RESEARCH COMMUNICATION

Serum Trace Element Levels in Patients with Bladder Cancer

İlhan Gecit[1]*, Servet Kavak[2], Halit Demir[3], Mustafa Güneş[1], Necip Pirinççi[1], Çiğdem Çetin[3], Kadir Ceylan[1], Erdal Benli[4], Ibrahim Yildiz[4]

Abstract

Trace elements are essential components of biological structures, but they can be toxic at concentrations beyond those necessary for their biological functions. In the present study, groups of 35 patients with bladder cancer and 34 healthy volunteer controls were measured for trace elements using a furnace atomic absorption spectrophotometer. Serum levels of Cd, Ni and Co were increased ($p<0.05$) and Mn and Zn were decreased ($p<0.05$) in patients with bladder cancer. In the present study, a relationship was seen between the level of trace elements and the occurrence of bladder cancer, suggesting that an increase in the serum level of Cd, Ni, Co and a decrease in the levels of Zn and Mn might be important causes of bladder cancer occurrence; however, defining such a cause-and-effect relationship needs several prospective studies to be done, which seems necessary with regard to the high prevalence of this cancer.

Keywords: Bladder cancer - trace element levels - patients - Turkey

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Introduction

Bladder cancer is a common tumor of the urinary tract (Macvicar et al., 2000). It is the fourth most common type of cancer in men in United States. The most common risk factors for bladder cancer are exposure to industrial carcinogens, cigarette smoking, male gender and possibly diet (Wynder et al., 1977; Zeegers et al., 2004). Another major etiological factor is infestation by the parasite Schistosoma haematobium (Wynder et al., 1977).

Bladder cancer is the most common urological cancer and is the sixth most common cancer in the United States. An estimated 46,400 new cases will develop and 10,400 deaths will occur in Americans in 1988 (Silverberg et al., 1988). A number of environmental risk factors for bladder cancer have been identified. These include cigarette smoking, radiation, schistosomal infections, and certain aromatic amines and amides (Morrison et al., 1982). The roles of saccharin (Hoover et al., 1980) and coffee (Cole et al., 1987) in human bladder cancer are uncertain at best. Foods are known to contain possible cancer preventive factors (Ames et al., 1987). Most studies of the preventive factors for bladder cancer have focused on retinol (vitamin A) and related compounds such as beta-Carotene (alfa-provitamin A compound) and synthetic retinoids (Moon et al., 1986). Alfa-tocopherol (vitamin E) and selenium are nutrients that act as antioxidants and have been found to have protective associations with other cancers (Kathy et al., 1989).

Trace elements are essential components of biological structures, but at the same time they can be toxic at concentrations beyond those necessary for their biological functions. In addition, the toxicity can be extended to other non-essential elements of very similar atomic characteristics that can mimic the reactivity of a trace element. To deal with this essentiality/toxicity duality, biological systems have developed the ability to recognize a metal, and deliver it to the target without allowing the metal to participate in toxic reactions (Gecit et al., 2011; Sayır et al., 2011). Proteins are primarily responsible for such recognition and transport, and most of the associations of trace element with other biomolecules lead to undesirable chemical modifications of these molecules. Oxidative processes occur most intensively in the background of an imbalance of trace elements incorporated into the structure of enzymes responsible for antioxidant protection (Hoekstra et al., 1974). Both an increase and a decrease of trace element ion content can affect the activity of the antioxidant enzymes (Yelinova et al., 1996). Some of these trace elements include: iron (Fe), copper (Cu), and zinc (Zn). Statistically significant differences from the normal distribution of Fe, Cu, and Zn have been reported to occur in patients with various forms of cancers (Spartz et al., 1992). In some studies, the Cu/Zn ratio in serum samples has been utilized as chemoprophylaxis (Li et al., 2004) and also as a way of evaluation and prognosis assessment in cancer patients (Kumar et al., 2003; Mazdak et al., 2010).

