RESEARCH COMMUNICATION

Variation of Urinary and Serum Trace Elements (Ca, Zn, Cu, Se) in Bladder Carcinoma in China

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Abstract

Backgrounds: Deficiency or excess of trace elements can induce body metabolic disorders and cellular growth disturbance, even mutation and cancerization. Since there are few studies of the effect of trace elements in bladder carcinoma in China, the aim of this study was thus to assess variation using a case control approach. Methods: To determine this, 81 patients with bladder carcinoma chosen as a study group and 130 healthy persons chosen as a control group were all assayed for urinary and serum trace elements (calcium [Ca], zinc [Zn], copper [Cu], selenium [Se]) using an atomic absorption spectrophotometer, and the results were analyzed by independent sample t tests. The correlative factors on questionnaires answered by all persons were analyzed by logistic regression. Results: The results showed urinary Ca, Zn and serum Cu levels of the study group to be significantly higher (P<0.05) than those of he control group. Serum Ca and Se levels of study group were significantly lower (P<0.05) than those of control group. Conclusion: There were higher urinary Zn and serum Cu concentrations in bladder carcinoma cases. Bladder carcinoma may be associated with Ca metabolic disorder, leading to higher urinary Ca and lower serum Ca. Low serum Se and smoking appear to be other risk factors for bladder carcinoma in China.

Keywords: Trace elements - atomic absorption - bladder carcinoma - risk factor - China

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Introduction

Many studies demonstrated that trace elements existed in various forms, and kept a dynamic balance status in human body (Navarro and Rohan, 2007). Deficiency or excess of trace elements can induce body metabolic disorder and cellular growth disturbance, even mutation and cancerization (Navarro and Rohan, 2007; Arslan et al., 2011; Demir et al., 2011).

Zinc deficiency has adverse consequences during embryogenesis and early childhood development, particularly on immune functioning (Prasad, 1995; Chasapis et al., 2011; Christudoss et al., 2012). Many reports have shown that the level of zinc in the serum and malignant tissues decreased in patients with some malignancies, such as carcinoma of the liver, or prostate (Vallee, 1995; Joazeiro and Weissman, 2000; Kadrmas and Beckerle, 2004). Studies have shown greatly elevated levels of Cu, Cu/Zn in cancer, such as breast, prostate and liver cancer (Daniel et al., 2004). In Wilson's and Menkes diseases, for example, copper transporters crucial to homeostasis are impaired, which causes potentially toxic copper accumulation (Uriu-Adams and Keen, 2005). 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 (Uriu-Adams and Keen, 2005). Higher levels of Se have been associated with a lower risk of many types of neoplasia, including prostate, lung, colorectal, and possibly bladder, although the data are inconsistent (Rayman, 2005; Brinkman et al., 2006). Se was thought to inhibit carcinogenesis through several different mechanisms, including reduction of oxidative stress and inflammation, enhancement of immune response, induction of apoptosis, cell cycle arrest, and transactivation of DNA repair genes (Smith et al., 2004; Rayman, 2005). Ca has long held an interest in cancer with essential messenger roles regulating cell cycle proliferation and apoptosis (Berridge et al.,1998; Capiod et al., 2007; Monteith et al., 2007; George et al., 2011). Ca consumption might also reduce the production of 1, 25-dihydroxyvitamin D, the hormonal form of vitamin D (Egan et al., 2008). Several prospective studies have suggested that greater intake of calcium or a higher level of serum calcium, is associated with aggressive, poorly differentiated, lesions or fatal prostate cancer (Skinner and Schwartz, 2008; Skinner and Schwartz, 2009).

Bladder carcinoma is a common cancer in the world, involved high recurrence rate. Bladder cancer is the forth

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and the tenth most common cancer in men and women, respectively (Salehi et al., 2011). Two-thirds of patients diagnosed with superficial bladder cancer experience a recurrence of their tumor (Jemal et al., 2007). About 92% of bladder carcinoma is transitional-cell or urothelial-cell carcinomas, while the others are adenocarcinomas, squamous-cell carcinomas, and small cell carcinomas. There are few studies and investigations of the effect of trace elements in bladder carcinoma in China. Therefore, it is worth to determine the urinary and serum concentrations of trace element Ca, Zn, Cu, and Se, and discussing their roles.

