Identification of Bacterial Type in Urinary Tract Infection Using 1H NMR Spectroscopy

Ashish Gupta[1], Mayank Dwivedi[2], Abbas A Mahdi[3], Chunni Lal Khetrapal[1], and Mahendra Bhandari[4]

[1]*Centre of Biomedical Magnetic Resonance, Lucknow, UP, India,* [2]*Departments of Microbiology, Lady Hardinge Medical College, New Delhi, New Delhi, India,* [3]*Department of Biochemistry, Chhatrapati Shahuji Maharaj Medical University, Lucknow, UP, India,* [4]*Henry Ford Hospital System, Vattikuti Urology Institute, Detroit, MI, United States*

SYNOPSIS: Quantitative analysis of 682 urine samples from suspected UTI patients, and 50 healthy volunteers was carried out to identify the differential biomarkers between gram negative bacilli (GNB) (*E. coli*, *P. aeruginosa*, *K. pneumonia*, *Enterobacter, Acinetobacter, Pr. mirabilis, Citrobacter frundii*) and gram positive cocci (GPC) (*Enterococcus faecalis, Streptococcus group B, Staphylococcus saprophyticus*) uropathogenic urinary tract infection (UTI) using ^{1}H NMR spectroscopy. Linear multivariate discriminant function analysis (DFA) reveals that ^{1}H NMR measured metabolites can differentiate not only between healthy controls and infected urine samples but also GNB and GPC type of uropathogenic microorganism.

INTRODUCTION: Urinary tract infections (UTIs) are among the most common bacterial infection of humans (1-2). UTI includes gram negative bacilli (GNB) and gram positive cocci (GPC) microorganisms (1-3). Identification of bacterial infection is currently achieved by conventional culture method and various biochemical tests. However, intrinsic limitations in these methods cause reporting delays and high chances of contamination, thus making them poor tools for rapid identification of infection and monitoring therapeutic response to treatment (4-5). To address the shortcomings of urine culture for the rapid identification of UTI, we applied ^{1}H-nuclear magnetic resonance (NMR) spectroscopy as a surrogate method for fast screening of GNB and GPC uropathogens, which differ in their morphology.

MATERIALS AND METHODS: We studied 682 urine samples from patients with suspected UTI and from 50 healthy volunteers. Each urine sample was divided into two parts of 1.0 ml each; one part was used for conventional culture and standard biochemical tests; the other was centrifuged for 5 min at 4°C and 9168*g* to remove all cell debris and other contaminants. The supernatant parts were decanted and subjected to ^{1}H-NMR experiments. Control urine samples were also prepared under similar conditions. The NMR spectra were obtained with Avance 400 MHz spectrometer with 5 mm Broad band Inverse probe (Bruker Biospin, India). The 0.6 ml of urine samples were transferred to NMR tubes. A sealed capillary, containing pre-calibrated 25µl of 0.75% trimethyl silyl propionic acid (TSP) deuterated at the CH_2 groups and dissolved in deuterium oxide, was inserted into the NMR tube. While TSP served as a chemical-shift reference as well as the quantitative standard for estimating metabolites, deuterium oxide served as the 'field-frequency-lock'. For all specimens, the one-dimensional ^{1}H-NMR experiments were done at 22°C by suppression of water resonance by pre-saturation. The parameters used were: spectral width, 8000 Hz; time domain points, 32 K; relaxation delay, 3s; pulse angle, 45°; number of scans, 64; spectrum size, 32 K and line broadening, 0.3 Hz. The Xwinnmr software 3.5 was used for the baseline correction.

The statistical significance for the NMR derived quantified metabolites (n=12) was determined by univariate analysis (one-way ANOVA) followed by a *post hoc* Student-Newman-Keuls multiple comparisons test, where a *p*-value of less than 0.05 indicated statistical significance in all. The data were subjected for multivariate discriminant function analysis (DFA) with a stepwise-forward variable selection procedure in order to define important variables for differentiation of infected group of patients from controls, followed by discrimination of two types of infection based on the discriminant function coefficient values. On the basis of DFA we have constructed a classification model comprising four separate sets of phases; (1) control vs GNB + GPC, (2) control vs GNB, (3) control vs GPC and (4) GNB vs GPC.

