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Increased Urinary Excretions of Oxidative Stress Biomarkers and Sialic Acid Associated with Severity of Bladder Tumors

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Abstract

Introduction: **Reactive oxygen species-induced damage to lipids, proteins and DNA plays a major role in carcinogenesis. This study aimed to measure urinary excretions of 8-hydroxydeoxyguanosine (8-OHdG), malondialdehyde (MDA) and total sialic acid (TSA) in patients with bladder tumor and to determine whether their excretory levels were associated with severity of the tumors.**

Materials and Methods: **Morning urine samples were collected from 48 patients with bladder tumor and 30 healthy subjects. Concentration of creatinine, proteins, 8-OHdG, MDA and TSA were measured in the urine samples. The severity of transitional cell carcinoma of the bladder (TCC) was assessed by urine cytology (grading C1 to C5). The bladder tumor patients were classified into 2 groups, patients with negative urinary malignant cells (C1 and C2; n = 28) and those with positive urinary malignant cells (C3 to C5; n = 20).**

Results: **Levels of urinary proteins, 8-OHdG, MDA and TSA in patients with bladder tumor were significantly higher than in healthy controls ($p = 0.002$, $p = 0.009$, $p < 0.001$ and $p < 0.001$, respectively). Urinary proteins, 8-OHdG, MDA and TSA levels in patients with C3 to C5 tumors were significantly higher than in those with C1 and C2 tumors ($p = 0.043$, $p = 0.007$, $p = 0.024$ and $p = 0.018$, respectively). In addition, urinary TSA levels in patients with C1 and C2 tumors were significantly higher than in healthy controls ($p = 0.004$).**

Conclusions: **The present study showed that urinary excretions of oxidative stress biomakers and TSA were significantly higher in bladder tumor patients and their levels correlated with the severity of the tumor. These noninvasive determinations may be clinically useful for diagnosing, prognosing and monitoring bladder cancer. Reduction of oxidative stress is recommended for primary prevention of bladder tumor.**

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Introduction

It is well known that reactive oxygen species[1] (ROS)-induced damage to lipids, proteins and DNA plays a major role in carcinogenesis and it is believed that change in the surface of tumor cells is important to their abnormal growth and behavior.

Sialic acid[2] is a common terminal sugar of the glycans of glycoproteins and glycolipids. A neoplasm frequently has an increased concentration of total sialic acid (TSA) on the cell surface, mostly in the form of sialoglycoproteins. These sialic acid-containing molecules are continuingly shaded and secreted, which consequently cause an increase of the concentration in the blood and urine.

8-hydroxydeoxyguanosine (8-OHdG)[3-5] is an oxidized nucleotide of DNA. Upon DNA repair, 8-OHdG is excreted in the urine. Malignant cells contain high concentration of the oxidized DNA, notably 8-OHdG. An increased urinary excretion of 8-OHdG has been reported in patients with bladder cancer. Malondialdehyde (MDA),[6] a lipid peroxidative damage product induced by ROS, has been implicated to mediate tissue damage in patients with bladder tumor.

The objective of this study was to investigate the urinary excretion of TSA, 8-OHdG and MDA in patients with bladder tumor and to evaluate their association with the progressiveness of the tumor.

Materials and Methods

Forty-eight patients with bladder tumor (male 34, female 14, mean age 65.3 ± 13.88 years) and 30 healthy people (male 19, female 11, mean age 50.1 ± 6.54 years) were recruited in the study. The patients were chosen from those admitted to the Division of Urology, King Chulalongkorn Memorial Hospital. Flexible cystoscope was employed to identify bladder tumor in the patients. Informed consent was obtained from all participants prior to specimen collection.

The morning urine samples were collected from all subjects and kept at -20 °C until testing. The concentration of creatinine (Jaffe method), protein (dye-binding method), 8-OHdG (competitive ELISA), MDA (spectrophotometric method) and TSA (Thiobarbituric assay) in the urine samples was measured.

Urine cytology was performed to classify the severity of bladder tumor. Urine sample was centrifuged at 2000 rpm for 10 min and the sediment was re-centrifuged in a Cytospin machine at 2000 rpm for 5 minutes. The cell smear was stained according to Papanicolaou's staining procedure. The smear was done in duplicate and examined separately by 2 pathologists. The criteria used for cytological grading is shown in Table 1.