Materials and Methods

Study Selection

81 patients (58 male, 23 female, average age 61±12) were chosen from the patients with bladder carcinomas hospitalized in Department of Urology, 1st Affiliated Hospital, China Medical University (CMU). 130 healthy persons (87 male, 43 female, average age 46±14) were chosen as a control group from the persons who had physical examinations in Department of Physical Examination, 1st Affiliated Hospital, CMU. After written informed consent was obtained, each of the patients and controls completed a questionnaire (Addendum 1), and contributed 1 ml blood and 1 ml urine.

Sample Collection and Treatment

Blood of patients were collected when they did blood tests in the morning after they had fasted overnight in hospital. Urine of patients were collected in the morning of the operation date after they had fasted overnight without the influence of diet and drug, and it was the most concentrated urine. Blood and urine of the healthy persons were collected in the morning when they did blood/urine tests after they had fasted overnight.

Blood samples were collected in appropriate sterile vials by arm venous puncture. Plasma and serum were separated by centrifugation at 4000 g for 5 minutes. Only serum was collected as sample after centrifugation.

All the serum and urine samples were placed in Eppendorf Pipes in order to prevent contaminate and degradation, and stored at -80 °C.

Sample Assay

The concentration of serum and urinary elements were analyzed by a atomic absorption spectrophotometer (180-80, Hitachi, Japan). Zn, Ca, and Cu were analyzed by Flame Synthesis. Se was analyzed by Graphite Furnace.

Statistical Analysis

The trace elements datas were analyzed by independent sample t tests of SPSS17.0 software, and the correlative factors were analyzed by logistic regression of SPSS17.0 software. And the significance was set at P<0.05.

Results

The Concentration Levels of Trace Elements in Urine

The mean concentrations of urinary Se, Zn, and Cu in 130 healthy persons and 81 bladder cancer patients are respectively Se: 16.74±6.22 vs 17.75±6.82, Zn: 0.46±0.43 vs 0.65±0.23, Cu: 0.20±0.10 vs 0.25±0.13, Ca: 62.51±31.50 vs 89.96±36.74. The concentration value of each trace element was shown in Table 1.

Table 1. The Concentration (μg/mL) Values of Urinary Trace Element

Group	Number	Ca	Se (μg/L)	Zn	Cu
control	130	62.5±31.5	16.7±6.2	0.4±0.4	0.2±0.1
cancer	81	89.9±36.7*	17.7±6.8	0.6±0.2*	0.2±0.1
P value		P=0.004*	P=0.541	P=0.04*	P=0.105

Asterisk (*) means statistical significance (P<0.05), each value is the mean±standard deviation

