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1H-NMR BASED URINE METABONOMICS AS A NOVEL APPROACH TO OVERACTIVE BLADDER SYNDROME

Hypothesis / aims of study

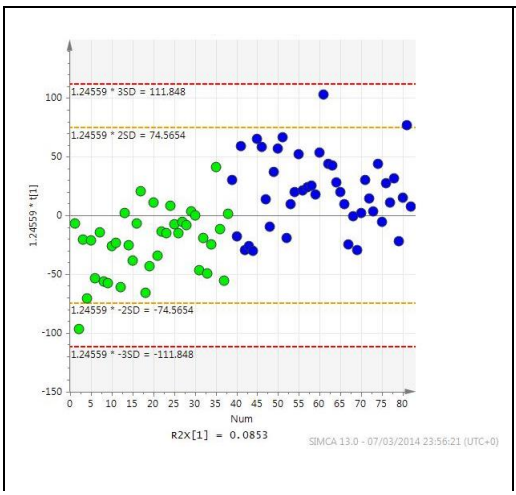
The underlying mechanism of overactive bladder (OAB) is poorly understood. This is likely a reflection of the fact that OAB has a multifactorial aetiology. Current diagnostic methods are limited in that they are often subjective by nature, invasive and costly. Moreover, treatment strategies are hindered by side effects and poor patient compliance. As a result, much recent research has been directed toward identifying novel, non-invasive biomarkers that have strong diagnostic and prognostic value. However, the majority of those identified lack specificity or have minimal prognostic benefit. Metabonomics offers an approach to quantitatively measure changes in metabolites and small molecules involved in biochemical processes thereby offering a direct assessment of systemic and local metabolic status [1]. This approach is already widely used in diagnosis, biochemistry, and identification of novel urinary bio-markers in many diseases. In this study we use high field proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) to investigate urinary metabolic changes associated with overactive bladder syndrome.

Study design, materials and methods

Women with and without lower urinary tract symptoms attending gynecology clinics in a tertiary referral center were recruited. All women were consented to participate in this study and local ethical approval was obtained. All patients completed the validated International Consultation on Incontinence – Female Lower Urinary Tract Symptoms Questionnaire [2] and provided mid-stream urine samples. Responses generally follow the format, never, occasionally, sometimes, most of the time, all of the time. Women with overactive bladder were defined as those reporting urgency ≥ sometimes, frequency ≥9-10 times, nocturia ≥ 2. Asymptomatic control patients were defined as those without lower urinary tract symptoms. Within 1 hour of collection, 1ml of whole urine was frozen at -80 C. Prior to processing the urine was defrosted on ice and 540µL added to 60 µL of buffer (1.5M KH_2PO_4/D_2O, 2mM NaN_3 and 0.1% 3-(trimethl-silyl)propionic acid-d_4). The mixture was then centrifuged at 13000 x *g* before 550µL was transferred into NMR tubes and metabolic profiles were acquired using ^{1}H-NMR. Standard 1D and 2D-JRES experiments were acquired. Unsupervised principal components analysis (PCA) was then used to examine data structure and identify outliers. Supervised orthogonal partial least squares discriminant analysis (OPLS-DA) was used to model class-related variability between patient cases and controls. Model performance was examined using the goodness of fit parameter (R^2_Y), and the predictive ability (Q^2_Y) was calculated by a seven-round internal cross-validation of R^2_Y. Correlation coefficient plots for the OPLS-DA models were used to identify peaks on the spectra that were used to differentiate between the two groups ant these were assigned to chemical identities using in house and publically available databases and structural information from the JRES.

Results

A total of 288 women (mean age 46, SD±15.7) were recruited to the study. OPLS-DA modeling of the urine metabolic data facilitated clear separation between cases and controls (R^2_Y=0.36, Q^2_Y=0.06; See Figure 1). Further analysis of the spectral data identified individual metabolities which discriminated between the groups which included lactate, citrate, creatine and creatinine (See Figure 2). There were no significant differences in demographic characteristics between the groups.



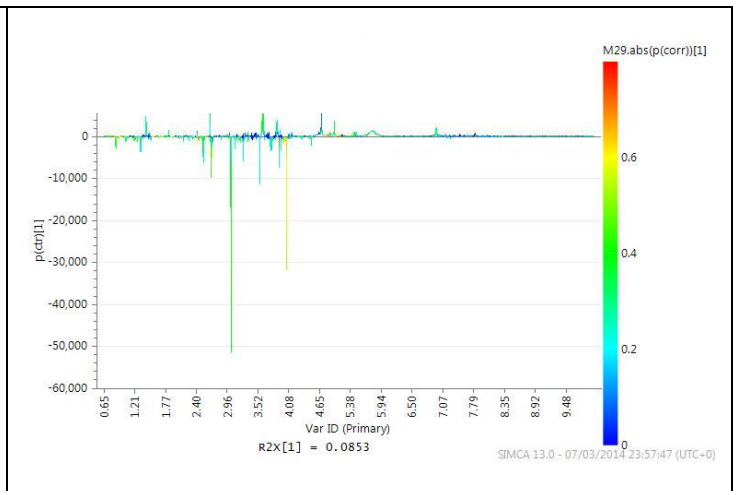


Figure 1: OPLS-DA modeling of ^{1}H-NMR urine metabolic profiles showing separation of those patients reporting OAB (blue) and controls (green).	Figure 2: Correlation coefficient plot for the OPLS model showing discriminatory peaks between the two groups. Metabolites increased in control patients above the line. Metabolites increased in cases below the line. Correlation graded by color scale.

Interpretation of results

Our results demonstrate that urinary metabolic profiles can be used to differentiate between women with and without overactive bladder syndrome. This approach shows potential as a diagnostic tool in urogynaecology as it objectively reflects measurable biochemical differences between the patient groups. It may result in biomarker discovery for diagnosis and prognosis, elucidate pathophysiology and identify of OAB subtypes allowing the development of new treatments and facilitate monitoring of therapeutic treatments.

Concluding message

This is the first work to demonstrate that urine metabonomics by ^{1}H NMR offers an efficient approach for identifying and characterizing underlying metabolic differences between patients with and without overactive bladder syndrome.

References

1. Nat Rev Drug Discov, 2003. 2(8): p. 668-76.
2. Am J Obstet Gynecol. 2004 Jul;191(1):73-82

Disclosures

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