Based upon urine cytology grading, the bladder cancer patients were classified into 2 groups; 1) patients with negative urinary malignant cells (C1 and C2 grading; low-severity grade), and 2) those with positive urinary malignant cells (C3, C4 and C5 grading; high-severity grade).

Descriptive statistics were used to summarize the characteristics of subjects. Two independent groups were compared by two-sample t-test or Mann-Whitney test where appropriate. Difference between 3 independent groups was assessed by ANOVA test followed by Bonferroni multiple-comparison test. Spearman's rank correlation test was performed to determine the association between 2 variables. Statistical analyses were performed using STATA version 8.0 (Stata Corp, College Station, TX). A two-sided $P < 0.05$ was considered statistically significant.

Results

Mean ages compared between patients with low- and high-severity grades were not significantly different, but their age were significantly higher than the mean age of healthy group (Table 2). Likewise, the urinary creatinine in bladder tumor patients was significantly lower than in healthy subjects. The level of urinary proteins in high-grade tumor patients was significantly higher than that in healthy controls (254.74 ± 227.99 vs. 57.58 ± 48.55 mg/L, $p < 0.001$).

Table 1 The criteria used for urine cytology grading

Grading	Urine cytology criteria
C1	Scant normal or benign urothelial cell or no cell
C2	Multiple benign or reactive urothelial cell (mild nuclear enlargement), inflammatory process
C3	Indeterminate
C4	Suspicious for malignancy
C5	Positive for malignant cell

Patients with high-severity grade excreted urinary MDA significantly higher than those with low-severity grade (p = 0.024) (Figure 1). Healthy subjects excreted urinary MDA significantly lower than patients with high-severity grade tumors (6.48 ± 4.69 vs. 16.07 ± 10.71 mM/g creatinine, p = 0.001), and not higher than patients with low-severity grade tumors. Similarly, urinary 8-OHdG level in patients with high-severity grade tumor was significantly higher than that in healthy controls (13.56 ± 11.07 vs. 4.47± 2.62 mg/mg creatinine, p <0.001) (Figure 2).

The comparison of urinary TSA between the 3 groups of subject is shown in Figure 3. The level of urinary TSA in healthy group was significantly lower than both groups of patients with low-severity (4.47 ± 2.62 vs. 7.01 ± 3.95 μg/g creatinine, p = 0.004) and high-severity grades (vs. 13.56 ± 11.07 μg/g creatinine, p <0.001).

To find the association between urinary TSA excretion and oxidative stress status in bladder tumor patients (n = 48), scatter plot and Spearman's rank correlation test were carried out. Figure 4 shows that the urinary excretion of TSA positively correlated with urinary 8-OHdG with Spearman's rho of 0.47 (p = 0.001). Also, urinary TSA was significantly associated with excretion of MDA in urine (Spearman's rho = 0.38, p = 0.010) (Figure 5). In addition to urinary 8-OHdG and MDA, a significant association between urinary TSA and urinary protein content was also observed (Spearman's rho = 0.33, p = 0.024) (Figure 6).

Discussion

In this study, we investigated urinary excretion of TSA, 8-OHdG, MDA and proteins in patients with

Table 2 Characteristics of the patients with bladder tumor and healthy controls

Variables	Bladder tumor patients		Healthy controls	p-value*
	Low-severity grade (C1+C2)	High-severity grade (C3+C4+C5)		
n	28	20	30	
Age (years)	63.5 ± 12.18	67.2 ± 15.58	50.1 ± 6.54	<0.001
Creatinine (g/L)	0.56 ± 0.40	0.53 ± 0.47	0.89 ± 0.56	0.013
Proteins (mg/L)	134.62 ± 180.17	254.74 ± 227.99	57.58 ± 48.55	<0.001