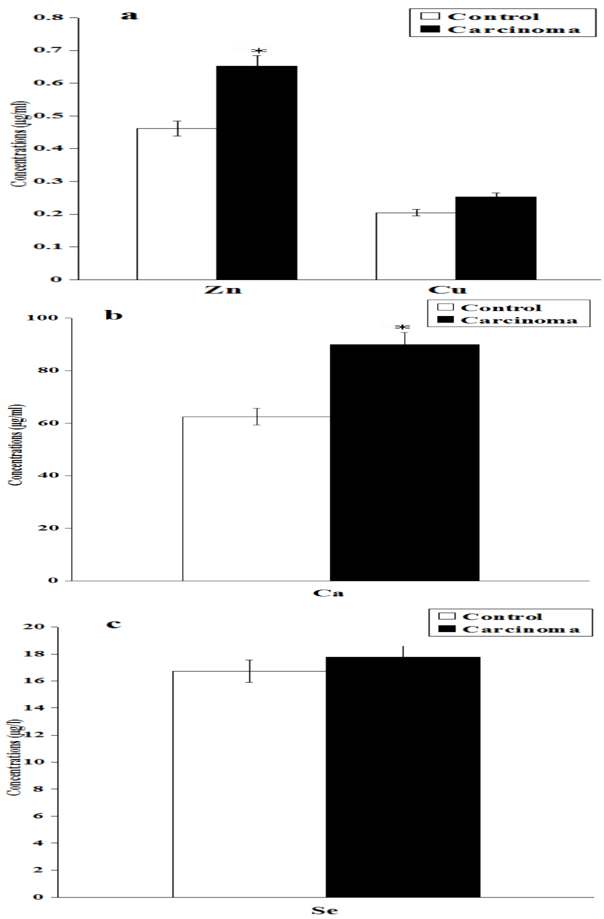

Figure 1. Trace Elements Zn, Cu, Ca, and Se Concentrations in Urine. Columns show mean concentrations and bars show standard deviation. Asterisk (*) means statistical significance (P<0.05). (a) The mean concentrations of Zn and Cu in urine were shown by histograms. (b) The mean concentration of Ca in urine was shown by histograms. (c) The mean concentrations of Se in urine was shown by histograms. Each mean concentration of these trace elements in urine listed in Table 1

The values of Ca (P=0.004) and Zn (P=0.04) in the urine of patients were significantly higher than those in the urine of control groups (P<0.05). And there was no difference for the values of urinary Cu (P=0.105) and Se (P=0.541) between study and control groups (P>0.05). (Figure 1)

The Concentration Levels of Trace Elements in Serum

The mean concentrations of serum Se, Zn, and Cu

Table 2. The Concentration (μg/mL) Values of Serum Trace Element

Group	Number	Ca	Se (μg/L)	Zn	Cu
control	130	89.7±6.2	0.06±0.01	1.1±0.3	0.8±0.1
cancer	81	83.7±7.1*	0.05±0.01*	0.9±0.1	0.9±0.1*
P value		P=0.002*	P=0.000*	P=0.11	P=0.002*

Asterisk (*) means statistical significance (P<0.05), each value is the mean±standard deviation

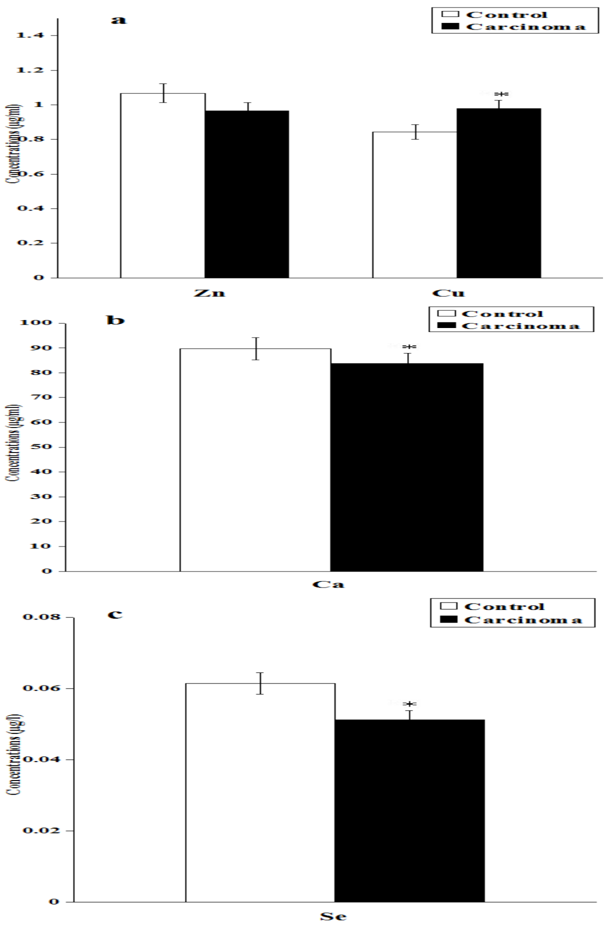

Figure 2. Trace Elements Zn, Cu, Ca, and Se Concentrations in Serum. Columns show mean concentrations and bars show standard deviation. Asterisk (*) means statistical significance (P<0.05). (a) The mean concentrations of Zn and Cu in serum were shown by histograms. (b) The mean concentration of Ca in serum was shown by histograms. (c) The mean concentrations of Se in serum was shown by histograms. Each mean concentration of these trace elements in serum listed in Table 2

in 130 healthy persons and 81 patients are respectively Se: 0.061±0.009 vs 0.051±0.006, Zn: 1.07±0.30 vs 0.97±0.15, Cu: 0.84±0.11 vs 0.98±0.19, Ca: 89.72±6.27 vs 83.73±7.13. The concentration value of each trace element was shown in Table 2.

