

SIGNIFICANCE OF TOTAL AND LIPID BOUND SIALIC ACID IN SERUM AND URINE AS MARKERS FOR BLADDER CANCER

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Abstract

The reliability of serum total sialic acid (TSA), serum lipid bound sialic acid (LSA), and urinary sialic acid / creatinine ratio (TSA/ Cr. ratio) as markers for bladder cancer for grading, staging and follow-up purposes was evaluated in 10 healthy controls and 70 patients with bladder cancer, 25 of them with superficial transitional bladder tumors were evaluated after treatment with TUR for follow up.

We found that TSA, LSA and TSA/ Cr. ratio levels were significantly higher in patients with bladder cancer (56.9 ± 8.9 mg / dl, 22.3 ± 2.3 mg/ dl and 37.2 ± 16.1 ug/mg creatinine respectively) than controls (48.8 ± 7.1 mg/dl, 16.9 ± 0.6 mg/dl and 11.9 ± 5.1 ug/mg creatinine respectively).

There was no significant difference between grade I, II and III, also between stage Ta, T1 & T2 and stage T3 & T4 bladder cancer patients regarding LSA and TSA, but there was a significant difference between these grades and stages regarding urinary TSA / Cr. Ratio (20.1 ± 16.1 , 40.6 ± 14.4 and 47.8 ± 11.9 ug/mg creatinine in grade I, II and III respectively and 32.1 ± 12.1 , 46.4 ± 15.1 ug/mg creatinine in stage Ta, T1 & T2 and stage T3 & T4 respectively). These findings suggest that TSA and LSA can not be used for grading and staging of bladder cancer patients but urinary TSA/Cr. ratio can be used for grading and staging of these patients. On comparison of superficial transitional bladder cancer patients before and after treatment regarding the studied parameters, we found marked drop of urinary TSA/cr. ratio in post-treatment patients (36.2 ± 15.1 ug/mg creatinine before treatment versus 17.5 ± 5.3 ug/mg creatinine after treatment) in contrary with serum TSA and LSA.

We conclude that : urine can be used as easily available physiologic fluid for evaluation of TSA / Cr. ratio by an easy.

inexpensive, photometric method as a marker for bladder cancer for grading, staging and follow - up of treatment in patients with bladder cancer.

Introduction

The urinary bladder is the most common site of cancer in the urinary tract. The male to female ratio of bladder cancer is 3 : 1. Bladder cancer is the fourth most common cause of cancer in men, accounting for about 9% of all cancer cases and is the fifth most common cancer in women, with an incidence comparable to that of ovarian cancer (Silverman et al., 1992 and Boring et al., 1994).

Early diagnosis of bladder cancer is very important for early management and follow-up of these patients. The limitations of the available clinical, roentgenographic and laboratory methods in diagnosing bladder cancer, as well as the considerable logistic problems and expense required for the follow-up of these patients have led to the search for tumor markers which may help in early detection and follow-up of bladder cancer patients (Erbil et al., 1986 and Akcay et al. 1994). Glycoproteins and glycolipids are cell surface constituents, important to cancer related properties, and

sialic acid is a common terminal saccharide of these glycoproteins and glycolipids. Sialic acid is the common name for acetylated derivatives of neuraminic acid (Spiro, 1963; Hakomori, 1974 and Alhadef, 1989).

A neoplasm often has an increased concentration of sialic acid on the tumor cell surface. In addition, both in vitro and in vivo studies indicate that tumors are capable of shedding cell surface antigens and suggest that the ability to do so may be related to the metastatic potential of the tumor, which increases its concentration in the blood (Bernacki and Kim, 1977; Kloppel and Morre, 1980 and Bolmer and Davidson, 1981). Therefore, total sialic acid in serum is of great interest as marker of malignancy as it was found to be elevated in many tumors e.g. breast, stomach, prostate and bladder cancer, although it has not been demonstrated to be specific for any type of cancer, in addition to the controversies regarding its quantitative changes in cancer patients (Moss et al., 1979; Harvey et al., 1981;

Dnistrian et al., 1982 and Shamberger, 1984).

Alterations in glycolipid metabolism are well documented in many tumors including human cancers (Hakomori, 1974). Furthermore, sialic acid containing glycolipids (lipid bound sialic acid) were found to have a relatively high retention time in plasma, a biophysical property that would allow them to accumulate in the blood (Barkai and DiCesare, 1975). Therefore, significantly elevated levels of serum lipidbound sialic acid (LSA) have been reported in patients with urogenital cancer, in addition to other types of cancer (Barkai and DiCesare, 1975 and Katopodis and Stock, 1980).

Only few reports could be found in the literature regarding urinary excretion of sialic acid in bladder tumors. It is postulated that desquamation of surface epithelial cells is the origin of normal sialic acid excretion in urine. In patients with bladder neoplasms, sialoglycoproteins and sialoglycolipids are shed or secreted by malignant cells which often have increased concentrations of sialic acid on their cell surface leading to its increased

concentration in urine (Akcay et al., 1994).