*p-values were obtained from one way ANOVA

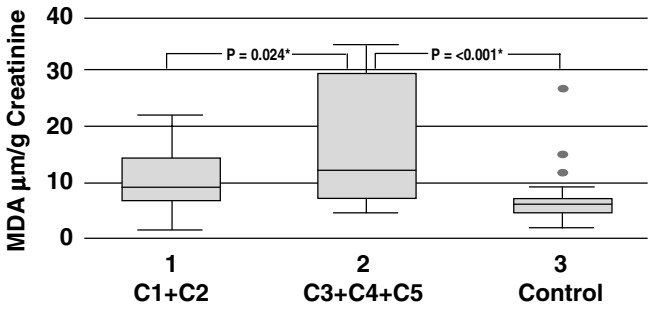

Figure 1 Box-Whisker plot compared levels of urinary MDA between bladder tumor patients with low-severity grade (C1+C2) and high-severity grade (C3+C4+C5) as well as healthy controls. Urinary level of MDA in healthy group was significantly lower than in patients with high-severity grade (p = 0.001). Patients with high-severity grade excreted urinary MDA significantly higher than those with low-severity grade (p = 0.024).

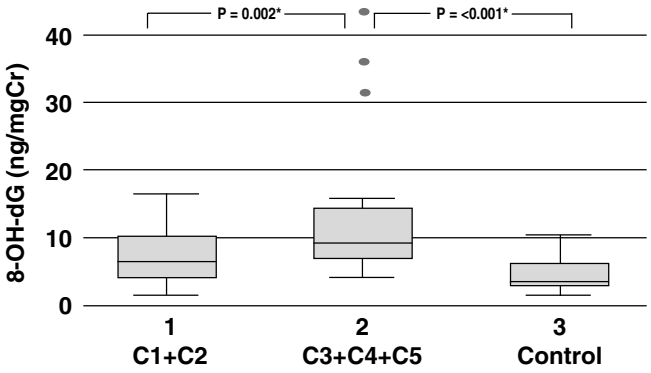

Figure 2 Box-Whisker plot compared levels of urinary 8-OHdG between bladder tumor patients with low-severity grade (C1+C2) and high-severity grade (C3+C4+C5) as well as healthy controls. Urinary level of 8-OHdG in healthy group was significantly lower than in patients with high-severity grade (p <0.001). Patients with high-severity grade excreted urinary 8-OHdG significantly higher than those with low-severity grade (p = 0.002).

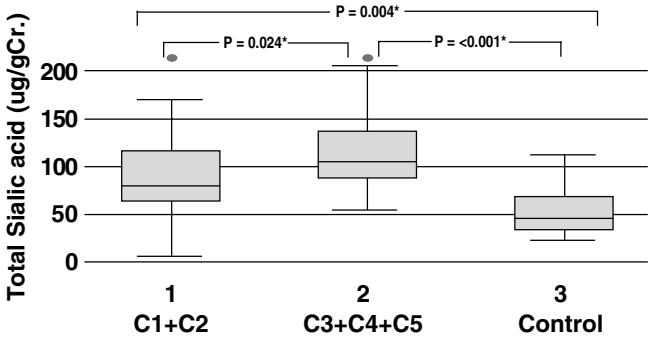

Figure 3 Box-Whisker plot compared levels of TSA between bladder tumor patients with low-severity grade (C1+C2) and high-severity grade (C3+C4+C5) as well as healthy controls. The level of urinary TSA in healthy group was significantly lower than both group of patients with low-severity (p = 0.004) and high-severity grades (p <0.001). Patients with high-severity grade excreted urinary TSA significantly higher than those with low-severity grade (p = 0.018).

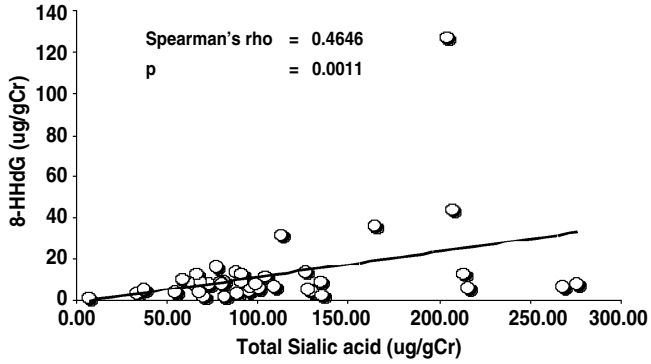

Figure 4 Bivariate analysis using scatter plot and Spearman's rank correlation test to assess the association between urinary excretions of TSA and 8-OHdG. Level of urinary TSA was positively correlated with urinary 8-OHdG (Spearman's rho = 0.47, p = 0.001).

