Determining Urinary Trace Elements (Cu, Zn, Pb, As, and Se) in Patients With Bladder Cancer

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Objectives: Trace elements are essential components of biological structures, but they can be toxic at concentrations beyond those necessary for their biological functions.

Methods: A study group of 30 patients with bladder cancer and a control group of 30 healthy volunteers were measured for trace elements using a graphite furnace atomic absorption spectrophotometer.

Results: Urinary zinc and selenium levels in patients were significantly ($P < 0.05$) higher

than those in controls, but urinary copper, arsenic, and lead were not significantly different.

Conclusion: This case–control study suggests that zinc and selenium concentrations are associated with the proliferation of bladder cancer cells because zinc and selenium are excreted in the urine of bladder cancer patients. *J. Clin. Lab. Anal.* 23:192–195, 2009. © 2009 Wiley-Liss, Inc.

Key words: trace elements; superficial bladder cancer; invasive bladder cancer

INTRODUCTION

Copper, zinc, and selenium are trace elements present in the body in very low amounts (micrograms/gram or less). Copper is vital to healthy, functioning organisms. In Wilson's and Menkes diseases, for example, copper transporters crucial to homeostasis are impaired, which causes potentially toxic copper accumulation. Studies have shown greatly elevated levels of copper in cancer tissues, and some diagnostics and treatments from Wilson's and Menkes diseases, such as copper chelation therapy, have been used in the treatment of cancer (1,2).

Zinc is involved in many metabolic processes: protein synthesis, immunological function, and growth and development. Zinc deficiencies induce growth failure, hypogonadism, impaired wound healing, and genetic disease. In various types of cancers with site tumors, such as breast, gallbladder, and prostate cancer, copper and zinc have been measured (3–7), and they (8–12) may protect against urothelial carcinomas.

Selenium is a component of glutathione peroxidase and iodothyronine-5' deiodinase. Selenium deficiencies may occur in long-term total parenteral nutrition patients with inadequate supplements, and where soil selenium levels are low. Serum selenium levels, which are dependent on factors such as nutritional habits, age, sex,

and geographical regions, differ in human beings. It has been proved that serum selenium levels correlate to cancer risk in human beings and many mammals. Serum selenium levels of various cancer patients were reported to be lower than those of healthy persons (13). Selenium can protect against urothelial carcinomas (14–18).

One study on trace element levels in humans with Blackfoot disease (BFD), a peripheral vascular disease (19), reports significantly higher amounts of urinary arsenic, copper, cadmium, manganese, and lead, and significantly lower urinary zinc and selenium than for healthy controls. Another (20) reports that the levels of arsenic, mercury, and lead in BFD patients were significantly higher than those in healthy controls. Many studies (21) in several countries have reported that arsenic causes a severe form of BFD but can protect against urothelial carcinomas (22). A deficiency in any of these trace elements leads to undesirable pathological conditions that can be prevented or reversed by adequate

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supplementation (23–26). About 90% of bladder cancer cases are transitional-cell or urothelial-cell carcinomas, and the rest are adenocarcinomas, small cell carcinomas, and squamous-cell carcinomas. The bladder cancer death rate in Taiwan is 3.3%, the number 15 cause of cancer deaths here. There are few studies on the current trend of urinary trace element levels in Taiwan bladder cancer patients. We have, therefore, determined the amount of copper, zinc, selenium, arsenic, and lead in 30 bladder cancer patients and 30 healthy adults.

MATERIALS AND METHODS

Study Participants

The urine samples of participants were obtained from the Department of Urology, Chi-Mei Medical Center, Tainan, Taiwan. The study group (patients) consisted of 30 patients with bladder cancer (28 men and 2 women; mean age: 63.8 ± 11.8 years; age range: 31–74 years). The control group (controls) consisted of 30 healthy volunteers (14 men and 16 women; mean age: 54.4 ± 13.4 years; age range: 42–83 years).

