# Taurine- a possible biomarker in urine of Urinary Bladder Cancer by 1H NMR Spectroscopy: A pilot study

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**INTRODUCTION:**

Urinary bladder cancer (UBC) is the fourth most common cancer in men and eighth in women[1]. An average of 260,000 new cases of urinary bladder cancer is diagnosed worldwide every year. Even, decrease in cigarette smoking, the most common cause of bladder cancer, did not arrest the steady increase in the incidence of bladder cancer[2]. Despite considerable efforts to develop safe, reliable, non-invasive screening strategies for bladder cancer, the identification of a single predictive diagnostic marker of the disease has remained elusive. The gold standard of diagnosing bladder cancer is histopathology along with urine cytology and transurethral cystoscopy and other advanced techniques include MRI-based virtual cystoscopy and Urovysion™ fluorescence in situ hybridization, CT, MRI, ultrasound and X-rays of the urinary tract. Cytology is very specific but suffers from low sensitivity and is highly invasive. There are newer urine bound markers for the diagnosis of bladder cancer viz. blood group antigens, tumor associated antigens, oncogenes, peptide growth factors and their receptors, cell adhesion molecules, tumor angiogenesis and angiogenesis inhibitors, and cell cycle regulatory proteins[3]. However, these markers are more sensitive but more expensive and time-consuming. The utility of $^1$H NMR spectroscopic urinalysis of UBC patients has been explored in this study, as metabolic profiling can be done in one-shot and thus, reducing the time for diagnosis.

**MATERIALS AND METHODS:**

All samples of urine from patients and controls were snap-frozen in liquid nitrogen immediately and were stored in –80°C until NMR experiments were performed. Prior to NMR analysis, samples were thawed and centrifuged to remove dead cells and RBC's present in urine. 500 microlitres of these samples were taken in NMR tubes and were subjected to NMR measurements. $^1$H NMR experiments were performed on Bruker BioSpin *Avance* 400 MHz FT-NMR spectrometer using 5mm Broadband Inverse probe equipped with z-gradient. A single-pulse $^1$H NMR and CPMG spectra were recorded with presaturation of water signal for semi-quantitative evaluation of metabolites. For NMR spectral assignment, one and two-dimensional experiments such as COSY, TOCSY and HSQC, were performed. Known concentration of TSP was used in a capillary for external reference as well as for quantitative estimation. Five metabolites were chosen for quantitative estimation because of overlapping of signals from various metabolites. All NMR raw data were recorded and processed using XWINNMR 3.5.

**RESULTS:**

Sixty-six cases were included in the study: twenty-eight belong to diseased group and thirty-eight to control group. A simple pulse and acquire spectrum showed resonances from lactate, alanine, citrate, dimethylamine (DMA), creatinine, glycine, taurine, phenylalanine (PA), tyrosine, hippurate and formate. Due to extensive peak overlap, resonances of citrate, DMA, taurine, PA and hippurate were semi-quantitated and significance level was calculated by Mann-Whitney U-test for citrate ($p< 0.002$), DMA ($p< 0.600$), taurine ($p< 0.001$), PA ($p<0.003$) and hippurate ($p<0.001$). Taurine, citrate, PA and hippurate have shown significant variations in their concentrations (Fig.1). However, concentration of citrate and phenyalanine were found to decrease, concentration of taurine which was not observed in control was found to be present in case of bladder cancer (Fig 2). The concentration of hippurate also showed significant alterations in bladder cancer.

**DISCUSSION:**

The $^1$H NMR spectroscopy has proved to be a powerful tool in exploring metabolic perturbations of various biofluids viz. cerebrospinal fluid, serum, urine, bile etc. in various pathological conditions. This study investigates the role of $^1$H NMR spectroscopy in urine analysis of pathological versus control state. The decreased level of citrate and phenylalanine goes in concordance with the fact that rate of TCA (Kreb's) cycle decreases in cancerous cell[4]. Increase in concentration of taurine also defines the malignant characteristics and its prominent presence in urine of patients suffering with bladder cancer may provide a promising diagnostic measure. Hippuric acid was found to be present in negligible amount in bladder cancer. The results of this study can be more promising at high field spectrometers with higher sensitivity and improved spectral dispersion. Thus, urinalysis by $^1$H NMR spectroscopy may provide a better non-invasive predictive measure along with the benefit of rapidity in diagnosis.

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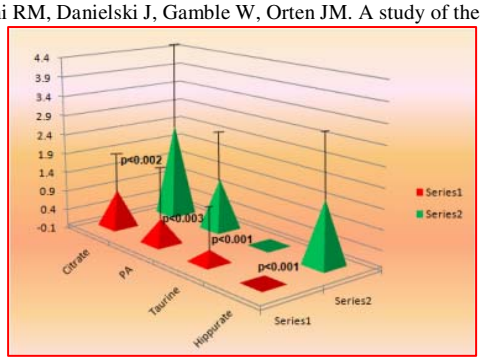


Figure 1 : Graph depicting concentrations(Mean±SD) of citrate, PA, taurine and Hippurate in UBC(Series 1) and control (Series 2) along with their respective p-values, where $p<0.05$ is significant.

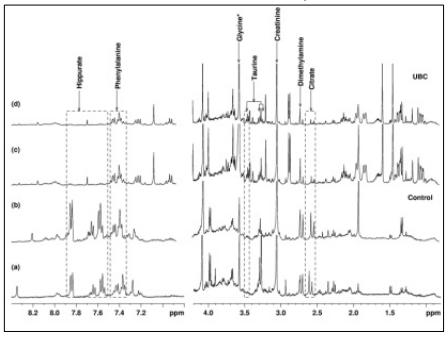


Figure 2 : Spectra of urine obtained from control{(a), (b)} and UBC {(c), (d)} cases. Taurine, citrate,hippurate and phenylalanine show significant variations in their concentrations. *Glycine is present as an impurity due to cystoscopy.