Comparison of Trace Metal Concentrations in Malign and Benign Human Prostate

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Received July 9, 2004

Imbalance in the composition of trace metals, recognized to be essential to normal human homeostasis, besides the accumulation of potentially toxic or nonessential trace metals, may cause disease. Thus, there is a need for their analysis in cancerous and noncancerous human tissues to examine the relationship between cancer and these elements. Trace metal concentrations including Cd, Ni, Zn, Cu, Fe, Mg, and Ca in both malign and benign prostate samples were determined by atomic absorption spectrophotometry. The tissues were digested by using microwave energy. In contrast to the literature data for zinc, the concentrations of calcium and zinc in the malign human prostate were found to be significantly higher than those in the benign human prostate ($p < 0.05$ for both metals). Similarly, the concentrations of iron, nickel, and magnesium in the malign prostate were also found to be higher than those in the benign prostate ($p \leq 0.1$). Therefore, it is understood that more studies are needed regarding the increase or decrease in the metal (particularly Ca and Zn) concentrations of malign prostate samples.

Introduction

Imbalance in the composition of trace metals, recognized to be essential to normal human homeostasis, besides accumulation of potentially toxic or nonessential trace metals, may cause disease. The essential trace elements have four major functions as stabilizers, elements of structure, essential elements for hormonal function, and cofactors in enzymes. As a result, the lack of essential trace elements will influence structure alone or will alter function of structure through the lack of stabilization, change of charge properties, or allosteric configuration.[1] It may be expected that the deficiency of essential trace elements as cofactors of enzymes could severely impair the host's resistance against carcinogenic stress.[2] From these elements, zinc is a component of over 300 proteins and over 100 DNA-binding proteins with zinc fingers. Zn and Cu are the prosthetic groups of some metalloenzymes containing superoxide dismutase (SOD), which is an important antioxidant enzyme for cellular protection from reactive oxygen species (ROS).[3] It is described that benign prostatic obstruction (BPO) is characterized by high Zn concentrations and prostate cancer (PCa) is characterized by low Zn concentrations.[4]

Cancer is a multietiological and multifactorial complex disease. The role of metals in the development and inhibition of cancer has a complex character and raises many questions. In the last 20 years, some metals, including cadmium, nickel, arsenic, cobalt, and chromium(VI), were recognized as human or animal carcinogens in addition to primary carcinogens such as radiation, viruses, and other chemicals.[5,6] Their carcinogenic potentials depend largely on factors such as oxidation states and solubilities.[7] The induction of oxidative DNA damage and the interaction with DNA repair processes were leading to an enhancement of genotoxicity in combination with a variety of DNA-damaging agents. Nucleotide excision repair (NER), which is the major repair system, is inhibited at low levels as well as at noncytotoxic concentrations of Ni^{II}, Cd^{II}, Co^{II}, and As^{III}. The repair of oxidative DNA base modifications is disturbed by Ni^{II} and Cd^{II}. One reason for repair inhibition appears to be the displacement of Zn^{II} and Mg^{II}.[8] Mg and Zn, essential elements that are cofactors for DNA polymerase, are effective protectors against carcinogenesis in vivo.

Table 1. Operating Parameters for FAAS

parameter	Cd	Ni	Cu	Zn	Fe	Mg	Ca
wavelength, nm	228.8	232.0	324.8	213.9	248.3	285.2	422.7
HCL current, mA	7.5	7.5	3.0	9.5	15	15	6
acetylene flow rate, L/min	0.5	0.5	0.5	0.5	0.5	0.5	4.2
N_2O flow rate, L/min							4.7
air flow rate, L/min	4.0	4.0	4.0	4.0	4.0	4.0	
slit, nm	0.5	0.2	0.5	0.5	0.2	0.5	0.5

The most common analytical technique used for trace metal analysis inbiological matrixes is atomic absorption spectrometry.[9−11] For improvedsensitivity of flame atomic absorption spectrometry (FAAS), a slotted tube atom trap (STAT) was used for some metals such as Cd and Pb.[12] In this study, the concentrations of different trace metals, including Cd, Ni, Cu, Zn, Fe, Mg, and Ca in malign and benign prostate tissues, were determined by atomic absorption spectrophotometry. For digestion of the tissues, a microwave oven was used.