Sample Collection and Storage

Because trace-element concentration is affected by diet, urine samples were collected from patients after they had fasted for 8 hr before surgery. Urine samples were collected from controls in the morning after they had fasted overnight, usually the most desirable time for a urinalysis because it provides the most concentrated urine. All the urine samples were placed in high-density polyethylene containers to prevent the trace elements from becoming contaminated and degraded. The containers were stored at -20°C until they were analyzed.

Determining Trace Elements

A graphite furnace atomic absorption spectrophotometer (GFAAS) (Z 5000 (Hitachi, Tokyo, Japan) with Zeeman background correction was used to determine the concentrations of urinary elements. The samples were first digested using the 65% nitric acid method. The operating parameters for the GFAAS were as follows. The wavelengths (nm) for zinc, copper, selenium, lead, and arsenic were 213.9, 324.8, 196, 283.3, and 193.7, respectively. Working solutions were prepared from 1,000 mg/L of stock standard solutions. Calibration curves were obtained by using the solutions of the studied elements at different concentrations.

Statistical Analysis

Results were analyzed using Student's *t*-tests. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The mean values for zinc and selenium were significantly higher in patients than in controls (Zn: 836 ± 153 $\mu\text{g/L}$ vs. 476 ± 82 $\mu\text{g/L}$, $P = 0.042$; Se: 4.49 ± 0.84 $\mu\text{g/L}$ vs. 2.46 ± 0.53 $\mu\text{g/L}$; $P = 0.047$) (Fig. 1A and B). No significant differences were found in mean copper (181 ± 76 $\mu\text{g/L}$ vs. 152 ± 61 $\mu\text{g/L}$, $P = 0.77$), lead (0.94 ± 0.12 $\mu\text{g/L}$ vs. 0.91 ± 0.13 $\mu\text{g/L}$, $P = 0.90$), or arsenic (1.68 ± 0.22 $\mu\text{g/L}$ vs. 1.62 ± 0.17 $\mu\text{g/L}$, $P = 0.83$) values between patients and controls. Lead levels were elevated in the urine of 40% of the patients. This agreed with other studies (27–29) in which copper, zinc, and lead levels were elevated in the urine of patients with bladder tumors. In our study and in a previous study (30), human zinc- α 2-glycoprotein was higher in the urine samples of patients with bladder cancer than in the urine samples of healthy controls.

The levels of copper were lower in the urine of the controls (152 ± 61 $\mu\text{g/L}$, $P = 0.64$) than in the urine of patients with superficial (170 ± 203 $\mu\text{g/L}$, $P = 0.46$) and invasive (211.00 ± 367 $\mu\text{g/L}$, $P = 0.82$) bladder cancer (Fig. 1A), which agreed with the earlier findings (31,32)

Fig. 1. Urinary copper (Cu), zinc (Zn), lead (Pb), arsenic (As), and selenium (Se) concentrations in the superficial and invasive bladder cancer group compared with healthy controls. Columns show mean concentrations; bars, standard deviation (* $P < 0.05$).

TABLE 1. Effect on Stage Concentration of Urinary Trace Elements in Bladder Cancer Patients

Bladder-cancer stage	Number of patients	Concentration ($\mu\text{g/L}$) of trace elements				
		Copper (Cu)	Zinc (Zn)	Lead (Pb)	Arsenic (As)	Selenium (Se)
<i>Superficial</i>						
Stage 0	5	32.9 ± 4.2	314 ± 140	1.13 ± 0.50	1.63 ± 0.42	5.48 ± 3.6
Stage A	8	108.0 ± 72	484 ± 66	0.83 ± 0.37	2.27 ± 0.63	6.02 ± 0.63
Stage B	9	202.0 ± 59	991 ± 406	1.08 ± 0.42	2.05 ± 0.83	5.32 ± 2.1
<i>Invasive</i>						
Stage C	6	117.0 ± 4.1	750 ± 296	0.96 ± 0.14	1.94 ± 0.31	1.64 ± 0.92
Stage D	2	131.0 ± 61	1069 ± 353	1.22 ± 0.81	1.14 ± 0.77	2.27 ± 1.48
Controls	30	152.0 ± 61	476 ± 82	0.91 ± 0.13	1.62 ± 0.17	2.46 ± 0.53

