NMR based metabonomics study on celiac disease in the blood serum

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ABSTRACT

Aim: The aim of this study is to look for the proper methods that would be a major step towards untreated CD diagnosis and seek the metabolic biomarkers causes of CD and compare them to control group.

Background: Celiac disease (CD) is a common autoimmune disorder that is not easily diagnosed using the clinical tests.

Patients and methods: Thirty cases and 30 controls were entered into this study. Metabolic profiling was obtained using proton nuclear magnetic resonance spectroscopy (^{1}HNMR) to seek metabolites that are helpful for the detection of CD. Classification of CD and healthy subject was done using random forest (RF).

Results: The obtained classification model showed an 89% correct classification of CD and healthy subject for the external test set. The metabolites that caused changes in people with CD were identified using RF; these metabolites include lactate, valine and lipid.

Conclusion: The findings of the present study reveal serum lactate, valin and lipid levels in CD patient are lower than healthy cohorts. This metabolite may provide diagnostic tools as well as insight into potential targets for disease therapy.

Keywords: Nuclear magnetic resonance spectroscopy, Celiac disease, Random forest, Metabonomics.

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Introduction

Celiac disease (CD) is a common systemic disorder, which can have multiple clinical manifestations. It has a multi factorial etiology with a complex genetics and histology. A comparison of recent studies in European and Middle Eastern countries has shown that CD is common in both areas, with an almost similar prevalence (1). Despite advances in investigation techniques, CD remains a challenging problem that often eludes diagnosis and receives sub-optimal attention (2). In this regard, metabonomics can provide powerful techniques for CD diagnosis.

Metabonomics is described as the quantitative measurement of the multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification(s) (3, 4). This quantitative measurement can provide multivariate

metrics of potential metabolic dysfunction in any living system. There are several available analytical spectroscopic methods to interpret the profiles of metabolism in the biological sample such as urine, plasma or tissue. In the biological systems, proton nuclear magnetic resonance spectroscopy (^{1}HNMR) is a useful method providing valuable data of the metabolites (5, 6). Various metabonomics studies are accomplished on CD. For instance, Bertini, et al. define the metabolic signature of CD through NMR of urine and serum samples of CD patients. In recent study, ^{1}H NMR metabolic profiling of their serum and urine samples examined a cohort of CD patients, before and after gluten free diet (GFD), and healthy controls. The results indicated that altered serum levels of glucose and ketonic bodies suggest alterations of energy metabolism, while the urine data point to alterations of gut microbiota(7). In another review, Bernini and co authors (8) address potential CD patients, defined as subjects who do not have, and have never had, a jejuna biopsy consistent with clear CD, and yet have immunological abnormalities similar to those found in celiac patients. They demonstrated that metabolic alterations may precede the development of small intestinal villous atrophy and provide a further rationale for early institution of GFD in patients with potential CD, as recently suggested by prospective clinical studies (8).

Leo Breiman has recently developed the random forests (RF) (9) that is based on classification and regression methods. RF reduces the variance and improves the prediction accuracy. In this study, we propose to apply RF for discriminating the control and CD subjects. In order to achieve this purpose, we seek the significance of metabolic biomarkers that can lead to the classification of these two groups.

Patients and Methods

Sample population

Thirty blood samples from adult CD patients (14 males and 16 females with mean age (±standard deviation) 34±11 years) and 30 healthy subjects (HS) were collected as described previously (10).

^{1}H NMR spectroscopy

^{1}H NMR experiments were acquired on a Bruker DRX 500 MHz spectrometer equipped with a 5mm NMR tube for analysis. The detail of this technique was presented in our previous study (11-13). Metabolites present in serum samples were identified on the basis of several previous studies (14-16).

Random forest (RF)

Random Forest (RF) classifiers are used to classify the serum samples of healthy and CD subjects and seeking the fundamental metabolites for desecrating (9). RF is a modified non-linear classification and regression trees (CART) method providing an importance ranking for the effectiveness of each metabolite. CART maximize the difference of heterogeneity, but the over fitting problem causes the classifier to have a high error of prediction in the test set whereas the bagging mechanism in RF algorithm can improve over fitting problem (18). The RF algorithm is illustrated in Ref (9). This algorithm builds every tree that is different owing to two factors. In first step, a best split is chosen at each node. This selection occurs from a random subset of the predictors rather than all of them. In next step, a bootstrap sample of the observations builds every tree. The out-of-bag (OOB) data are one-third of the observations. They can be used to estimate the prediction accuracy. Finally, based on averaging over all the trees is calculated overall prediction.

RF package is a readily accessible implementation of the RF algorithm and can be downloaded from the website. The data preprocessing and the modeling was executed utilizing MATLAB (version 6.5.1, The Math works, Cambridge, UK). RF has been applied on mean-centered data set and validated by predicting the classes of test set not used in the training set (17).

