Low- and High-Grade Bladder Cancer Determination via Human Serum-Based Metabolomics Approach

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SYNOPSIS: A novel approach of ^{1}H NMR spectroscopy of serum metabolite profile and multivariate statistical approach—orthogonal partial least-squares discriminant analysis (PLS-DA) was carried out to identify differential biomarkers of urinary bladder cancer (UBC) comprising, low grade (LG) and high grade (HG). The study was carried out on 67 UBC patients and 32 healthy volunteers to differentiate among healthy control (HC), LG and HG. PLS-DA-derived serum metabolomics were able to precisely discriminate 95% of cases of BC with 96% sensitivity and 94% specificity when compared to HC and 98% of cases of LG from HG with 97% sensitivity and 99% specificity.

INTRODUCTION: Urinary bladder cancer (UBC) is the 5th most common cancers and one of the leading causes of death worldwide[1]. Even after treatment, the recurrence rate of BC is significantly higher. The current comprehensive examinations include cytology, imaging, and highly expensive and invasive cystoscopy[2-4]. These methods are subjective, costly, have inter-observer variability, and poor sensitivity and specificity especially for LG tumors[5-7]. To address the shortcomings of urine cytology and cystoscopy for probing and grading of UBC, we applied 1H-nuclear magnetic resonance (NMR) spectroscopy as a surrogate method for fast screening and grading of BC.

MATERIALS AND METHODS: A 2 ml peripheral venous blood sample was obtained and serum was separated using standard protocol. The total 99 serum samples; 67 from UBC patients, and 32 HC were collected and analyzed using ^{1}H NMR spectroscopy. Final diagnosis was achieved after consideration of histological investigations. The NMR experiments were performed on a Bruker Avance 800 MHz spectrometer using a 5-mm broad band inverse probe head with a Z-shielded gradient. 400µl of serum samples were taken in 5-mm NMR tubes. A co-axial insert containing 0.006 mg trimethyl silyl propionic acid sodium salt (TSP) deuterated at CH2 groups was used for deuterium lock, external reference, and the standard signal for absolute quantitative estimation of metabolites. For all the specimens, one-dimensional ^{1}H NMR measurements were performed using a Carr-Purcell-Meiboom-Gill (CPMG) sequence with water suppression by pre-saturation at 25°C. The parameters used were: spectral sweep width, 16500 Hz; data points, 64 K; pulse angle, 90°; total relaxation delay, 5s; number of scans, 128; and line broadening, 0.3 Hz. Multivariate chemometric analysis was applied on the data generated from the CPMG sequence with the help of 'Unscrambler X' software (version 10.0.1, Camo USA, Norway). The data were subjected for supervised- orthogonal partial least-squares discriminant analysis (PLS-DA) to check grouping trends[8] and to builds a model which can be used to detect potential biomarkers related to the discrimination among LG, HG, and HC cohorts. To evaluate the clinical utility of biomarkers derived from PLS-DA model, ROC analysis was also performed.

RESULTS: Fig. 1 shows various typical 1H NMR spectra of serum samples, revealing a diverse metabolic profile and chemical shift assignments of different resonances along with histopathological analysis of urinary bladder tissue samples from different groups. PLS-DA and ROC analysis reveals that the combination of dimethylamine (DMA), malonate, lactate, glutamine, histidine, and valine were able to accurately classify 95% of BC with 96% sensitivity and 94% specificity when compared to HC. On the basis DMA, lactate, glutamine, histidine, and valine, 97% of LG cases were able to segregate with 95% sensitivity and 99% specificity when compared to HC. Similarly, the combination of DMA, malonate, lactate, and histidine were able to segregate 98% of HG from HC with 97% sensitivity and 99% specificity. The permutation of DMA, glutamine, and malonate were able to differentiate 98% of LG cases from HG with 97% sensitivity and 99% specificity. Our study showed that LG and HG of BC not only generate signature biomarkers, but also that these biomarkers can be used to differentiate them.

DISCUSSION: This study exhibited the role of 1H NMR spectroscopy in serum analysis of different grades of BC with their respective controls. The increased level of lactate not only justifies by the fact that the rate of anaerobic glycolysis increases in BC cells but also generates excessive branched-chain amino acids such as valine[9]. The elevated malonate and DMA level goes with the fact that the fatty acid metabolism and rate of glycerophospholipid is significantly higher in BC cells to lodge the excess amount of these metabolites[10]. The altered level of glutamine in LG is in accordance with the fact that the rate of glutaminolysis increases in BC cells to meet the requirement of energy source needed for abandoned proliferation[11]. Inflammation is the key event of many cancers and this fact is also rationalized in our study too by observing the escalate level of histidine (only source of histamine)[12]. ^{1}H NMR-based serum metabolic screening appears to be a promising, rapid, and least-invasive approach for probing and grading of BC in contrast to the tedious conventional approach. This approach may be used to predict early and accurate identification of BC which is crucial to start tailored treatment, leading to prompt and appropriate care of BC patients.

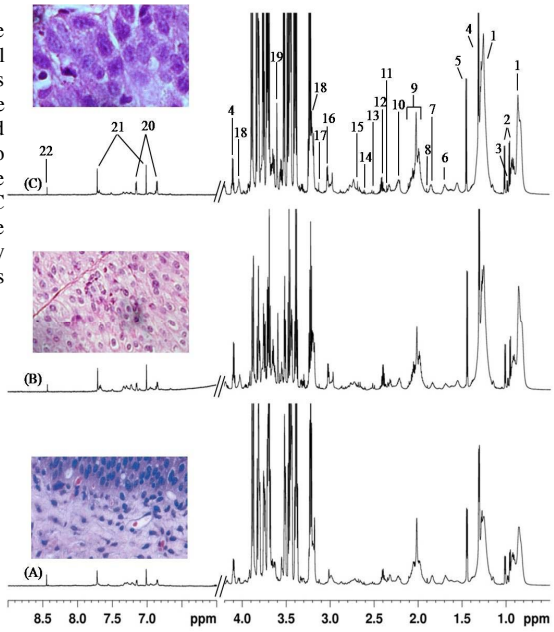

Fig.1: The histopathological analysis of urinary bladder tissue samples from different groups viz: (A) healthy control, (B) Low grade BC, (C) High grade BC patient is shown alongside of each 1H NMR spectra of corresponding human serum samples. Key: 1, HDL+LDL+VLDL; 2, valine; 3, isoleucine; 4, lactate; 5, alanine; 6, lysine; 7, proline; 8, acetate; 9, N-acetylglycoprotein; 10, glutamate; 11, pyruvate; 12, glutamine; 13, citrate; 14, hypotaurine; 15, dimethylamine; 16, creatinine; 17, malonate; 18, choline; 19, glycine; 20, tyrosine; 21, Histidine; 22, format.

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