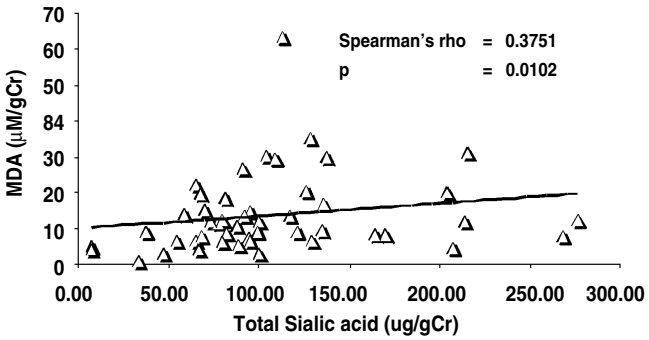

Figure 5 Bivariate analysis using scatter plot and Spearman's rank correlation test to assess the association between urinary excretions of TSA and MDA. Level of urinary TSA was positively correlated with urinary MDA (Spearman's rho = 0.38, p = 0.010).

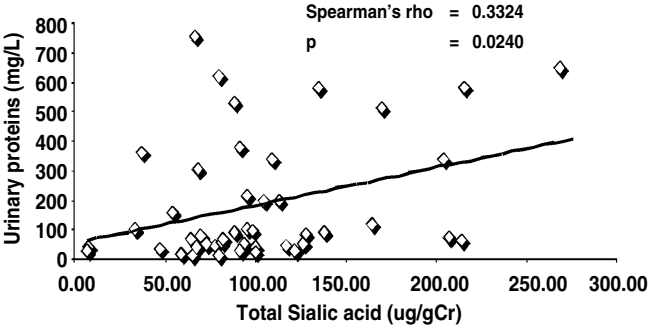

Figure 6 Bivariate analysis using scatter plot and Spearman's rank correlation test to assess the association between urinary excretions of TSA and proteins. Level of urinary TSA was positively correlated with urinary protein (Spearman's rho = 0.33, p = 0.024).

bladder tumor. The bladder cancer patients excreted these cellular substances significantly higher than healthy controls. In addition, these urinary biomarkers were associated with the degree of severity of tumors. We also found that urinary TSA in patients with low-severity grade was significantly higher than in healthy controls.

Urinary 8-OHdG and MDA, indicators of oxidative DNA damage and lipid peroxidation respectively, provided a potential to become oxidative stress biomarkers for bladder tumor, since their levels in bladder tumor patients were significantly greater than in healthy subjects. Furthermore, their urinary levels could be used to distinguish the patients with high-severity grade from those with low-severity grade. Urinary 8-OHdG and MDA compared between patients with low-severity grade and healthy subjects were not significantly different, indicating that these biomarkers had a low differentiating power for these 2 populations.

The present finding showed that urinary excretion of TSA in bladder tumor patients with high-severity grade was significantly higher than in low-severity grade patients and in the healthy controls, respectively. Thus, urinary TSA seemed to be a potential marker to differentiate the bladder tumor patients with high-severity grade from those with low-severity grade as well as to distinguish the low-severity grade patients from healthy population. We suggested that determination of urinary TSA in patients with bladder

tumor (C1-C5) may be clinically useful to follow the patients after treatment. Additionally, urinary TSA might be helpful to predict the presence of malignant cells in the urine.

We found significant association of urinary TSA with urinary oxidative stress biomarkers. In addition, urinary TSA was linearly related to urinary protein contents. These may imply that increased ROS generation in malignant cells, indicated by increased urinary 8-OHdG and MDA, contributed to the over-production of sialoglycoproteins on their cell surface. However, this hypothesis needs further investigation.

In conclusion, the noninvasive determinations of urinary TSA, 8-OHdG and MDA may have a potential for the diagnosis and prognosis of the transitional cell carcinoma of the bladder as well as monitoring the patients after treatment. Reduction of oxidative stress is recommended as a very important approach to prevent the development and progression of bladder cancer.

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