To our knowledge, changes in the serum level of Fe, Cu, and Pb in bladder cancer patients has never been determined. Therefore, in the present study, the concentrations of Mn, Cd, Ni, Co and Zn were changed in

*[1]Department of Urology, [2]Department of Biophysics, [3]Department of Chemistry, Division of Biochemistry, Faculty of Science, Yuzuncu Yil University, Van, [4]Department of Urology, Bingol State Hospital, Bingol, Turkey *For correspondence: ilhan_gecit@hotmail.com*

Table 1. Some Mineral Concentrations in the Bladder Cancer and Control Patient Groups (n = 34/35)

Elements			Control					Patients			
	Median	Mean	S. Dev.	Min.	Max.	Median	Mean	S. Dev.	Min.	Max.	p
Mn	0.0539	0.0750	0.0492	0.0380	0.1771	0.0481	0.0475*	0.0158	0.0226	0.0858	0.027
Cd	0.0330	0.0229	0.0153	0.0032	0.0374	0.0349	0.0322*	0.0089	0.0040	0.0407	0.049
Cu	0.6100	0.6525	0.1555	0.4356	0.8558	0.6498	0.7089	0.1875	0.3449	1.0852	0.433
Pb	0.0319	0.0281	0.0248	0.0022	0.0721	0.0473	0.1322	0.2617	0.0000	0.8933	0.249
Fe	1.2254	1.1939	.5367	.5071	2.3843	1.1344	1.0911	0 .4041	.4092	1.7567	0.572
Ni	0.2333	0.2746	0.1213	0.1222	0.5319	0.5387	0.5552*	0.0870	0.4109	0.7112	0.001
Co	0.0088	0.0223	0.0207	0.0023	0.0479	0.0360	0.0455*	0.0257	0.0182	0.0978	0.025
Zn	0.7117	0.9566	0.3886	0.6111	1.6378	0.6691	0.6157*	0.3263	0.1436	1.6533	0.021

The results were expressed as mean ± standard deviation (SD). *denotes a significant difference (P < 0.05) between bladder cancer and control patient groups

the serum of patients with bladder cancer in comparison to the healthy subjects.

Materials and Methods

Subjects

Thirty-five (mean age of 64.24 ± 2.4) men patients with bladder cancer were enrolled in the present cross-sectional study. All the patients were lifetime none-smokers and free of drug, alcohol, antioxidant supplement consumption and any metabolic disease. None of the patients had any other significant disease or malignancies except bladder cancer and only the newly diagnosed patients with no prior chemotherapeutic treatment were included in this study. Controls consisted of 34 healthy (men of age 62.8 ± 4.6) randomly selected from a group of healthy non-smoker volunteers with no history of previous disease, drug or alcohol consumption. We took meticulous caution against the possibility of any previous consumption of antioxidant agents by these volunteers, for this could have interfered with the study.

The patient and control groups were of similar socioeconomic status. As for tumor staging, 28 patients were diagnosed with non-invasive tumor (Ta–T1) where as 7 patients with invasive tumor (T2–T4). Patients were classified into three groups with respect to tumor grading where 21 patients had well differentiated (G1), 9 had intermediate (G2), and 5 had poorly differentiated (G3) tumors. The superficial urothelial papillary tumors were graded according to the 2004 World Heath Organization (WHO) grading system into low grade and high grade papillary neoplasms

The study protocol was carried out in accordance with the Helsinki Declaration as revised in 1989. All participants were informed about the study protocol and the written consent was obtained from each one.

Blood collection

Following 12 h of fasting period, blood samples were obtained in the morning, collected into empty tubes and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min. Serum samples for measurement of trace element levels were stored at −20°C until they were used.

Measurements of Mineral-Heavy Metal Levels

Exactly 2.0 mL of the mixture of HNO3/H2O2 (2:1) was added to 0.7 g of the serum samples. The mixture was placed into the water bath at 70˚C for 30 min and stirred occasionally. Then, 1.0 mL of the same acid mixture was added, and the mixture was transferred into a Teflon vessel bomb for the microwave oven. The bomb was closed, and the solution was placed inside the microwave oven. Radiation was applied for 3 min at 450 W. After addition of 0.5 mL of the same acid mixture, radiation was repeated for 3 min. After cooling for 5 min, 2.0 mL of 0.1 mol/L HNO_3 was added, and the solution was transferred into a Pyrex tube. After centrifugation, the clear solution was used for the determination of Mn, Cd, Cu, Pb, Fe Ni, Co and Zn (11, 12). They were measured by Atomic Absorption Spectrophotometer measurements using a UNICAM-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK).