Each trace element value is the mean $\pm$ standard deviation.

reporting greatly elevated levels of copper in the serum and tissue of patients with bladder cancer. Mean urinary zinc concentrations were significantly higher in patients with superficial ($753 \pm 685 \mu\text{g/L}$, $P = 0.01$) and invasive ($833 \pm 443 \mu\text{g/L}$, $P = 0.76$) bladder cancer than in controls ($476 \pm 82 \mu\text{g/L}$, $P = 0.01$). In fact, the mean zinc concentration of $793 \mu\text{g/L}$ was closely consistent with that reported in another study (33): $762 \mu\text{g/L}$ in the serum of 38 patients with bladder cancer. The mean values for urinary selenium were $5.42 \pm 5.01 \mu\text{g/L}$ in patients with superficial bladder cancer and $2.46 \pm 0.53 \mu\text{g/L}$ in controls ($P = 0.01$). This does not agree with previous studies (15,34). Most studies report low serum selenium levels in bladder cancer: $65.7 \pm 6.5 \text{ mg/L}$ ($n = 10$) vs. $94.1 \pm 12.5 \text{ mg/L}$ (13), and $45.09 \pm 16 \text{ mg/L}$ ($n = 27$) vs. $70.3 \pm 13 \text{ mg/L}$ (34) for case and controls, respectively. The metabolism of selenium is thought to be involved in many biochemical pathways (15). Selenium can be methylated and excreted from the body as trimethylselenonium ions in the urine. The selenium levels in urine were lower than in serum because urine selenium varies with intake, and volatile forms of selenium are exhaled. Serum has many selenium-containing proteins; selenium is the form of selenocysteine such as glutathione peroxidase, and selenoprotein P, which have been assessed for the measurement of selenium status. The TNM staging system stands for Tumor, Node, Metastasis. We use a staging scale of 0, A–D but some reports use a scale of 0–4 to describe the cancer. Superficial bladder cancer is early transitional-cell bladder cancer. This includes Stages 0, A, and B. Transitional-cell bladder cancer can become invasive (Stages C and D). Zinc concentrations significantly increased with the stage, whereas selenium concentrations decreased with the stage (Table 1).

Gender differences affect the risk of developing bladder cancer. Male patients had higher, but statistically nonsignificant, concentrations of copper, lead, selenium, and arsenic, and a lower concentration of zinc

than female patients did. To determine the association between arsenic and BFD, we measured the amount of arsenic in urine from patients with BFD. Urinary arsenic levels in patients who lived in a BFD area ($1.19 \pm 0.20 \mu\text{g/L}$) were statistically nonsignificantly lower than for those who did not live in a BFD area ($1.01 \pm 0.58 \mu\text{g/L}$). Finally, patients taking Chinese herbs ($0.91 \pm 0.14 \mu\text{g/L}$) had higher concentrations of urinary lead than those not taking Chinese herbs ($0.57 \pm 0.19 \mu\text{g/L}$). One possible explanation for this is that several patent medicines imported from China, and probably used by the patients in our study, contained high levels of lead.

CONCLUSION

The possible contributions of copper, zinc, selenium, lead, and arsenic concentrations to the development and progression of bladder cancer have been examined in this study. After comparing the urinary concentrations of these trace elements in patients with bladder cancer with those of healthy controls, the data showed statistically significantly higher levels of urinary zinc in patients than in controls. The urinary zinc concentrations gradually and significantly increased from bladder cancer stage 0 to stage D in the patient group. Differences in the concentrations of the other trace elements were not statistically significant.

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