Results and Discussion

Calibration curves were obtained by using the solutions of the studied elements at different concentrations.

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10.1021/jm0494568 CCC: $30.25
Published on Web 12/22/2004

Table 2. Trace Metal Concentrations in the Malignant and Benign Prostate Tissues[a]

	Cd, ng/g		Ni, ng/g		Cu, mg/kg		Zn, mg/kg		Fe, mg/kg		Mg, mg/kg		Ca[b], mg/kg	
tissue	malign	benign	malign	benign	malign	benign	malign	benign	malign	benign	malign	benign	malign	benign
prostate	124 ± 13	33 ± 4	1300 ± 100	75 ± 8	0.9 ± 0.1	0.7 ± 0.1	119 ± 13	35 ± 4	26 ± 3.0	15 ± 2	360 ± 40	255 ± 32	1650	1200
prostate	100 ± 12	130 ± 15	200 ± 28	500 ± 60	2.5 ± 0.3	0.8 ± 0.1	136 ± 16	54 ± 6	35 ± 4	12 ± 1	160 ± 25	111 ± 13	1300	200
prostate	80 ± 9	108 ± 12	130 ± 13	100 ± 9	1,1 ± 0.1	0.8 ± 0.1	50 ± 6	40 ± 5	85 ± 8	14 ± 1	400 ± 53	200 ± 29	1400	820
prostate	65 ± 7	20 ± 2	400 ± 46	450 ± 55	0.7 ± 0.1	0.6 ± 0.1	69 ± 7	64 ± 6	20 ± 2	17 ± 2	200 ± 24	62 ± 8	1740	271
prostate	82 ± 7	74 ± 8	500 ± 58	400 ± 52	0.3 ± 0.04	0.6 ± 0.1	59 ± 6	29 ± 3	15 ± 1	17 ± 1	98 ± 12	110 ± 13	540	388
prostate	49 ± 5	105 ± 11	480 ± 50		0.9 ± 0.1	0.5 ± 0.1	78 ± 8	70 ± 8	46 ± 5	37 ± 4	67 ± 8	146 ± 17	2350 (1700−2900)	2100 (1700−2500)
prostate	65 ± 5		630 ± 70		0.9 ± 0.1		43 ± 5		25 ± 3		93 ± 11		2200 (750−3100)	
prostate	66 ± 6		440 ± 50		0.5 ± 0.1		47 ± 5		20 ± 2		122 ± 14		2100 (1400−2800)	
prostate	56 ± 5		301 ± 30		0.7 ± 0.1		45 ± 5		12 ± 2		502 ± 62		1350	
prostate[c]	90 ± 10	85 ± 8	500 ± 63	350 ± 40	0.3 ± 0.03	0.2 ± 0.03	60 ± 5	40 ± 4	20 ± 1	14 ± 2	175 ± 17	122 ± 15	590	527
prostate	25 ± 3		330 ± 32		0.2 ± 0.03		46 ± 4		21 ± 2		133 ± 18		641	
average	69 ± 29	79 ± 40	473 ± 310	267 ± 203	0.8 ± 0.6	0.6 ± 0.2	68 ± 31	47 ± 15	30 ± 21	18 ± 9	210 ± 144	144 ± 64	1441 ± 646	787 ± 675
range	25−124	20−130	130−1300	75−500	0.2−2.5	0.2−0.8	43−136	29−70	12−85	12−37	67−502	62−255	540−2350	200−2100
urinary bladder			750 ± 70		1.3 ± 0.1		32 ± 3		507 ± 40		30 ± 5		1866 ± 1553 (600−3600)	
urinary bladder[c]	11 ± 1	13 ± 1	1300 ± 650	825 ± 35	0.4 ± 0.1	0.3 ± 0.1	19 ± 1	11 ± 1	33 ± 4	21 ± 2	270 ± 10	205 ± 5	1200 ± 288	830 ± 70