This study was carried out to estimate the levels of serum total sialic acid (TSA), lipid bound sialic acid (LSA) and urinary sialic acid/creatinine ratio (TSA/ Cr.) ratio and evaluate their potential clinical significance as early biochemical markers for early detection, grading, staging and monitoring the efficacy of therapy in bladder cancer patients.

Subjects and Methods

This study was carried out at Benha university hospitals and included 70 patients with bladder cancer and 10 apparently healthy subjects without clinical evidence of the disease as control. All patients with bladder cancer were evaluated with complete clinical examination, radiological and laboratory investigations as well as cystoscopy and biopsy of their lesions and the diagnosis was established according to the results of histopathologic examination of their biopsy materials. Bladder tumors were clinically staged according to TNM staging system and graded according to the World Health Organisation (WHO) grading system.

Twenty five patients with clinically proved superficial transitional bladder tumors (stage Ta and T1) were selected out of the patients with bladder neoplasms and treated with complete transurethral resection (TUR) of their tumors followed by intravesical instillation of BCG (Bacillus Calmette-Guerin) vaccine as immunotherapy for prophylaxis against tumor recurrence in high risk cases (e.g. multiple tumors, high grade tumors). These patients were followed up and reevaluated at 3 month interval after treatment of their tumors by urinary cytology and cystoscopy and their clinical status was evaluated.

Blood and 24-hour urine samples were obtained from all subjects included in the study and twice from patients with superficial bladder cancer before TUR and 3 months after intravesical treatment with BCG.

The 24-hour urine samples were collected in clean containers for complete urine examination. Creatinine concentration and then stored at -20°C for urinary sialic acid assay. Blood samples were obtained by venous puncture and allowed to be clotted to

separate serum for determination of urea & creatinine concentration, then stored at -20°C for total and lipid bound sialic acid assay.

Determination of total sialic acid (TSA) in serum and urine :

TSA was estimated by thiobarbituric acid method described by Warren (1959). The procedure used the following solutions: Sodium periodate (meta) 0.2 M in 9 M phosphoric acid. sodium arsenite 10% in a solution of (0.5M sodium sulfate $-0.1\text{ N H}_2\text{So}_4$). Thiobarbituric acid. 0.6% in 0.5 M sodium sulfate. Cyclohexanone. (All aqueous solutions were prepared with warming). All chemicals were purchased from sigma chemical company.

Procedure :

To a sample volume of 0.2 ml was added 0.1 ml of the periodate solution, the tubes were shaken and allowed to stand at room temperature for 20 minutes, arsenite solution 1 ml was added and the tubes were shaken until a yellow brown colour disappeared, thiobarbituric acid solution 3 ml was added and the tubes were shaken, capped and then heated in a vigorously boiling water

bath for 15 minutes, tubes were removed and placed in cold water for 5 minutes, during cooling the red colour faded and the solution often became cloudy, this did not affect the final reading of this solution. 1 ml was transferred to another tube, which contains 1 ml of cyclohexanone, the tube was shaken twice and then centrifuged for 3 minutes in the centrifuge, the upper cyclohexanone phase was red, the optical densities of the organic phase were determined at 459 nm in a spectrophotometer.

- TSA in serum (mg/dl) = $0.075 \times 30.9 \times 0.D$ at 459.
- TSA in urine (ug/dl) = $0.075 \times 30.9 \times 1000 \times 0.D$ at 459.
- Urinary sialic acid/ creatinine ratio was calculated.

***Determination of lipid bound sialic acid (LSA) in serum :** by an improved method described by Katopodis and Stock, (1980).

Chemicals used: chloroform, methanol, phosphotungstic acid, n-butanol, butyl acetate, resorcinol reagents (stock solution was 2% in water) and n-acetyl neuraminic acid for standard curve (all chemicals were purchased from sigma chemical co.).

Procedure :

50 μ of serum was extracted with 3 ml of chloroform : methanol 2 : 1 (V/V), the lipid extract was partitioned with 0.5 ml water, LSA was purified by phosphotungstic acid precipitation, after removal of the supernatant, the sialic acid in the precipitation was determined by the resorcinol method of Svennerholm, (1975) as modified by Miettinen and Takki-Luukkainen, (1959), the final blue color was read at 580 nm in a spectrophotometer and the amount of lipid bound sialic acid was determined by use of a standard curve developed from a standard sample of n-acetyl neuraminic acid and the use of this formula:

$$\text{LSA (mg/ 100 ml serum)} = \frac{\chi \times 100000}{\gamma \times 50 \times 1000}$$

χ : y NANA read from standard curve for the sample.

γ : 1 ml of supernatant :
volume of entire supernatant.

Results

The present study included 70 patients with bladder cancer (42 males and 28 females, mean age 52 years \pm 16) and 10 healthy controls (7 males and 3 females, mean age 44 years \pm