Non-invasive Diagnosis of Type II Diabetes Based on Non-glucose Regions of 1H NMR Spectrum of Urine: A Metabolomics Approach

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INTRODUCTION: Diabetes is a chronic life threatening disease in which the body does not properly produce or respond to insulin, and characterized by increased levels of blood glucose leading to severe damage to vital organs such as heart, eyes and kidneys. Traditional methods of monitoring the blood glucose concentration of an individual require that blood be taken by venipuncture. This method can be painful, inconvenient and poses risk of infection. A non-invasive method for measuring glucose involves urine analysis; however, this may not reflect the correct status of the patient's blood glucose, because glucose appears in the urine only after a significant period of elevated levels of blood glucose. In diabetes, there may be metabolic alterations in serum and urine other than the changes in the levels of glucose, and identification of such metabolites in urine will be useful for the development of non-invasive methods for the diagnosis of diabetes [1]. Metabolomics combines NMR spectroscopy with pattern recognition methods to generate complex, high data density fingerprints of biological tissues and fluids, which can be used to fingerprint certain physiological and pathological status of an organism [2]. In spite of the potential for non-glucose NMR diagnosis of diabetes, published studies reveal an overlap in the levels of these metabolites. This overlap of individual concentrations makes it impossible to develop a reliable clinical diagnosis of diabetes based on conventional analysis of NMR spectra. It has become obvious that a reliable diagnosis of diabetes based on NMR evaluation of non-glucose metabolites could only be developed with a statistical classification strategy (SCS) – an approach that takes into account either the whole spectrum, or at least large parts of the spectrum [3].

MATERIALS AND METHODS: Urine specimens were obtained from two groups of subjects: control and type II DM patients. The control group consisted of 50 subjects (30 females, 20 males; mean age = 35 years; range = 23 - 67 years). The type II DM group was made up of 71 patients (41 females, 30 males; mean age = 54 years; range = 25 – 75 years). The DM group was selected so that all subjects present glucosuria. The NMR spectra were recorded on a Bruker Avance DRX 400 MHz spectrometer using a 5 mm inverse detection multinuclear probe equipped with gradients on the z-axis. A volume of 0.9 mL of urine, with 0.1 mL of TSP/D_2O was used for the analysis. The ^{1}H-NMR spectra were recorded with water presaturation. The pulse sequence used 32 scans, a 90º pulse, 30 s relaxation delay, 3 s CW irradiation, 4 s acquisition time, 4790 Hz spectral window, collecting 38 K data points, with a resolution of 0.13 Hz. The SCS analysis was performed on spectral regions that excluded the glucose signals. For this analysis, the data was partitioned into training (normal = 42, diabetes = 44) and test sets (normal = 8, diabetes = 27) randomly. In order to exclude any interference with glucose, only the following two spectral regions were used for the classifier development: 0.755 – 2.80 ppm (2,800 points) and 6.400 – 9.468 ppm (4,200 points).

RESULTS & DISCUSSION: Detection of glucose in urine from diabetic patients will not be an early method for the non-invasive diagnosis of diabetes mellitus, because we can only observe glucose in urine when the serum glucose levels are very high. In the present study, we have used non-glucose parts of the ^{1}H NMR spectra for the classification of urine samples from diabetic and non-diabetic subjects. Figure 1 shows ^{1}H NMR spectra of urine samples from a healthy control and DM patient. The middle part of the spectrum (3.0-5.5 ppm) in the DM spectrum is dominated by the glucose signals, making the assessment of other metabolites difficult. Using the SCS, subdividing the data set into 50 different training/test splits and finding which spectral regions were selected most often, our optimal region selection algorithm identified 5 spectral regions as being discriminatory. The 5 spectral regions selected by the algorithm and used for the classification were 8.93 - 8.86, 8.16 – 8.01, 7.09 – 6.84, 2.32 – 2.24, and 2.10 – 2.00 ppm. These regions could be assigned to metabolites such as NADH, tyrosine, glutamate, and glutamine [1]. The sensitivity, specificity and overall accuracy in the training set were 86.4%, 97.6%, and 91.9% respectively. The sensitivity, specificity and overall accuracy in the test set were 81.5%%, 100%, and 85.7% respectively making this a reliable medical diagnostic approach.

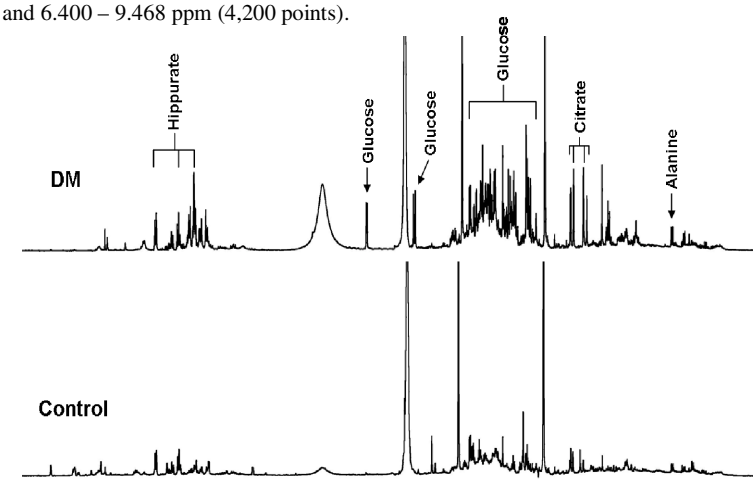

Figure 1: ^{1}H NMR spectra (400 MHz) of urine samples from a healthy control and a diabetic mellitus (DM) patient.

CONCLUSION: With the help of a statistical classification strategy, the non-glucose region of the ^{1}H NMR of urine can be of diagnostic value. Given the fact that glucosuria occurs at a later stage in the development of diabetes, identifying the changes in other metabolites may offer an opportunity for early diagnosis and hence early intervention.

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