[a] The results are mean values (fresh weight basis), $n = 3$. Every malign and benign tissue belongs to a different person [b] Standard deviations for the Ca results of prostate are in the range 15−50%. [c] The malign and benign tissues in this line are belong to the same person.

The graphs obtained were linear in the concentration range described follow, and the equations of the curves were as follows

$$Y = 1.11X + 0.4 \qquad R^2 = 0.99$$

for Cd (10−140 ng/mL by STAT-AAS)

$$Y = 85X + 0.5 \qquad R^2 = 0.99$$

for Ni (0.25−2.0 mg/L)

$$Y = 0.21X + 0.5 \qquad R^2 = 1.0$$

for Cu (50−500 ng/mL by STAT-AAS)

$$Y = 302X + 0.75 \qquad R^2 = 0.99$$

for Zn (0.1−1.0 mg/L)

$$Y = 64X + 0.43 \qquad R^2 = 0.99$$

for Fe (0.20−3.0 mg/L)

$$Y = 515X + 7.0 \qquad R^2 = 0.99$$

for Mg (0.25−2.0 mg/L)

$$Y = 305X + 39 \qquad R^2 = 0.99$$

for Ca (0.25−2.0 mg/L).

The accuracy of the method was studied by examining the recovery of the metals from prostate samples fortified with various amounts of the studied metals. The following metal amounts were added: 50 ng/g of Cd, 100 ng/g of Ni, 0.5 mg/kg of Cu, 40 mg/kg of Zn, 10 mg/kg of Fe, 100 mg/kg of Mg, and 200 mg/kg of Ca. After digestion by microwave oven, the recoveries were found to be at least 93% for all studied metals. In addition, the standard additions method was used to investigate possible interferences caused by the matrix. The slopes of the calibration curves for all studied elements were compared with the slopes obtained by the standard additions method. The slopes of the calibration curves were found to be the same as those obtained with the standard additions method. In other words, all of the standard additions curves were parallel to the calibration curves. These results indicate an absence of chemical interference.

Comparison of Metal Levels in the Malign and Benign Tissues. Data related with carcinogen effects of cadmium and nickel have been detailed in the literature.[8,13] Occupational exposure to Cd is associated with lung cancers in humans, whereas other sites, potentially including the prostate, are not definitively established.[14] Cadmium can also cause prostatic proliferative lesions, including adenocarcinomas after systemic or direct exposure.[14] As seen from Table 2, high Cd concentrations were found in both malignant and benign prostate tissues. The average Ni contents in the studied malign prostate samples were found to be significantly higher ($p < 0.1$) than those in the benign samples.

Zinc. It has been known that Zn concentration in the prostate gland is much higher than in other human tissues.[4] In addition, there is some evidence that an increase of Zn content in benign prostatic hyperplasia (BPH) and a decrease in prostatic carcinoma (PCA), as compared to normal tissues, occurs,[15,16] but the information about the mechanism of the accumulation of Zn in prostate is too incomplete to draw any conclusions regarding its importance.[17,18] However, Zn and Se deficiency were considered as possible cancer risk factors for prostate.[19]

In contrast to the above literature information, it is seen from Table 2 and Figure 1 that the Zn levels in the malign prostate tissues were significantly higher than the Zn levels in the benign prostate tissues ($p < 0.05$). One cause of these results may be Zn accumulated in apoptotic cells because cell homeostasis was destroyed. Reactive oxygen substances (ROS) could produce DNA damage with toxic free hydroxyl radicals in vivo and lead to cancer. The prostate converts testosterone to dihydrotestosterone, a key substrate for downstream hormone metabolism. Withdrawal of testosterone by surgical or medical castration is a well-known treatment for prostate cancer and is effective in

