NMR Spectroscopy of Urine for the Detection of Urinary Tract Infection (UTI) in Children

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INTRODUCTION: Urinary tract infection (UTI) is the most common non-epidemic bacterial infection in adults and children. Infections to the kidneys and scarring are well established complications of infection of the upper urinary tract in children and can lead to hypertension and renal failure [1]. The reference standard for confirmation of UTI in children is growth of a single organism on a culture of urine obtained by urethral-catheterization/suprapubic aspiration. *Escherichia coli* is the most common uropathogen causing UTI in children, accounting for up to 78% [2]. However, other pathogens such as *Proteus* and *Klebsiella* species, and some *Staphylococcus* and *Streptococcus* species may also cause UTI. The culture method has the disadvantage of taking at least 48 hours to give a result. Therefore, more rapid methods are desirable. Currently, the most widely used rapid tests are dipsticks (leukocyte esterase and nitrite). Dipstick tests have the advantage of being quick and easy to perform, however, they are associated with false negative results for bacteria which do not produce nitrite and false positive results in the presence of ascorbic acid, drug interference and overgrowth with nitrite producing bacteria. For routine purposes, the ideal method for microbial characterization would require minimum sample preparation, be rapid, automated and inexpensive. In this study, we have tested if ^{1}H NMR spectroscopy will be of value in differentiating metabolite profiles of urine samples with and without UTI.

MATERIALS AND METHODS: 33 urine samples (Control: 16; Infection: 17) were collected from children (ages: 1- 16 years) who were referred to the Diagnostic Imaging Department of the Children's Hospital in Winnipeg for a voiding cystourethrogram (VCU). A sample of the urine taken from the bladder was sent for urine dipstick, microscopy and culture, and a portion of the sample was allocated for the NMR spectroscopic study. The "gold standard" for confirmation of UTI was the presence of pyuria (dipstick or microscopy) and growth of a single organism $>10^8$/L. After measuring pH, 500 μl of the urine sample was taken in a 5 mm NMR tube with a re-usable co-axial capillary tube (sealed on both ends) containing TSP dissolved in D_2O. The TSP was used for chemical shift referencing (0.00 ppm) and for the alignment of spectra during data processing. All samples were run on an Avance 360 MHz Spectrometer (Bruker Instruments) with no spinning. The temperature was set to 298 K and lock performed on the deuterium signal. The following acquisition parameters were employed in all experiments: NS (number of scans) = 32; P1 (90° pulse) = 6 μsec, PL9 (presaturation power) = 60 dB, TD (number of points in time domain) = 32k, D1 (relaxation delay) = 5.0 s, SW (spectral width) = 4990 Hz, and AQ (acquisition time) = 2.8 s.

RESULTS & DISCUSSION: Figure 1 shows ^{1}H NMR spectra of urine samples from (a) control and (b) UTI patient showing alteration in the levels of key urinary metabolites- creatinine, creatine, trimethylamine-N-oxide (TMAO), and an unidentified signal at 3.71 ppm. From Fig. 1, it is clear that TMAO, creatine, and the unidentified signal at 3.71 ppm have been elevated whereas creatinine is relatively decreased. Of the 17 UTI patients, 15 showed either one or more of the above features (see Figure 2 for more details).

In total, TMAO was elevated in the urine of 9 patients, creatine was elevated in 8 patients and the metabolite at 3.71 ppm was elevated in 8 patients. The spectral patterns of the remaining 2 urine samples (from UTI patients) resembled those of the control group, however, one of them showed the presence of additional signals in the region 0.8 – 1.6 ppm. Composition of urine is altered due to the bacterial invasion of bladder epithelial cells as well as host-defence against the invading bacteria. The pathogens identified in this cohort included *E. coli, Proteous mirabilis, Klebsiella oxytoca and Gram negative rods*. Further studies using a large patient-cohort may provide an idea about causative organisms responsible for the UTI.

Once data from a larger number of samples is collected, we will employ a statistical classification strategy developed in-house and successfully used in other applications to analyze the data [3]. Metabolic profiling using ^{1}H NMR spectroscopy and pattern recognition methods will be valuable in the high-throughput analysis of urine samples for the differentiation of spectral patterns and identification of biochemicals responsible for UTI.

Figure 2: Venn diagram representing urine samples (n=15) from UTI patients with elevated levels of three metabolites.

Figure 1: ^{1}H NMR spectra of urine samples obtained from (a) Control and (b) UTI patient showing elevated levels of creatine, TMAO, and an unknown signal at 3.71 ppm in the UTI patient.

CONCLUSION: Due to the longer diagnostic wait time required for the culture method, dipstick methods are commonly used for the quick diagnosis of UTI. However, dipstick methods are commonly associated with false negative and/or false positive results. ^{1}H NMR based metabolic profiling of urine samples could be valuable in the accurate diagnosis of UTI.

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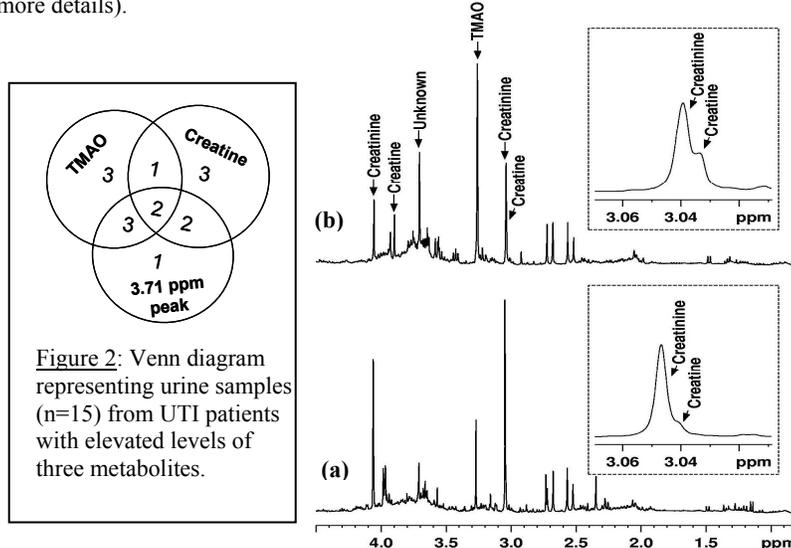3. Bezabeh T, Somorjai R, Dolenko B et al., *NMR Biomed*. 2009; **22**: 593-600.