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IS METABOLOMIC PROFILING USEFUL IN THE ASSESMENT OF WOMEN WITH LOWER URINARY TRACT SYMPTOMS?

Hypothesis / aims of study

Metabolomics is a high-throughput technology that quantitatively measures metabolites within a biological sample. Metabolites in urine represent end products of normal and pathological cellular processes that are closely linked to disease states. Urine metabolic profiles may thus be regarded as functional signatures of the physiological state and can be exploited for diagnosis and for better understanding of the biochemical changes associated with bladder function pathophysiology [1]. It is already widely used in diagnosis, biochemistry, and identification of novel urinary bio-markers in many diseases. The aim of our study was to assess whether metabolic profiling of urine samples by high field proton nuclear magnetic resonance spectroscopy (H^1-NMR) and subsequent multivariate analyses would be useful to characterize metabolic perturbations associated with lower urinary tract symptoms, starting with urgency.

Study design, materials and methods

Women with and without lower urinary tract symptoms attending gynaecology clinics in a tertiary referral centre 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 who reported urgency 'all of the time' were taken as cases. Asymptomatic control patients were defined as those with normal scores for all storage and incontinence symptom items. 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 acquired using ^{1}H-NMR. Standard 1D-NOESY 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.

Results

288 women with a mean age 46 (SD ±15.7) were recruited. OPLS-DA modelling of the urine metabolic data facilitated clear separation between cases and controls (R^2_Y=0.87, Q^2_Y=0.24; See Figure 1). Further spectral analysis identified discriminatory metabolites between the two groups (See Figure 2).

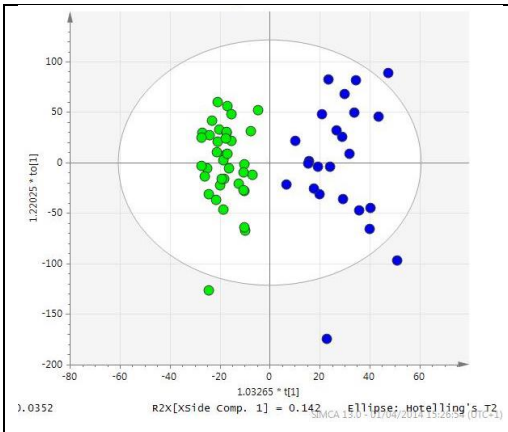

Figure 1: OPLS-DA modeling of the ^{1}H-NMR urine metabolic profiles enabled separation of those patients reporting urgency (blue) and controls (green).

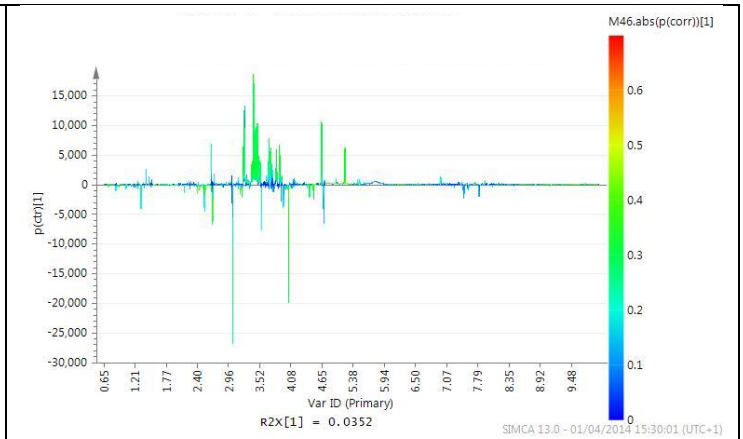

Figure 2: Correlation coefficient plot for the OPLS model showing discriminatory peaks between the two groups. Metabolites increased in control patients above the line. Metabolites increased in cases below the line. Correlation graded by color scale.

Interpretation of results

The metabonomics approach described in this study reveals clear differences in the urinary metabolic profiles between patients with urgency and controls. This likely represents differences in underlying biochemical processes that may be directly linked to pathology- or represent system responses- to underlying pathology of urgency. Identification of metabolites whose concentration differs between control and patient populations may lead to improved understanding of pathophysiology and represent novel urine based biomarkers useful for diagnosis and stratification of patients suffering from urgency.

Concluding message

Our findings reveal the potential strength of metabolic profiling for future diagnostic and prognostic applications in our field. Further work focusing on the application of metabonomics to other lower urinary tract symptoms and symptom complexes is warranted.

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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