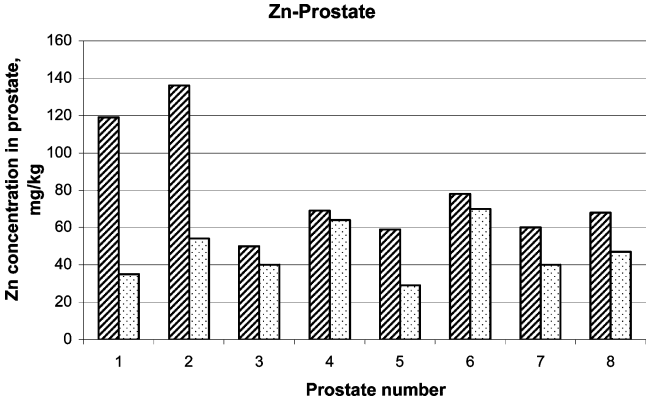

Figure 1. Comparison of Zn concentrations in malign and benign prostate tissues. Dark bars, malign; light bars, benign. The results given for number 7 belong to the same person for both malign and normal tissues. The results given for number 8 are the average values of the studied samples.

75−80% of patients with metastatic prostate cancer.[19] Zn can inhibit transforming of testosterone to dihydrotestosterone at low and also at high concentrations too.[18] Therefore, the second cause of high Zn concentration in the malign prostate may be the result of prostate cancer due to inhibition of the transformation of testosterone to dihydrotestosterone.

The differences between the literature in which Zn levels in malign prostate were lower than those in the benign prostate and our values that show Zn levels in malign prostate were higher than those in the benign prostate may be caused for the following reasons: (a) Zn is not uniformly distributed throughout the different anatomical regions of the prostate. For example, the Zn levels in the lateral lobe and anterior lobe were 211 and 84 mg/kg on a wet tissue basis, respectively.[15] (b) The literature values were either too old (up to 35 years)[15] or were given on a dry weight basis.[16]

Iron. Reducer active metal ions, such as Fe and Cu, play a role in the increase of the ROS production (Fenton reaction) in biological systems.[20] Although Fe is an essential nutritional element for all life forms, it is known that excess iron, like iron deficiency, also leads to oxidative DNA damage.[21] Similar to zinc, iron levels in the malign prostate tissues were found to be higher ($p < 0.1$) than the levels in the benign prostate tissues (Figure 2).

Copper. Although copper is an essential element for humans and animals, high concentrations of Cu (above normal) could induce growth proliferation and cancer by damaging DNA with toxic free hydroxyl radicals.[20] In this study, Cu levels were found to be slightly higher ($p < 0.2$) in the malign prostate samples than in the benign prostate samples (Table 2 and Figure 3). Unfortunately, we did not measure the ROS levels in the studied prostate tissues. Therefore, there is a need for studies regarding the ROS production and the trace element levels.

Magnesium and Calcium. As similar to Zn, Fe, and Cu, the magnesium levels were also found to be higher ($p = 0.1018$) in the malign prostate samples than in the benign prostate samples (Figure 4). Last, calcium levels in the malign prostate samples were found to be 2 times higher ($p < 0.05$) than those in the benign samples

