Reports of Biochemistry & Molecular Biology
Vol. 2, No. 2, Apr 2014

Original article

www.RBMB.net

A Study of Lipid- and Protein- Bound Sialic Acids for the Diagnosis of Bladder Cancer and Their Relationships with the Severity of Malignancy

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Abstract

Background: The gold standard for detection of bladder cancer is cystoscopy, which is an invasive and complicated procedure. Our study was conducted to find a tumor marker with high specificity, sensitivity, and accuracy for the diagnosis of bladder cancer.

Methods: Serum samples were collected from 58 bladder cancer patients and 60 healthy control subjects. Levels of lipid-bound sialic acid (LBSA), and protein-bound sialic acid (PBSA) were measured spectrophotometrically by Aminoff's method.

Results: Mean levels of both markers were found to be significantly higher in the patients than the healthy controls. Positive correlations were observed between serum levels of lipid- (r=0.283, $p<0.05$) and protein-bound (r=0.56, $p<0.05$) sialic acids and the grade of malignancy. To differentiate patients with bladder tumors from healthy controls, cut-offpoints were determined for each of the two parameters based on Receiver Operating Characteristic (ROC) curve analysis (LBSA=21.25 mg/dL, PBSA=6.15 mg/dL). The data showed good sensitivities (LBSA=89%, PBSA=79%), specificities (LBSA=70%, PBSA=70%) and accuracies (LBSA=83%, PBSA=81%) for both markers.

Conclusion: Measuring serum LBSA and PBSA by this simple, reproducible, noninvasive, and inexpensive method can accurately discriminate cancer patients from healthy individuals.

Keywords: N-Acetylneuraminic Acid, Tumor Markers, Urinary Bladder Neoplasms

Introduction

Cancer is a major public health problem in most of the world. One of every four deaths in the United States is due to cancer. Bladder cancer is the second most common urogenital malignancy in humans (1). The most common method of diagnosis and initial staging of bladder cancer is cystoscopy plus transurethral resection (TUR). This procedure is the gold standard for bladder cancer detection, but is expensive and invasive (2). Therefore, the possibility of screening and following patients with bladder tumors using noninvasive, inexpensive, and repeatable serum tests would be a significant step forward (3, 4). Cell surfaces change during malignant transformation. The analysis of these changes could be considered a kind of biochemical biopsy, which simplifies the diagnosis of organ abnormalities (5, 6).

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Received: Jan 5, 2014; Accepted: Feb 9, 2014

It has been demonstrated that glycosylation patterns of cells change during tumorigenesis (7). Sialic acid (N-acetylneuraminic acid) is a nine-carbon α-keto acid commonly found at the ends of carbohydrate chains of glycoproteins and glycolipids (8). During cancer progression, the sialic acid content of cell surfaces increases and this sialic acid is released into the blood as glycoproteins and glycolipids. A positive correlation exists between the increased levels of lipid- and protein- bound sialic acids and the clinical activity of some diseases, including cancer (9-11). The current study aimed to find a noninvasive and inexpensive way to diagnose bladder tumors and help patients experience less discomfort in post-treatment examinations than is currently possible.

Materials and Methods

Serum samples from suspected bladder cancer cases who were admitted to one of our three research hospitals were collected and stored at -70° C. After examinations, 58 patients who were histopathologically confirmed as having superficial or invasive bladder tumors were enrolled in the study. Smokers and patients with infections, diabetes, or thyroiditis were excluded. Tumor grades and stages were determined according the ISUP/WHO categorizing system. In addition, serum samples were collected from 60 healthy non-smoking age- and sex-matched controls. The controls were judged to be healthy by reviewing their medical histories, and they had no evidence of disease.

Table 1. The clinical details of bladder cancer patients

Stage (n)	Grade (n)		
	G1: n (%)	G2: n (%)	G3: n (%)
Ta	4 (6.89)	4 (6.89)	1 (1.72)
T1	4 (6.89)	17 (29.31)	3 (5.17)
T2	2 (3.44)	4 (6.89)	10 (17.24)
T3	-	1 (1.72)	3 (5.17)
T4	-	-	5 (8.62)
Total(%) = 58 (100)	10 (17.24)	26 (44.82)	22 (37.93)

Data are presented as number of patients (percent). Ta: Non-invasive papillary carcinoma, T1: The tumor has grown from the layer of cells lining the bladder into the connective tissue below, T2: The tumor has grown into the muscle layer, T3: The tumor has grown through the muscle layer of the bladder and into the fatty tissue layer that surrounds it. T4: The cancer has spread to lymph nodes or to sites such as the bones, liver, or lungs. G1: slow growing and look similar to normal cells, G2: slow growing and unlikely to spread, G3: more quickly growing and more likely to spread.