Results

A total of 42 different training and 18 test sets were built by random splitting for NMR spectra. Test set contain about 1/3 of the samples. In the classification model, these descriptive variables are the integral at the difference of the chemical shift in NMR spectra while the class numbers of the different samples were employed as response. According to RF model, we concluded that using three descriptors, CD and control groups could be classified. These metabolites include lactate, valine and lipid. Table 1 presents the summaries of the metabolite level distributions which are considerably (P-value < 0.001) different between CD patients and control group. Table 1 depicts serum lipid, valine and lactate levels in CD patient are lower than healthy group.

Table 1. Metabolites present in serum samples of celiac patients and control α

Metabolite	Assignment	^{1}H chemical shift (ppm)	CD group
Lactate	βCH3	1.32	↓
Valine	δCH$_3$	1.03	↓
Lipid	CH$_2$CH$_2$CO	1.56	↓

α The arrows (↓) indicate decrease of metabolites levels in CD group.
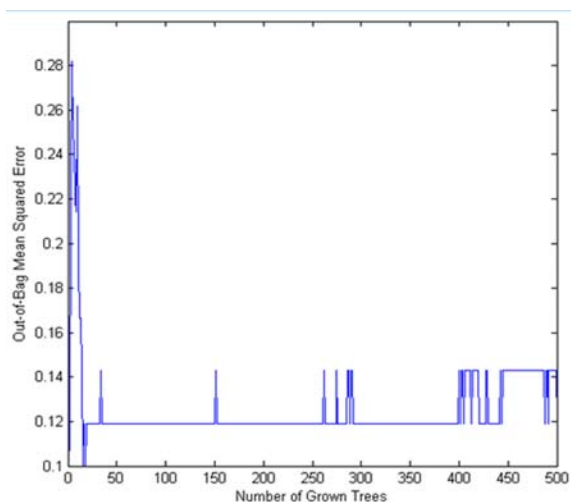

Figure 1. Plot of OOB error for RF classification of CD and control group

Samples of the training set were classified using RF in which 500 trees were grown. The

OOB data was used to estimate the prediction accuracy of classification. Figure 1 presents the OOB error rate. The RF algorithm considers how much prediction error increases. At this time OOB data for that variable is permuted while all other variables are left unchanged. By this method the significance of a variable can be estimated. Confusion matrix is a tool to illustrate the relations between real class and predicted classes. Table 2 presents confusion matrix of the RF model for the training and test set. In detecting CD patients of test set, as it is clear from Table 2, RF model has an accuracy of 0.89. With respect to these results, RF model has great chance in diagnosis of CD.

Table 2. Confusion matrix for training and test set.

		Predicted	
	Observed	CD class	Healthy class
Training set	CD class	20	1
	Healthy class	2	19
Test set	CD class	8	1
	Healthy class	1	8

Table 3 reports the classification specificity and other classification parameters for each individual in the training and test set. Another evidence for capability of RF model in CD diagnosis is the high non-error rate in the external test set.

Table 3. The calculated error and non-error rates of the classification index and the classification performances of training and test sets

Set	Error rate	Non-error rate	specificity	sensitivity	accuracy
Training	0.07	0.93	0.95	0.91	0.93
Test	0.11	0.89	0.89	0.89	0.89

Discussion

Lipids are a group of naturally occurring molecules such as types of vitamins, monoglycerides, and others. Lipids act as structural components of cell membranes. The majority of lipids in biological systems include

energy storage (18, 19). Krums and et.al stated lipid metabolism was evaluated in patients with CD (20). The reason of this function in patients with CD is disorders of lipid metabolism in the small intestine. Lewis and co-authors investigated cholesterol profile in people with newly diagnosed CD (21). They suggested that untreated CD is associated with lower total cholesterol than in the general population. Bertini, et al. (7) expressed that lipid oxidation should be increased in CD. In these cases intake of lipids is reduced because of malabsorption in CD patients. They explained that the lower levels of lipids in sera to be due to an enhanced lipids oxidation and malabsorption. Also they found valine and lactate levels in the serum of healthy individuals to be less than those found in CD patients. Our classification results are better than results of Bertini and coauthors (7). They found the classification accuracy of CD and healthy control groups was 79.7-83.4% for serum and 69.3% for urine. We applied the RF method for classification that has optimization parameters which are less than support vector machines (SVM) (applied method in Ref (7)).

An essential amino acid is valine that must be ingested. Valine both in structure and function is closely related to leucine and isoleucine. These amino acids are important for supplying energy to muscles and increase endurance and aid in muscle tissue recovery and repair. Hernanz, et.al analyzed amino acid concentrations in plasma from control and treated and untreated patients with CD (22). They found both treated and untreated cohorts had significantly decreased plasma concentrations of citrulline, tyrosine, valine, isoleucine, and leucine compared with control cohorts. In another review, Bernini et.al stated that glycolysis is somehow impaired in CD explaining both a lowering of lactate levels and an increase of glucose levels in blood (8). The body produces lactate in throughout the day. It is actually an important fuel used by the muscles during prolonged exercise. Also Bertini and coworkers defined metabonomics for CD in

three components including malabsorption, energy metabolism and the third related to alterations of gut microflora (7).

In Conclusion, metabonomics and analysis of the important above mentioned metabolites in serum is applied widely in early stage of CD disease. Due to the results RF proved to be quite powerful in discriminating between CD and healthy subjects.

Acknowledgements

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