The values of Ca (P=0.002) and Se (P=0.000) in the serum of patients were significantly lower than those in control groups (P<0.05), but Cu (P=0.002), is higher (P<0.05). Only Zn (P=0.11) was not different (P>0.05). (Figure 2)

Analysis of Correlative Factors about Bladder Carcinoma

According to the questionnaires, nine factors (occupation, contactant, food, drug, surroundings, smoking, drink, urination and disease) were analyzed through logistic regression. Regression coefficients of occupation (0.068), contactant (0.524), drug (2.036), smoke (1.412), drink (0.710), and disease (1.065) were positive. Regression coefficients of food (-0.308), surroundings (-21.576) and urination (-0.037), were negative. Smoking (P=0.000) and drug (P=0.004) were the risk correlative factors of bladder carcinoma by statistical analysis (P<0.05). Others were not the risk relational factors (P>0.05). The result of logistic regression was shown in Table 3.

Table 3. The Correlative Factors of Bladder Carcinoma

Factors	Regression Coefficient	Standard Error	Wald	P Value
Occupation	0.06	1.01	0.004	0.947
Contactant	0.5	0.4	1.3	0.25
Food	-0.3	0.4	0.5	0.455
Drug	2	0.7	8.2	0.004*
Surroundings	-21.5	16621.6	0	0.999
Smoking	1.4	0.3	14.3	0.000*
Drink	0.7	0.4	2.5	0.108
Urination	-0.03	0.4	0.01	0.926
Disease	1.06	0.5	3.2	0.074

Asterisk (*) means statistical significance (P<0.05)

Discussion

Bladder cancer is the forth and the tenth most common cancer in men and women, respectively. Two-thirds of patients diagnosed with superficial bladder cancer experience a recurrence of their tumor (Jemal et al., 2007). The tumorigenesis mechanism of bladder carcinoma is controversial and has not been clearly revealed yet (Han et al., 2011). Oxidative processes are one of the mechanisms involved in both incidence and recurrence of bladder carcinoma (Knight, 1998; Akcay et al., 2003). Oxidative stress causes severe damage to biologic macromolecules and dysregulation of normal metabolism and physiology (Hoekstra et al., 1974; Toyokuni, 1999). The role of oxidative stress in the etiology of cancers is supported by epidemiologic studies in a way that decreasing the oxidative reaction has been offered as one of the primary measures in preventing the formation of bladder carcinoma (Willett and MacMahon, 1984; Nelson, 1992; Salganik et al., 1994).

Oxidative processes occur most intensively in the background of an imbalance of trace elements incorporated into the structure of enzymes responsible for antioxidant protection. Both the increase and decrease of trace element ion content can affect the activity of the antioxidant enzymes, including Ca, Cu, and Zn and Se (Yelinova et al., 1996). Statistically significant differences from the normal distribution of Se, Cu and Zn have been reported to occur in patients with various forms of cancers (Trush and Kensler, 1991; Spartz and Bloom, 1992). Variation in the urine and serum level of Ca, Se, Cu, and Zn in bladder carcinoma patients of China has few been determined.

In this study, the concentration values of urinary Ca and Zn in patients was obviously higher than that in control