The sialic acid content of serum glycoproteins was measured by a modification of Aminoff´s method (12, 13). LBSA was determined by the method described by Katopodis and Stock (14). The data were analyzed using the Statistical Package for Social Sciences (version 18.0; SPSS Inc., Chicago, USA). The Student's t-test was used to compare mean levels. A χ^2 test for quality variables and one-way ANOVA with post-hoc tukey were used to compare the mean levels of quantitative variables in different tumor stages and grades. Receiver Operating Characteristic curves were constructed to estimate the diagnostic value of the markers. To evaluate the association of the markers with the tumor stages and grades, Spearman's correlation coefficient was administrated. A *p*-value below 0.05 was considered as statistically significant.

Table 2. Mean levels of sera LBSA and PBSA in case and control groups

	Patients	Controls	*P*-value
LBSA (mg/dl)	28.66±6.42	20.34±5.77	<0.001
PBSA (mg/dl)	7.2±1.34	5.56±1.12	<0.001

Data are presented as Mean±SD. LBSA: lipid bound sialic acid, PBSA: protein bound sialic acid. The differences between groups were assessed by Independent t-test. P<0.05 was considered as significant.

Results

The case and control groups were age- and sex-matched (p=0.093 and p=0.405 respectively). In the case group, there were 47 men and 11 women with a mean age of 62.53±12.52 years. The control group contained 52 men and 8 women with a mean age of 59.47±6.19 years. Demographic data of the case group are shown in Table 1. There was a significant difference between the mean levels of LBSA (p<0.001) and PBSA (p<0.001) in the case and control groups (Table 2). Significant differences were seen between the mean levels of PBSA in G1 and G2 (p<0.05), G1 and G3 (p<0.001), and G2 and G3 (p<0.05) (Table 3). These results mean that the PBSA concentrations increased as the tumors grew. Positive correlations were observed between LBSA and PBSA alterations with the histopathological grades of the tumors (r=0.283, p<0.05 and r=0.56, p<0.05

respectively). To evaluate the diagnostic value of these markers, ROC curves were constructed (Figures 1 and 2). The best cut-off points, specificities, sensitivities, and other diagnostic values were calculated (Table 4). The best cut-off point is the point that most accurately differentiates healthy subjects from cancer patients (15). The areas under the curves (AUC) (AUC±SE) for LBSA and PBSA were 0.845±0.036 and 0.825±0.037, respectively. These results revealed that approximately 84% and 82% of randomly selected subjects in the case group would have higher LBSA and PBSA concentrations, respectively, than randomly selected subjects from the control group.

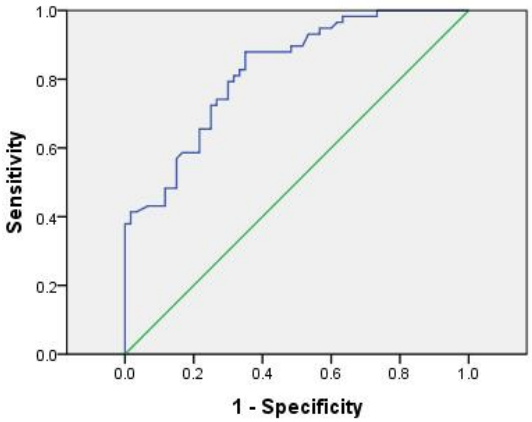

Fig. 1. ROC curve for protein bound sialic acid (PBSA) marker

Discussion

Cell surface glycoconjugates, which include glycoproteins and glycolipids, play important roles in a variety of biological events such as cell-cell interactions, cell-matrix adhesion, cell-cell recognition, antigenicity, and tumor progression (7). The properties and contents of carbohydrate moieties of glycoproteins and glycolipids change during tumorigenesis (16, 17). Sialic acid is a nine-carbon sugar found at the terminal ends of glycoconjugates. It is widely distributed and has important functional and biological roles in the body (8, 9).

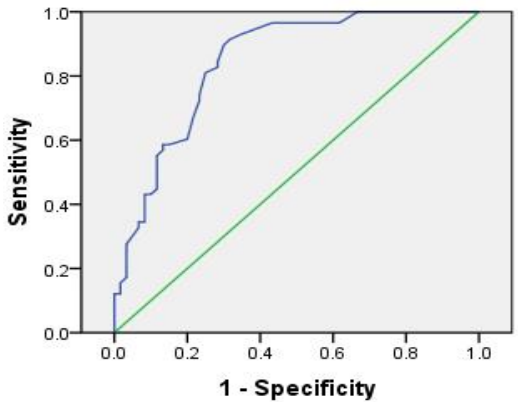

Fig. 2. ROC curve for lipid bound sialic acid (LBSA) marker

Table 3. Mean levels of LBSA and PBSA in different stages and grades of malignant tumor and the number of elevated markers

Histopathological data	LBSA (mg/dL)		PBSA (mg/dl)	
	Mean±SD	Positive (>21.25)†	Mean±SD	Positive (>6.15)†
G1	25.1±1.15	9/10	5.85±0.245	4/10
G2	28.63±1.23	22/26	7.04±0.203	21/26
G3	30.32±1.41	21/22	8.01±0.282	21/22
Ta+T1	28.37±6.23	30/34	6.83±1.11	25/34
T2	27.27±5.63	14/15	7.47±1.08	13/15
T3+T4	32.14±6.64	8/9	8.14±1.99	8/9