RESULTS: Among several resonances, twelve metabolites (acetate, lactate, ethanol, succinate, formate, creatinine, trimethyl-amine (TMA), citrate, trimethyl-amine-N-oxide (TMAO), glycine, urea and hippurate) were quantified from their respective resonances (viz., 1.91, 1.33, 1.07, 2.41, 8.46, 3.01, 2.88, 2.55, 3.27, 3.56, 5.78, and 7.84 ppm) and subjected to univariate and multivariate statistical analysis. Out of 12 NMR measured metabolites, when only acetate, lactate, succinate, and formate were chosen based on their discriminant function coefficients, and DFA was performed for classification of GNB+GPC (all UTI) samples and controls, overall 99.5% of UTI cases (GNB+GPC) were effectively classified with 99.3% sensitivity and 99.5% specificity (Wilks' Lambda, 0.632; $p<0.0001$) (Fig. 1A). For classification of control vs GNB UTI urine samples, among 12 metabolites when only acetate, lactate, ethanol, succinate, and formate were chosen based on their discriminant function coefficients and the DFA was carried out, and the result exhibited 98.3% cases could be successfully classified, with a sensitivity of 99.5% and a specificity of 98% (Wilke's Lambda, 0.485; $p<0.0001$). Similarly, when GPC infected cases were compared with the controls; acetate, lactate and formate were chosen based on their discriminant function coefficients and DFA was performed, and the results exhibited that 95% of GPC cases were classified with 99.5% sensitivity and 92% specificity (Wilke's Lambda, 0.451; $p<0.0001$). Similarly, when GNB cases were compared with GPC, results reveal that succinate, lactate, and ethanol were able to classify 96% of GPC cases with 96% sensitivity and 96% specificity (Wilke's Lambda, 0.607; $p<0.0001$) (Fig. 1B).

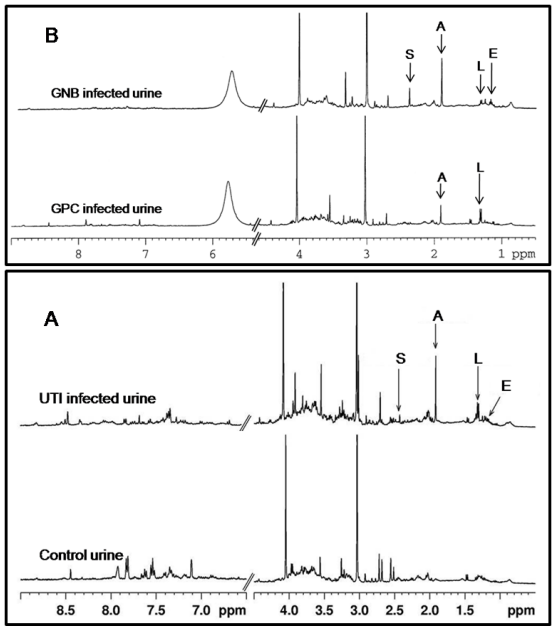

Fig.1: Typical ^{1}H NMR spectra of human urine samples. (A) Control vs UTI urine samples and (B) GPC vs GNB infected urine samples. Key: E, ethanol; L, lactate; A, acetate; S, succinate.

DISCUSSION: This study exhibited the NMR observed metabolite concentrations as a 'method of choice' for a quick differential diagnosis of UTI. DFA derived classification model defined that acetate, lactate, succinate, and formate were necessary to separate out the UTI (GNB+GPC) cases from the control group. These metabolites along with ethanol were able to segregate GNB infected urine samples from controls. Acetate, lactate and formate were vital metabolites to segregate GPC infected urine samples from the controls and succinate, lactate, and ethanol were the vital descriptors for the segregation of GNB from GPC case. In order to check the accuracy of the prediction of this DFA model, the prediction possibility of the classifications was assessed with a 75/25 data split, by using 75% of the data in each category as training sets and the remaining 25% as test sets. The classifications of 99.3, 99.1, 98.9, and 96.6% were obtained for the test set groups containing control vs. GNB + GPC, control vs. GNB, control vs. GPC, and GNB vs. GPC, respectively, followed by classifications of 99.1, 98.8, 99.1, and 92.2% for the training set groups, respectively. Results indicated that the prediction accuracy of GNB and GPC infection using NMR variables is very good and is simple, rapid, sensitive and comprehensive identification method which is useful for elderly, recurrent, chronic and severe UTI.

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