Statistical Analyisis

Descriptive statistics for the studied traits were expressed as median, mean, standard deviation, minimum and maximum values. Mann-Whitney U test was used for comparison of groups. In the study, 5 % level was considered to statistically significant differences between groups and SPSS Statistical package program (Ver.13) was used for the all statistical computations.

Results

The demographic and clinical data of bladder cancer and control groups are shown in Table 1. There were no statistically significant differences between bladder cancer patients and controls in respect to age and BMI (all p > 0.05) (Table 1).

In addition, the trace element levels in two groups were analyzed. Cu, Pb and Fe were measured 0.70± 0.15; 0.03± 0.20 and 1.19± 0.54 in group control to 0.71± 0.19; 0.13± 0.16 and 1.09± 0.40 in group bladder cancer, respectively no significant. The Cd, Ni and Co element levels were significantly higher in bladder cancer group (P < 0.005). Nevertheless, Cd, Ni and Co elements, with the values of 0.023± 0.015, 0.2746± 0.1213 and 0.0223± 0.0207 in group control, and 0.0322± 0.0089, 0.5552± 0.087 and 0.0455± 0.026 in group bladder cancer, respectively, had significantly lower values in group bladder cancer (P < 0.005). Furthermore, Mn and Zn levels were found 0.0750± 0.049 and 0.956± 0,39 in group control to 0.0475± 0.016, and 0.6157± 0.3263 0.24 in group bladder cancer, respectively. The amounts of these elements were decrease between two groups (P< 0.005).

Discussion

Trace elements are essential components of biological structures, but their roles in the development and inhibition of cancer is complex and have raised many questions because of their essential and toxic effects at concentrations beyond those necessary for their biological functions on human health. Literature on this subject demonstrates conflicting results (Piccinini et al., 1996; Zowczak et al., 2001).

Our study demonstrated that concentrations Mn and Zn in the serum the patients with bladder cancer decreased compared to the control group. Mn is an essential element that is required for the activity of several enzymes. Mn is one of the essential trace elements that plays an important role in antioxidant defense and forms a part of SOD enzyme (Johnson et al., 2001). So, low serum concentration of Mn with the mechanism of antioxidant disturbance can sensitize the target organs to the carcinogens. The data of the present study showed that the Mn concentration in the serum of the patients with bladder cancer was lower than that of the control group. Our findings were in accordance with those of a study carried out by Mazdak et al, in 2009 (Mazdak et al., 2010). According to our hypothesis, and Mn as the trace elements affecting the oxidation status can result in this oxidant/antioxidant imbalance in bladder cancer patients.

Zinc plays an anticarcinogenic role by stabilizing the structure of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and ribosome. Zinc is also essential to the function of several transcription factors and proteins that recognize certain DNA sequences and regulate gene transcription. Zinc protects cells from free-radical injury (Wu et al., 2004). Lower serum Zn concentrations in patients with ovarian and cervical cancers have been reported (Cunzhi et al., 2003; Yaman et al., 2007). It seems that determination of zinc levels in ovarian cancer patients and comparison with a normal controls without ovarian pathology is necessary to fully elucidate the relationship between the Zn level and ovarian cancer. Additionally, normal or elevated concentration of zinc in blood serum identifiable only in cases of primary cancer of the liver (matched by a decrease in blood serum zinc level in the remaining cases) provides a differential diagnosis sign suggesting transformation of hepatocirrhosis into primary cancer of the liver (Karlinskiĭ et al., 1985). Our study demonstrated decrease Zn concentrations in the bladder cancer patients with control patients, the differences were statistically significant, which is in agreement with the findings of Mazdak et al. (Mazdak et al., 2010). They found that the serum Zn concentration was lower in the serum of the patients with bladder cancer compared to the control group.