† Data are presented as the number patients with positive values/total number of patients. LBSA: lipid bound sialic acid, PBSA: protein bound sialic acid.,Ta: Non-invasive papillary carcinoma, T1: The tumor has grown from the layer of cells lining the bladder into the connective tissue below, T2: The tumor has grown into the muscle layer, T3: The tumor has grown through the muscle layer of the bladder and into the fatty tissue layer that surrounds it. T4: The cancer has spread to lymph nodes or to sites such as the bones, liver, or lungs. G1: slow growing and look similar to normal cells, G2: slow growing and unlikely to spread, G3: more quickly growing and more likely to spread.

Table 4. Diagnostic indices of LBSA and PBSA markers in bladder cancer patients

Tumor marker	Sensitivity	Specificity	Positive predictive value (%)	Negative predictive value (%)	Accuracy	Cut-off point (mg/dL)	Area under curve (AUC±SE)
LBSA	89%	70%	76%	90%	83%	21.25	0.845±0.036
PBSA	79%	70%	78%	84%	81%	6.15	0.825±0.037

LBSA: lipid bound sialic acid, PBSA: protein bound sialic acid.

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This sugar can modify the ligand-receptor binding process by either creating or masking recognition sites at the cell surface. For example, it can mask epitopes of malignant cells, weaken immune response reactivity, and help cells evade cellular defense systems (10). Oversialylation of a malignant cell is a phenomenon of considerable biological importance that has been reported in many types of cancer such as liver (15), prostate (18), breast (19), oral cavity (4), and brain (5). The negative charge of sialic acid causes repulsion between cells. This repulsion enhances and facilitates cell detachment due to the increasing negative charge. Thus, it promotes motility and increases cell adhesion and invasion to the endothelium, which is a crucial step in metastasis. Moreover, hypersialylation probably extends the life span of tumor cells in the blood stream (10, 20).

Some studies suggest that sialic acid levels increase even before the emersion of cancer clinical symptoms (10). Aberrant glycosylation is one of the most prominent characteristics of malignant tissues (18).

In our study, LBSA and PBSA were significantly higher in cancer patients (p<0.001) than normal controls, which is in accordance with some earlier reports. We found significant increases in both markers in higher grades of malignant tumor and significant correlations between tumor grades and LBSA and PBSA concentrations. In addition, the numbers of patients with elevated markers above their cut-off points were greater in higher grade and stage tumors than in lower grade and stage tumors.

In a study performed by Lagana et al, no significant increases were observed in mean levels of total sialic acid (TSA) and LBSA in 22 patients with bladder tumors in comparison to 20 normal subjects; however, high sensitivity and specificity was reported for free sialic acid (FSA) (21). In contrast, in a report by Oztokati et al (22) LBSA was significantly increased in bladder cancer patients compared with healthy controls

Significant elevation of LBSA and PBSA had been reported previously by Erbil K et al. In this study sensitivity and specificity for LBSA were 70 and 90%, and for PBSA, 65 and 87.5%, respectively (23).

Higher concentrations of sialic acid in the urine of patients with bladder tumors than in healthy individuals have been previously observed (24, 25). Konukoglu et al (24) reported a massive decrease in urinary excretion of sialic acid after treatments.

It has been documented that the expression of α2-6-linked sialic acid proteins in many human tumors is a common phenomenon, which correlates with poor prognosis and enhances the invasive characteristics of tumor cells (26).

Increased activity of circulatory sialyltransferase in cancer patients has been reported (16, 10). Increased turnover and secretion or shedding of sialic acid into the blood have been suggested as other causes for elevated sialic acid in serum.

According to previous reports, elevated sialic acid in blood is associated with poor prognoses and resistance to treatments (17). Some sialic acids also have roles in signaling, some of which result in angiogenesis and tumor growth (9). Current therapies require cystoscopies every 3-6 months to monitor and manage the disease due to high bladder cancer recurrence rates (27). An important objective of a bladder cancer blood-screening test with high sensitivity and specificity is to find a simple and non-invasive method for early detection and treatment monitoring. Altered cell glycosylation is a characteristic manifestation of a number of cancers, including bladder carcinoma (16).

The challenge of finding reliable serum tests for cancer detection continues (3). According to our results, PBSA and LBSA can discriminate bladder

cancer patients from healthy controls with relatively high sensitivity, specificity, and accuracy.

In conclusion, we believe that sialic acid can be a useful tumor marker for bladder cancer detection. However, further investigations are needed to determine whether this approach has clinical application as a single biomarker or as an adjunct to traditional approaches in detection and monitoring of bladder tumors.

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Acknowledgements

This study was financially supported by Tehran University of Medical Sciences (Project No: 6658). We appreciate the personnel of Emam Khomeini, Milad and Labbafinejad hospitals, and Iranian Blood Transfusion Organization for their active collaboration with our group. The authors declare that they have no conflict of interests.

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