(P<0.05). The reason may be the renal metabolism or carcinoma autocrine. During collection of urine and blood samples, the effect of diet and drugs was eliminated. If the reason is renal metabolism, the body metabolism suffering from carcinoma should be abnormal and urinary Ca and Zn excreted by kidney would be increased. So serum Ca and Zn should be reduced. According to the results of serum trace elements concentrations, the values of Ca in patients were obviously lower than that in control (P<0.05), but Zn was not different. It indicated that the variation of Ca may be induced by excessive excretion of kidney and Zn was not related with metabolism of patients. The increasing of urinary Zn may be induced by carcinoma autocrine. Due to Zn-relation transportprotein change of tumour cell comparing with normal cell, intracellular Zn level become unbalanced and Zn is excreted out of cell. Lower serum Ca and higher urinary Ca may suggest that suffering from carcinoma may induce Ca ion metabolism abnormal. However there is always calcification feature when bladder carcinoma necrosis and calcium deposited is observed on cystoscope. So higher Ca concentration in urine was also related with calcification of carcinoma. In addition to metabolism and calcification, carcinoma autocrine is not absolutely denied. Many experiments showed that in bladder carcinoma Ca-relation transportproteins are varied, then the balance of Ca level between intracell and extracell is broken.

Low level of serum Se is the risk factor of cancer, which is indicated in many literatures (Wallace et al., 2009). In this experiment urinary Se did not change, but the concentration level of serum Se in patients was lower than that in control (P<0.05). It also shows that low serum Se may correlate with carcinomaigenesis. Se in blood is combinded with some proteins and plays an important role in eliminating free radical and antioxidation. Low serum Se may induce normal cells to canceration. The level of urinary Se was obviously higher than the level of serum Se. The reason was that the level of urinary Se was closely related with diet. Its significance was less important than serum Se (Lin et al., 2009). In this experiment the level of serum Cu was increased in bladder carcinoma which agreed with the previous studies (Huang et al., 1999; Hronek et al., 2000; McMillan et al., 2000; Mross, 2000; Valko et al., 2006). In many cancers, for example hepatoma and breast cancer, serum Cu/Zn also rises, which was same as that in this study (Marco et al., 2001; Zhai et al., 2003).

In this study, male patients were nearly two times more than female. It also accords with the literature that shows bladder carcinoma is more common for man. The age of patients was divided into three stages: youth (< 45), mid-life (45-59) and old (>60). Youth was 8, mid-life was 32, and old was 41. It shows that bladder carcinoma focus on the age of mid-life and old. The older is it, the riskier is it. According to the questionnaires answered by patients and healthy persons, smoking and drug were the significant risk factors of bladder carcinoma (P<0.05). Others were no significance. But occupation and contactant should be the risk factors. Some occupation, for example electroplating industry or chemical industry, and working with carcinogen, such as benzene, lead and nickel, can greatly increase the risk of bladder carcinoma. In this study there was a patient who was a painter contacting pigment all day, suffering from bladder carcinoma for six times. However, among all the patients, there were only a few people with the specificity of these occupations chosen in this study. So there was no statics significance for them. Smoking is considered as a risk factor of numerous cancers, such as lung, liver, stomach, kidney and bladder, because it can decompose into aldehydes, phenols, amine, cyanide, benzene, nicotine and CO, which are all strong carcinogens. As is shown in many experiments smoking was a risk factor of bladder carcinoma (Zeegers et al., 2004). Drug, in this study, was considered as a risk factor too. It should be not directly correlative with bladder carcinoma, and it may be false positive. Most of the controls were absolutely healthy persons that never have long-term drug histories. On the contrary, some of patients suffered from hypertension or diabetes, and had to take drugs every day. So, in spite that there was statistics significance for drug, it still may cause false positive. The regression coefficient of urination was -0.037 which indicated that it was a protective factor without statistics significance. Less urinating has to make metabolite residual long-time in bladder, which may contain several special substance inducing canceration. Holding back urine may be the potential risk factor of bladder carcinoma.

Variation of urinary and serum trace elements, Ca, Zn, Cu, and Se, in bladder carcinoma patients of China can be an evidence of oxidative stress mechanism for bladder carcinoma tumorigenesis. The relative transport-proteins of those trace elements may be the potential pathognomonic biomarkers of bladder carcinoma. Further, the mechanism of trace elements carcinogenesis and variation of the relative transport-proteins in bladder carcinoma are worth to attention and study.

In conclusion, There were higher urinary Zn concentration and serum Cu concentration in bladder carcinoma. Bladder carcinoma patients may have Ca metabolic disorder, leading to higher urinary Ca and lower serum Ca. Low serum Se and smoking should be the risk factors of bladder carcinoma.

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