Our study also demonstrated that concentrations Cd, Ni, and Co in the serum the patients with bladder cancer increased compared to the control group. The elevated levels of carcinogenic elements may be associated with a number of physiological disorders in humans. It has been reported that Cd is a mutagen in mammalian (Snow et al., 1997; Hayes et al., 2007) and enhanced concentration of Cd may result in prostate, renal and lung cancers (Drasch et al., 2005). Moreover, in the last decade, injection of cadmium metal powder and various cadmium compounds has been reported to produce sarcomas locally (WHO, 2010) and interstitial cell testicular tumors systemically (WHO, 2000) in experimental animals. Our findings were in accordance with those of a study carried out by Kellen et al. (Kellen et al., 2007). They found that serum Cd concentration was higher in the serum of the patients with bladder cancer compared to the control group. Our study suggests that individuals with increased exposure to cadmium have an increased risk of bladder cancer. Ni is another mutagen (Snow et al., 1992), which is also associated with lung and nasal cancer (Hayes et al., 2007). Among the other metal, Co have been linked to lung cancer (Schnorr et al., 1995).

They have been tested for their anti-tumor activity in cell culture experiments, animal models and human clinical trials (Tandon et al., 2005). Co is a natural element found throughout the environment. Respiratory effects are also the major effects noted from chronic exposure to cobalt by inhalation, with respiratory irritation, wheezing, asthma, pneumonia, and fibrosis noted. Furthermore, cardiac effects, congestion of the liver, kidneys, and conjunctiva, and immunological effects have also been noted in chronically-exposed humans (Calabrese et al., 1991). Animal studies have shown that Co accumulates in the myocardium and causes myocytolysis. Detrimental effects on sperm production and fertility have been observed in rats (Anderson et al., 1992). Higher serum Ni and Co concentrations in patients with prostate cancer have been reported (Ozmen et al., 2006).

This study also demonstrated that levels Cu, Pb and Fe in the serum of the patients with bladder cancer were not significantly different compared to the control group. However, in a study carried out by Mazdak et al, the analysis of serum Cu level demonstrates that in the bladder cancer group, the serum Cu level is significantly and remarkably higher than the control group (Mazdak et al., 2010).

Cu is an essential nutrient that plays a role in the production of hemoglobin, myelin, collagen, and melanin (Sayır et al., 2011). Recent evidence also suggests that adequate intake of Cu is necessary for normal immune function (Gecit et al., 2011; Sayır et al., 2011). Cu deficiency affects various physiological functions that may be important in immunological defense to pathogenic challenge (Stabel et al., 1993). It was reported that serum levels of Cu, Fe and ceruloplasmin activity decreased in Cu-deficient animals (Andersen et al., 2007).

Early life inorganic lead exposure induces testicular teratoma and renal and urinary bladder preneoplasia (Erik et al., 2010). Iron is an essential trace element that is crucial to normal cell functioning and its deficiency or excess is associated with several disease states (Dayani et al., 2004). In our study, the performed analysis indicates that patients having bladder were not significantly different compared to the control group. Our results are different from those obtained from most of the earlier studies that investigated iron in other types of cancer such as lung cancer, hepatocellular carcinoma, and colorectal cancer (Tsunehiro et al., 2003). In these studies the serum iron level is higher than the normal group. Dayani et al. showed that the mechanisms of pathogenesis could be mediated by direct effect of iron overload on the formation of hydroxyl-free radicals from hydrogen peroxide and superoxide via the Fenton and Haber–Weiss reaction (Dayani et al., 2004). Moreover, Ilker et al. (Ilker et al., 2003) showed that serum iron levels in patients with invasive bladder cancer were found to be significantly lower when compared with non-invasive group. Anemia as a result of chronic blood loss and chronic hematuria is one of the mechanisms that may cause a lower iron level in the bladder cancer group (Olinski et al., 2003).

In conclusion, the presence of an association between bladder cancer and trace elements is observed in the present study. We also suggest that a increase Cd, Ni and Co levels may play an important role in bladder cancer induction. However, further prospective cohort studies concerning the causes of changes in Mn and Zn concentration in blood serum of patients with bladder cancer seems to be well ground. Further studies are required to clarify the association between the different stages of bladder cancer and serum trace element levels.

