using in-vitro NMR spectroscopy, (b) to analyze the metabolome using multivariate analysis to determine the disease pattern and investigate the putative biomarkers for differentiation of CeD from controls.

Patients and Methods: Twenty patients with CeD (n=20); mean age 28.2±10.4yrs) and twelve DC (n=12; mean age 35.4±9.5yrs) were recruited for this study. Eleven healthy controls (n=11; mean age 28.8±6.4yrs) were also included in the study. An informed consent was taken and the Institute Ethics Committee approved the study. All subjects were treated according to standard treatment regimen. The diagnosis of CeD was made on the basis of European Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN). Blood samples were collected in morning pre-prandial and centrifuged (2000g, 10 min at 4 °C) and plasma was separated and stored at -80 °C until NMR spectroscopic analysis**.** Formate (0.05mM) was added and used as an internal reference at δ 8.46 ppm. ^{1}H NMR spectra of plasma samples were carried out at 700 MHz (Agilent, U.S.A.) spectrometer at 298K using 1D CPMG with presaturation. Typical paprameters for 1D were : spectral width 9000 Hz, 32 K data points, time to echo, τ= 15 ms, number of scans= 64, relaxation delay =70 seconds. Comparison of metabolites in CeD patients and controls were carried out using student't' test. Probability values of 5% were considered significant (p<0.05). Partial least squares-discriminant analysis (PLS-DA) was performed using Unscrambler 10.2 (CAMO Software,Oslo, Norway).

Results: Figure 1 shows the representative proton NMR spectra of blood plasma of a patient with CeD, DC and HC. In all 40 metabolites were assigned using 1D and 2D NMR. The concentration of metabolites that showed statistical difference with HC is presented in Table 1. PLS-DA showed clear distinction among CeD patients, DC and HC (Fig.2).

Discussion: Our results revealed significantly higher concentration of glucose (Glc) and acetoacetate (Act) in blood plasma of CeD patients compared to HC suggesting the use of ketone bodies as energy source in CeD patients[2]. Our data also showed higher concentration of gluconeogenic amino acids like alanine (Ala) and glycine (Gly) in CeD patients compared to HC. These findings indicated that Ala and Gly amino acids probably might not have been utilized for gluconeogenesis in CeD and thus were seen in elevated concentration. Utilization of ketone bodies have been shown to inhibit gluconeogenesis[3] which is in agreement with our findings. Our results also showed significant increase in the concentration of glutamine (Gln) in CeD. Gln regulates the proliferation of T-lymphocytes and release of cytokines. It was reported that cytokines activate intraepithelial lymphocytes that leads to inflammation of intestinal mucosa and thereby resulting into the villous abnormality in CeD[4]. Thus elevated levels of Gln may probably indicate the involvement of Gln in the pathogenesis of CeD through proliferation of T lymphocytes. Involvement of Gln has also been reported in pathogenesis of autoimmune disease like type-1 diabetes[5]. Our results also showed decreased in level of creatinine in CeD which may reflect malabsorption of protein in CeD.

Conclusion: Our results revealed that PLS-DA differentiates between CeD and HC. Significantly elevated levels of Gln, Ala, Gly and Acet in patients with CeD were observed which may have the potential to serve as putative biomarker for differentiation of CeD from controls.

Fig1: Representative ^{1}H NMR spectra of celiac disease patient CeD, DC and HC.

Metabolites	CD (n=20) Mean±SD	DC (n=12) Mean±SD	HC (n=12) Mean±SD
Ala	885.6±190.7	859.8±166.3	708.9±181.2 *
Acetoacetate	322.4±193.6	236.5±103.4	227.2±85.5*
Glu-Gln	4054.2±903.3	3936.6±825.0	3326.4±884.6*
Gln	1395.9±420.6	1293.7±325.4	1116.4±318.0*
GPc	130.8±27.7	137.6±58.6	104.1±17.6*
Gly	497.1±161.4	455.3±206.6	321.6±128.7*
Creatinine	271.9±107.8	581.1±169.1	472.9±136.4*
Glu	3221.4±2135.8	2430.1±1471.6	1519.1±654.2*

Table 1: Concentration (μM/L) of metabolites in CeD patients, DC and HC. (* denotes, p<0.05 between CeD and HC)

Fig2: PLS-DA plot for the (A) CeD patient (blue) & DC (red). (B) CeD patient & HC (green).

References:

[1] SharmaU, Mewar S, Makharia GK et al. Metabonomics of Gastrointestinal Mucosa in Celiac Disease Using in-vitro proton MR Spectroscopy. Proc ISMRM 20, 2013; 1546; [2] Bertini I, Calabrò A, De Carli V et al. The metabonomic signature of celiac disease. J Proteome Res. 2009; 8 (1):170-77; [3] Müller MJ, Paschen U, Seitz H J. Effect of ketone bodies on glucose production and utilization in the miniature pig. J Clin Invest. 1984; 74(1): 249–261; [4] Garrote JA, Gómez-González E, Bernardo D et al. Celiac disease pathogenesis: the proinflammatory cytokine network. J Pediatr Gastroenterol Nutr. 2008; 47 (Suppl 1):S27-32; [5] Wu G, Field CJ , Marliss EB et al. Elevated glutamine metabolism in splenocytes from spon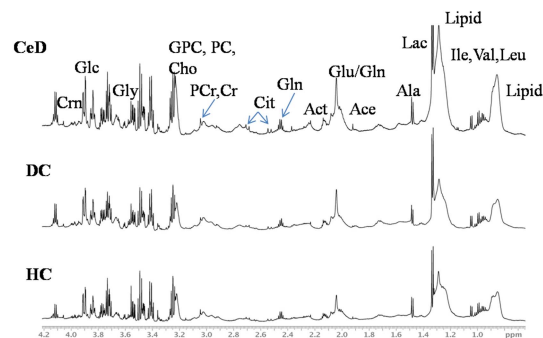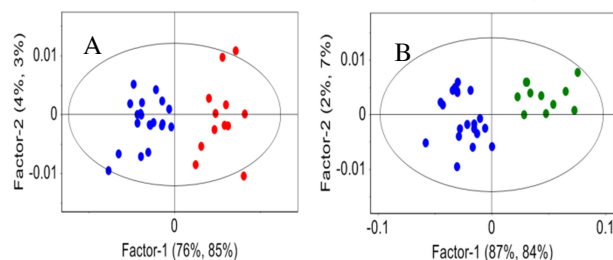taneously diabetic BB rats. Biochem J. 1991; 274(Pt-1):49-54.