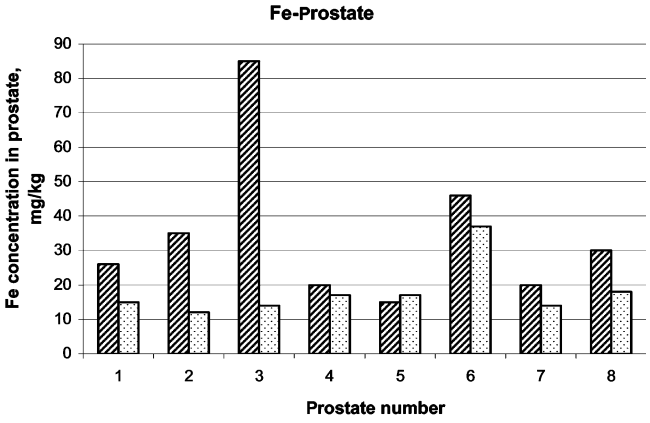

Figure 2. Comparison of Fe concentrations in malign and benign prostate tissues. Dark bars, malign; light bars, benign. The results given for number 7 belong to the same person for both malign and normal tissues. The results given for number 8 are the average values of the studied samples.

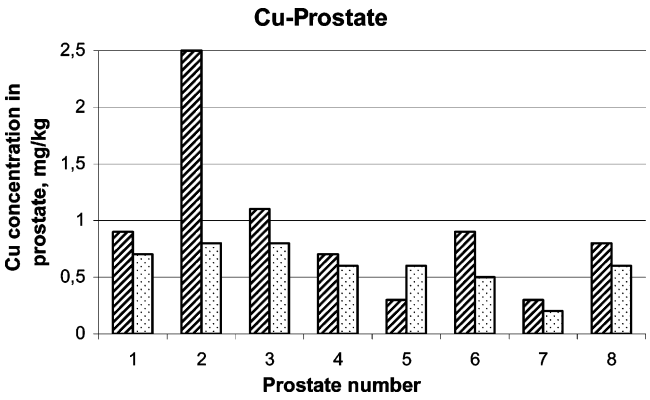

Figure 3. Comparison of Cu concentrations in malign and benign prostate tissues. Dark bars, malign; light bars, benign. The results given for number 7 belong to the same person for both malign and normal tissues. The results given for number 8 are the average values of the studied samples.

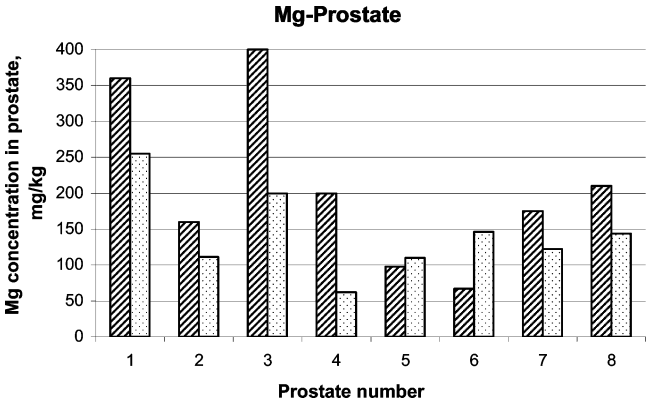

Figure 4. Comparison of Mg concentrations in malign and benign prostate tissues. Dark bars, malign; light bars, benign. The results given for number 7 belong to the same person for both malign and normal tissues. The results given for number 8 are the average values of the studied samples.

(Figure 5). This suggested that the stored calcium in the cells was not departing from the cells because the cell functions were disturbed in the malign cancer.

In Table 2, there are also results belonging the same person for malign and benign samples. It was observed that similar results to the above were found for these samples.

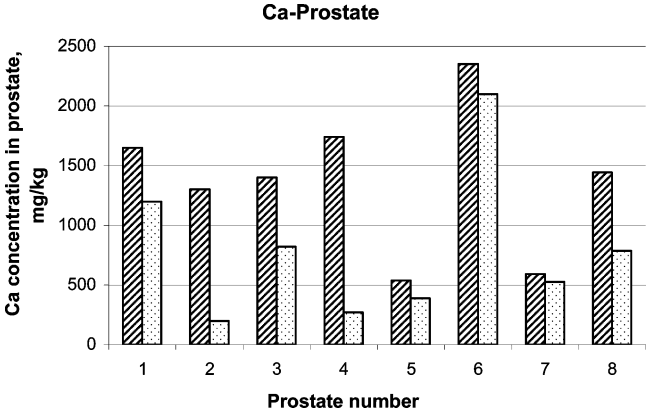

Figure 5. Comparison of Ca concentrations in malign and benign prostate tissues. Dark bars, malign; light bars, benign. The results given for number 7 belong to the same person for both malign and normal tissues. The results given for number 8 are the average values of the studied samples.

