348

Bray R[1], Jimenez B[2], MacIntyre D[3], Cartwright R[4], Franklin-Nembhard L[1], Digesu A[1], Fernando R[1], Holmes E[2], Bennett P[3], Khullar V[1]

1. Department of Urogynaecology, St Mary's Hospital, Imperial College, London, 2. Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, 3. Department of Surgery and Cancer, Parturition Research Group, Institute of Reproduction and Developmental Biology, Imperial College London, 4. Dept of Urogynaecology, St Mary's Hospital, London & Dept of Epidemiology & Biostatistics, Imperial College London

HIPPURIC ACID: A BIOMARKER FOR BLADDER PAIN SYNDROME

Hypothesis / aims of study

Bladder pain syndrome affects an estimated 3.3 million U.S. women. It is associated with profound physical and psychological impact, and significant economic costs. While the aetiology remains largely unknown, inflammation is thought to play an important role. The use of biomarkers in the diagnosis of lower urinary tract symptoms is controversial as diagnosis is largely based on patient's symptoms. Despite this several biomarkers have been identified in patients with overactive bladder syndrome, but none have been studied in patients with bladder pain syndrome. Metabolites in urine represent the end products of normal and pathological cellular processes. Therefore they can be used as biomarkers of disease and exploited for better understanding of the biochemical changes associated with bladder function [2]. High field proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy is a high-throughput technology that allows us to simultaneously and quantitatively measure all of the metabolites within a biological sample, such as urine. The resulting profiles can provide unique metabolic signatures for disease. The aim of this study was to evaluate the urinary metabolic profiles associated with bladder pain syndrome using ^{1}H-NMR) spectroscopy.

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 and provided mid stream urine samples. Those women bladder pain syndrome as defined by the international continence society were included in the symptomatic group [1]. Asymptomatic control patients were defined as those without lower urinary tract symptoms including bladder pain. 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 modeling of the urine metabolic data facilitated separation between cases and controls (R^2_Y=0.70, Q^2_Y=0.08; See Figure 1). Further analysis of the spectral data identified hippuric acid as a primary discriminatory metabolite between the two groups with levels consistently reduced in patients reporting bladder pain compare to controls (See Figure 2). There were no significant differences in demographic characteristics between the groups.
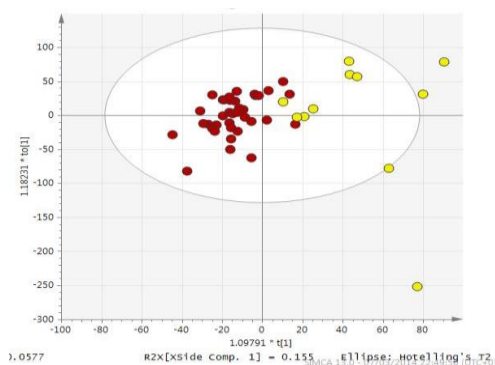

Figure 1: OPLS-DA modeling of the ^{1}H-NMR urine metabolic profiles enabled separation of those patients reporting bladder pain (yellow) and controls (red).
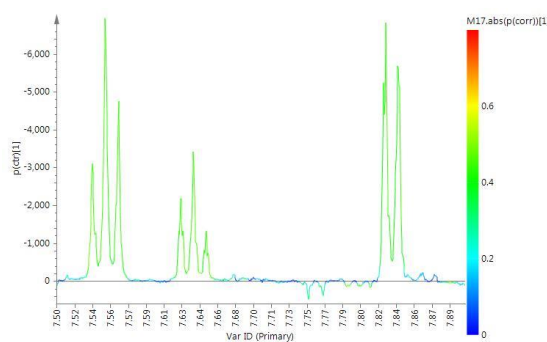

Figure 2: Correlation coefficient plot for the OPLS model showing hippuric acid peaks and a positive correlation of high hippuric acid in control patients.

Interpretation of results

Our results demonstrate that urinary metabolites have the potential be used as biomarkers to differentiate women with bladder pain syndrome from asymptomatic controls. We show specifically that women with bladder pain syndrome have reduced urinary hippuric acid. Synthetic hippuric salts in the form of methenamine hippurate have been widely used for treatment of bladder pain, where they regulate urine pH and have an anti-inflammatory role. Endogenous hippuric acid production is dependent on intestinal bacteria, and alterations in gut microbial metabolism have been identified as the cause of reduced urinary levels in other inflammatory conditions, such as Crohns disease [3]. It is possible that these alterations could also be involved in the pathogenesis of bladder pain syndrome, especially as these conditions often have overlapping symptomatology. The fact that patients with bladder pain syndrome have low levels of hippuric acid may confirm the theory that inflammation plays an important role in pathogenesis and provide novel routes for further investigation.

Concluding message

Our findings reveal that hippuric acid may be a potentially useful biomarker in a clinical research setting to evaluate women with bladder pain syndrome. Further studies in larger cohorts are required to confirm our preliminary results and investigate the role of hippuric acid in the pathogenesis of bladder pain syndrome.

References
1. Neurourol Urodyn. 2012;31(4):415-21.
2. Nat Rev Drug Discov, 2003;2(8):668-76
3. BMC Gastroenterol. 2010 Sep 17;10:108.