References

Ames BN, Magaw R, Gold LS (1987). Ranking possible carcinogenic hazards. *Science*, **236**, 271-80.

Andersen HS, Gambling L, Holtrop G, McArdle HJ (2007). Effect of dietary copper deficiency on iron metabolism in the pregnant rat. *British J Nutr,* **97**, 239-46

Anderson MB, Pedigo NG, Katz RP, George WJ (1992). Histopathology of testes from mice chronically treated with cobalt. *Reproductive toxicology,* **6**, 41-50.

Calabrese EJ, Kenyon EM (1991). Air Toxics and risk assessment. Lewis Publishers, Chelsea, MI.

Cole P (1971). Coffee-drinking and cancer of the lower urinary tract. *Lancet*, **I**, 1335-7.

Cunzhi H, Jiexian J, Xianwen Z, et al (2003). Serum and tissue levels of six trace elements and copper/ zinc ratio in patients with cervical cancer and uterine myoma. *Biol Trace Elem Res,* **94**, 113-22.

Dayani PN, Bishop MC, Black K, Zeltzer PM (2004). Desferoxamine (DFO)-mediated iron chelation: rationale for a novel approach to therapy for brain cancer. *J Neurooncol,* **67**, 367–77.

Drasch G, Schopfer J, Schrauzer GN (2005). Selenium/Cadmium rations in human prostrates. Indicators for prostate cancer risk of smokers and non-smokers and relevance to the cancer protective effects of selenium. *Biol Trace Elem Res,* **103**, 103–7.

Tokar EJ, Diwan BA, Waalkes MP (2010). Early life inorganic lead exposure induces testicular teratoma and renal and urinary bladder preneoplasia in adult metallothionein-knockout mice but not in wild type mice. *Toxicology,* **276**, 5-10.

Gecit I, Kavak S, Meral I, et al (2011). Effects of shock waves on oxidative stress, antioxidant enzyme and element levels in kidney of rats. *Biol Trace Elem Res*, **144**, 1069-76.

Hayes, RB (1997). The carcinogenicity of metals in humans. *Cancer Causes Control,* **8**, 371–85.

Hoekstra WG, Suttie JW, Ganther HG, Mentz W (1974). Trace elements metabolism in animals, vol 2. University Park Press, Baltimore, 61.

Hoover R, Stasser PH (1980). Artificial sweeteners and human bladder cancer. *Lancet*, **19**, 837-40.

Ilker A, Akçay T, Çakatay U, et al (2003). Relation between bladder cancer and protein oxidation. *Int Urol Nephrol,* **35**, 345–50,

Johnson S (2001). The possible crucial role of iron accumulation combined with low tryptophan, zinc and manganese incarcinogenesis. *Med hypothesis*, **57**, 539-643.

Karlinskii VM, Bogomolova GG (1985) Change in zinc metabolism in malignant neoplasms. *Vopr Onkol*, **31**, 25-9.

Helzlsouer KJ, Comstock GW, Morris JS (1989). Selenium, lycopene, alpha-tocopherol, beta-carotene, retinol, and subsequent bladder cancer. *Cancer Res,* **49**, 6144-8.

Kellen E, Hond E den, Buntinx F (2007). Blood cadmium may be associated with bladder carcinogenesis: the Belgian case-control study on bladder cancer. *Cancer Detect Prev,* **31**, 77-82.

Kumar SV, Kumar AT, Sureshwer SP, Chandra PM, Ravindra NM (2003). Micronutrients, antioxidants, and carcinoma of the gallbladder. *J Surg Oncol,* **84**, 31–5.

Li Y, Xue Q, Chen L, et al (2004). Research on relationships of gastric cancer with serum trace elements, Helicobacter pylori and COX-2 in gastric tissue. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi,* **21**, 107–10.

Macvicar AD (2000). Bladder cancer staging. *BJU Int,* **86**, 11–22.

Mazdak H, Yazdekhasti F, Movahedian A, Mirkheshti N, Shafieian M (2010). The comparative study of serum iron, copper, and zinc levels between bladder cancer patients and a control group. *Int Urol Nephrol,* **42**, 89–93

Moon RC, Mehta RG (1986). Anticarcinogenic effects of retinoids in animals. Adv Exp *Med Biol*, **206**, 399-411.

