Elevated Levels of Acetate in ^{1}H NMR of Urine Could Have Diagnostic Utility in Pediatric Urinary Tract Infection

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INTRODUCTION: Urinary tract infection (UTI) is the most common community acquired bacterial infection in adults (especially women) and children. Uropathogenic *Escherichia coli* (UPEC, strain CFT073) is the most common uropathogen causing UTI, accounting for up to 70 – 90% [1]. However, other pathogens such as *Proteus* and *Klebsiella* species, and some *Staphylococcus* and *Streptococcus* species may also cause UTI. The gold standard for the confirmation of UTI in children is the growth of a single organism on a culture of urine obtained by urethral-catheterization/suprapubic aspiration. The culture method has the disadvantage that it requires more than 48 hours to give a result. Therefore, more rapid tests are being used. Currently, the most widely used rapid tests are dipsticks (leukocyte esterase and nitrite). Dipstick tests have the advantage of being quick, but they are associated with high false negative and/or 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 regard, ^{1}H NMR-based methods have been developed by us and others [2,3]. In this study, we have tested if the quantification of acetate in urine samples using ^{1}H NMR can be of value in the diagnosis of pediatric UTI.

MATERIALS AND METHODS: A total of 108 urine samples [Controls: 87; UTI: 13; Asymptomatic bacteriuria (ABU): 8] 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. Patients with ABU are the ones with positive culture but without measurable inflammation to suggest UTI. 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 a known concentration of TSP dissolved in D_2O. The TSP was used for chemical shift referencing (0.00 ppm), the alignment of spectra during data processing and as an external reference for the quantification of acetate levels in the urine samples. All samples were run on an Avance 360 MHz Spectrometer (Bruker Biospin) 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 typical ^{1}H NMR spectra of urine samples from control, a patient with asymptomatic bacteriurea (ABU) and an UTI patient, showing relative levels of some major urinary metabolites - acetate, lactate, creatinine/creatine, trimethylamine-N-oxide (TMAO) and urea. From Fig. 1, it is clear that acetate levels were highly elevated in UTI compared to the asymptomatic bacteriuria and control subject. Acetate was detected only in 47/87 control subjects (21±28 µM, mean±S.D.), but it was detected in all of the 13 UTI patients (1202±1329 µM), and 7/8 patients with asymptomatic bacteriuria (479±770 µM). We compared the mean values of acetate in the above three groups using a Student's t-Test, and found that the control and UTI groups were significantly different (P = 0.007). However, the comparisons between control and ABU groups as well as the UTI and ABU groups were not statistically significant (P = 0.14 and 0.13 respectively).

In a recent study, Gupta et al. [3] have reported the presence of acetate, lactate, succinate, and ethanol as key metabolites of bacterial degradation in urine samples from adult UTI patients. They attributed the presence of the above metabolites to glucose metabolism. In our study comprising children with UTI, we detected only acetate, lactate, and succinate but not ethanol. Therefore, we speculate a different mechanism for the elevated levels of only acetate and for the absence of ethanol in the urine samples from pediatric UTI patients.

Urine is a dilute mixture of amino acids and small peptides, quite similar to tryptone broth [4]. D-serine is one of the most abundant amino acids present in human urine with a concentration ranging between 3 to 115 µg/mL. It is well known that growing cells in tryptone media sequentially utilize serine and then aspartate, secreting acetate. This order of nutrient preference also holds true for *E. coli*. Since UPEC is the major uropathogen responsible for UTI, it adapts to D-serine metabolism resulting in the production of acetate [4]. This could be the reason for the elevated levels of acetate in the urine samples from UTI patients in our study. Although the current observation is promising, it needs to be validated on a large number of samples.

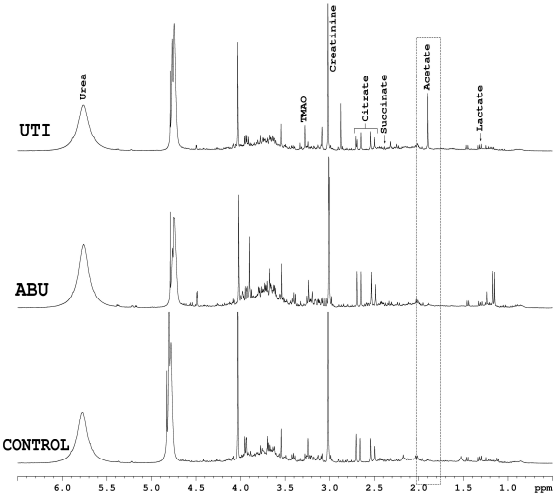

Figure 1: ^{1}H NMR spectra of urine samples obtained from (a) Control, (b) Patient with asymptomatic bacteriuria (ABU) and (c) UTI patient, showing significantly elevated levels of acetate (signal at 1.92 ppm) in the UTI patient.

CONCLUSION: Elevated levels of acetate in urine indicate underlying uropathogenic *E. coli* infection and the quantification of urinary acetate could be valuable in the diagnosis of UTI in both children and adults. Since the gold standard "culture method" requires longer diagnostic wait time and dipstick methods are commonly associated with false negative and/or false positive results, development of biomarker-based methods will be helpful in the accurate diagnosis of UTI.

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