Table 3. List of Significant and Tendentious Elements in Malign Prostate

status	prostate	*p*
significant	Ca(+)	0.0315
	Zn(+)	0.0395
	Fe(+)	0.0610
	Ni(+)	0.0982
	Mg(+)	0.1018
tendentious	Cu(+)	0.1540

In the Table 3, significant and tendentious elements are listed. The positive sign is used to illustrate the accumulation of the element in malign prostate, and the minus sign is used to indicate the depletion of the element in malign prostate.

In conclusion, we have found that zinc, iron, copper, nickel, magnesium, and calcium concentrations in malignant prostate samples were higher than those in the benign prostate samples, in contrast to the literature data for zinc. We think that the increase in Ca levels and its heterogeneous distribution in malign samples are very important for the investigation of cancer mechanisms. Therefore, it is understood that more studies are needed regarding the increase or decrease in the Zn concentrations in malign prostate samples. A slotted tube atom trap (STAT) as an accessory was used to improve the sensitivity to copper and cadmium.

In addition, the tissue digesting by using closed-vessel digestion with a microwave oven was found to be simple, rapid, and practical besides having very low blank values and reduce the risk of metal loss or contamination.

Experimental Section

Apparatus and Reagents. An ATI UNICAM 929 model flame atomic absorption spectrophotometer (FAAS) equipped with ATI UNICAM hollow cathode lamps was used for the metal determinations. The optimum conditions for FAAS are given in Table 1. A slotted tube atom trap (STAT) was used to increase the Cd and Cu sensitivity by FAAS. A domestic microwave oven (Kenwood) was used for the digestion of the tissues.

Unless stated otherwise, all chemicals used were of analytical reagent grade. Throughout all analytical work, doubly distilled water was used. All glass apparatus (Pyrex) were kept permanently full of 1 M nitric acid when not in use. In the digestion procedures, concentrated nitric acid (65%, Merck) and hydrogen peroxide (35%, Merck) were used. Stock solutions of metals (1000 mg L^{-1}) were prepared by dissolving their salts (Merck) in 1.0 M nitric acid.

Preparation of Samples. The samples were obtained in the formaldehyde solution from private pathology laboratories and the pathology laboratories of Firat University in Elazig, Turkey. Eleven samples of the cancerous (malignant) and six samples of the noncancerous (benign) prostate samples were taken from patients of different sex, age, and living conditions except one sample. In addition, two urinary bladder samples were taken. The tissue samples were cut into small pieces with a stainless steel knife and transferred to a beaker.

Digestion by Using Microwave Oven. Exactly 1.0 mL of the acid mixtures of HNO_3/H_2O_2 (2:1) was added to 0.5 g of samples. The mixture was placed into the water bath at 70 °C for 30 min with stirring 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. The radiation was applied for 3 min at 450 W. After the addition of 0.5 mL of the same acid mixture, the radiation was repeated for 3 min. After 5 min of cooling, 2.0 mL of 0.1 M HNO_3 was added, and the solution was transferred into a Pyrex tube. After centrifugation, the clear solution was measured by FAAS. The blank digests were carried out in the same way.

Acknowledgment. We thank to Professor Dr. Hayrettin Yekeler from Firat University, Medicine Faculty, Department of Pathology for his assistance. This study was supported by the Scientific Investigate Projects of Firat University (FÜBAP 575).

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JM0494568