Morrison AS, Cole P (1982) Urinary tract. In: Schottenfeld and Fraumeni (eds.), Cancer Epidemiology and Prevention, 925-37.

Olinski R, Gackowski D, Rozalski R, Foksinski M, Bialkowski K (2003). Oxidativ DNA damage in cancer patients: a cause or a consequence of the disease development? *Mutat Res,* **531**, 177-90.

Ozmen H, Erulas AF, Karatas F, Cukurovali A, Yalcin O (2006). Comparison of the concentration of trace metals (Ni, Zn, Co, Cu and Se), Fe, vitamins A, C and E, and lipid peroxidation in patients with prostate. *Clin Chem Lab Med* , **44**, 175–9.

Piccinini L, Borella P, Bargellini A, Medici CI, Zoboli A (1996). A case- control study on selenium, zinc, and copper in plasma and hair of subjects affected by breast and lung cancer. *Biol Trace Element Res,* **51**, 23-30.

Sayır F, Kavak S, Meral İ, et al (2011). Effects of crush and axotomy on oxidative stress and some trace element levels in phrenic nerve of rats. Brain research bulletin.

Schnorr TM, Steenland K, Thun MJ, Rinsky RA (1995). Mortality in a cohort of antimony smelter workers. *Am J Ind Med,* **27**, 759–70.

Silverberg BS, Lubera BBA (1988). Cancer statistics. *J Cancer Pract*, **38**, 5-22.

Snow ET (1992). Metal carcinogenesis: mechanistic implications. *Pharmacol Ther,* **53**, 31-65

Spartz L, Bloom AD (1992). Biological consequences of oxidative stress: implications for cardiovascular disease and carcinogenesis. Oxford University Press, New York, pp 138–61.

Stabel JR, Spears JW, Brown TT (1993). Effect of copper deficiency on tissue, blood characteristics and immune function of calves challenged with infectious bovine rhinotracheitis virus and *Pasteurella hemolytica*. *J Anim*

Sci, **71**, 1247-55.
Tandon WR, Sharma S, Mahajan A, Bardi GH (2005). Oxidative Stress : A Novel Strategy in Cancer Treatment. Jk Scıence Vol. 7 No. 1.
Tsunehiro O, Toshihiro K, Koji M (2003). A case-case study comparing the usefulness of serum trace elements (Cu, Zn and Se) and tumor markers (CEA, SCC and SLX) in non-small cell lung cancer patients. *Anticancer Res,* **23**, 605–61.
World Health Organızatıon IARC (1993) IARC monographs on the evaluation of the carcinogenic risk to man: beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. Working Group views and expert opinions. IARC Press, Lyon.
World Health Organızatıon Regional Office for Europe (2000) Air quality guidelines, 2nd edn. World Health Organization, Copenhagen, Denmark.
Wu T, Sempos CT, Freudenheim JL, Muti P, Smit E (2004). Serum iron, copper and zinc concentrations and risk of cancer mortality in US adults. *Ann Epidemiol* , **14**, 195-201.
Wynder EL, Goldsmith R (1977) The epidemiology of bladder cancer: a second look. *Cancer,* **40**, 1246-68.
Yaman M, Kaya G, Simsek M (2007). Comparision of trace elements concentrations in cancerous and noncancerous human endometrial and ovary tissues. *Int J Gynecol Cancer*, **17**, 220-8.
Yelinova V, Glazachev Y, Khramtsov V, et al (1996). Studies of human and rat blood under oxidative stress: changes in plasma thiol level, antioxidant enzyme activity, protein carbonyl content, and fluidity of erythrocyte membrane. *Biochem Biophys Res Commun,* **221**, 300-3.
Zeegers MP, Kellen E, Buntinx F, Van den Brandt PA (2004). The association between smoking, beverage consumption, diet and bladder cancer: a systematic literature review. *World J Urol,* **21**, 392-401.
Zowczak M, Iskra M, Torlinski L, Cofta S (2001) Analysis of serum copper and zinc concentration in cancer patients. *Biol Trace Elem Res*, **82**, 1-8.