Copper, Lead, Zinc and Cadmium levels in serum of prostate cancer patients by polarography in Iran

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ABSTRACT

Cancer of the prostate is an important and potentially fatal disease in humans, but its etiology is yet undefined. Previous observations have shown that heavy metals play various roles in human health. The heavy metals zinc, cadmium, lead, and copper (Zn, Cd, Cu, and Pb) are known to be associated with prostate cancer, but their functions are unclear. This study examined and compared serum levels of these metals in prostate cancer patients with those of a control group. In total, 72 subjects (36 controls and 36 prostate cancer patients) were included in this study. Blood samples were collected from 36 newly-diagnosed prostate cancer patients and 36 healthy men who were matched by age, sex, race, and smoking and drinking status. After samples were prepared, serum concentrations of zinc, cadmium, lead, and copper were determined using polarography. Serum levels of cadmium and copper were significantly higher in patients with prostate cancer than in controls (p<0.05). No significant association was found between the serum levels of zinc and lead in prostate cancer patients compared with the controls (p>0.05); however, the mean concentrations of zinc and lead were higher in patients. This study supports the hypothesis that cadmium exposure increases prostate cancer risk. These findings can accelerate the diagnosis and treatment of prostate cancer. No distinct association was found between serum levels of zinc and lead and prostate cancer. More studies with larger sample sizes are needed to identify the role of heavy metals in prostate cancer.

Key words: Prostate cancer; Heavy metal; Urologic Disease; Differential pulse polarography

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INTRODUCTION

Prostate carcinoma (PCa) is a public health problem that is currently the most common neoplasm and the second leading cause of cancer-related deaths in males of western populations[1,2]. Prostate cancer rates vary markedly both within and between populations [3]. Understanding the underlying causes of the variations is important for cancer prevention. Numerous risk factors for prostate cancer have been identified. Age, race, and family history of prostate cancer are equal risk factors for this malignancy, but there are a lot of probable risk factors for prostate cancer [4]. Zinc, cadmium, lead, and copper are heavy metals and are frequently detected in the environment. Many studies have shown that metals are associated with adverse health effects, pointing to their relevance as a public health concern [5]. Zinc (Zn) is an essential metal. Zinc toxicity is uncommon and occurs only at very high exposure levels. The association between zinc and cancer incidence rates is unclear [6]. Oral zinc supplements do not appear to have significant effects on the incidence of cancer. In fact, zinc deficiency may be associated with increased risk of cancer in humans [7]. Cadmium (Cd) is a major toxic metal. Cadmium compounds are considered to be human carcinogens [8]. Early human studies indicated that cadmium may be linked to prostate cancer, but this hypothesis has not been confirmed by more recent work. Evidence showed that the prostate can be a target of

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cadmium carcinogenesis in rats [9]. Lead (Pb) is a toxic metal that is widely used by humans. The belief in the association of lead exposure with increased human cancer risk was strengthened by recent studies [10]. A study of 20700 workers co-exposed to lead and engine exhaust found a 1.4-fold increase in overall cancer incidence [7]. Copper (Cu) is an essential element widely distributed in nature. Increased serum and tumor tissue levels of copper are also observed in several cancers, although little is known about how the metal might promote disease progression at the molecular level [11]. Epidemiological studies have found no distinct relationship between copper exposure and cancer [7]. The above-discussed factors are frequently inconsistent, showing a positive association with prostate cancer in some studies and no or even a negative association in others. There is currently no study about the relationship of serum concentrations of zinc, cadmium, lead, and copper in prostate cancer patients in Iran. The current study is the first of its type. This study aimed to assess the relationships between these metals (Zn, Cd, Pb, and Cu) and prostate cancer. Because of its accuracy, repeatability, low detection limit, and lack of usual chemical intervention, polarography was used to determine the levels of these metals in blood samples.

EXPERIMENTAL SECTION

Reagents and solutions
All solutions were prepared from analytical reagent grade materials in deionized water. Water was purified by a high purity water system ASTM 2 type (TKA, Germany). Zinc, lead, cadmium, and copper standard solutions were obtained from Merck Company, Germany. The mercury used in the dropping mercury electrode was obtained from Metrohm (Herisau, Switzerland).

Selection criteria
This study was conducted on 36 newly-diagnosed prostate cancer patients and 36 controls who matched in age, sex, race, smoking and drinking status, family history of prostate cancer, and time of blood sampling. All patients had biopsy–confirmed, clinically localized prostate cancer. Informed consent was obtained from all study participants.

Blood sampling and preparation
Three ml of blood samples were collected from the subjects in the morning. Collected blood samples were left at the ambient temperature for 30 minutes to coagulate, next centrifuged at 3000 rpm for 5 minutes, and then serums were separated by sampler. Afterward, 0.5 ml of serum and 8 ml nitric acid were added to the digestion vessels, which were then placed into a Microwave Digestion and Extraction MDS-10 (Sineo, China). After digestion, samples were immediately stored at 4ºC.

Apparatus
Polarography is an electroanalytical technique based on recording current-voltage curves using dropping mercury as the working electrode. It can be used for investigations of both reductions and oxidations of inorganic and organic species. Polarography was used in this study because of its accuracy and reproducibility in the detection of minute quantities of heavy metals. The determination limits of polarography for zinc, lead, cadmium, and copper are shown in Table 1 [12].

Table 1 Determination limit of polarography for Zinc, Cadmium, Lead and Copper

Element	Determination Limit	Concentration Max
Zn	1/0 µg/L	50 mg/L
Cd	0/1 µg/L	50 mg/L
Pb	0/1 µg/L	50 mg/L
Cu	1/0 µg/L	50 mg/L

The polarographic measurements were performed using a Metrohm 797 VA Computrance (Herisau, Switzerland). The three-electrode configuration consisted of the mercury drop electrode (HMDE)hanging from the multi-mode electrode (Metrohm) as the working electrode, an Ag/AgCl reference electrode with a 3M KCL filling solution, and a platinum wire as auxiliary electrode. For all measurements, the differential pulse (DP) mode was used. Stirrer speed, purge time, equilibration time, and pulse amplitude were 2000rpm, 300s, 10s, and 50mV, respectively, and Voltage step, Voltage step time, and Sweep rate were 6 mV, 0.1 s, and 6mV/s, respectively, for all measurements. Start potential and end potential were -1.15 mV and 0.05 mV for all measurements. Peak potential for zinc, cadmium, lead, and copper were -0.98 V, -0.56 V, -0.38 V, and -0.10 V respectively. Table 2 shows the optimized typical parameters in DPP detection.

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Table 2 Optimized and instrumental parameters for the determination of Zinc, Lead, Cadmium and Copper by using DPP detection

Working electrode	HMDE	Start potential	- 1.15 V
Calibration	Standard addition method	End potential	0.05 V
Number of replications	2	Voltage step	0.006 V
Stirrer speed/RDE	2000 rpm	Voltage step time	0.1 s
Measurement Mode	DP	Sweep rate	0.06 V/s
Purge time	300 s	Peak potential (Ep)	- 0.60 V
Pulse amplitude	0.05 V	Peak potential (Zn)	- 0.98 V
Deposition potential	- 1.15 V	Peak potential (Cd)	- 0.56 V
Deposition time	90 s	Peak potential (Pb)	- 0.38 V
Equilibration time	3 s	Peak potential (Cu)	- 0.10 V

Preparation of standard solution

Samples of 1mg L^{-1} mixed standard of zinc, cadmium, lead, and copper were prepared for Voltammetric analysis from the stock solution of 1000 mg L^{-1}. Polarogram of the standard solutions are shown in Figure 1.
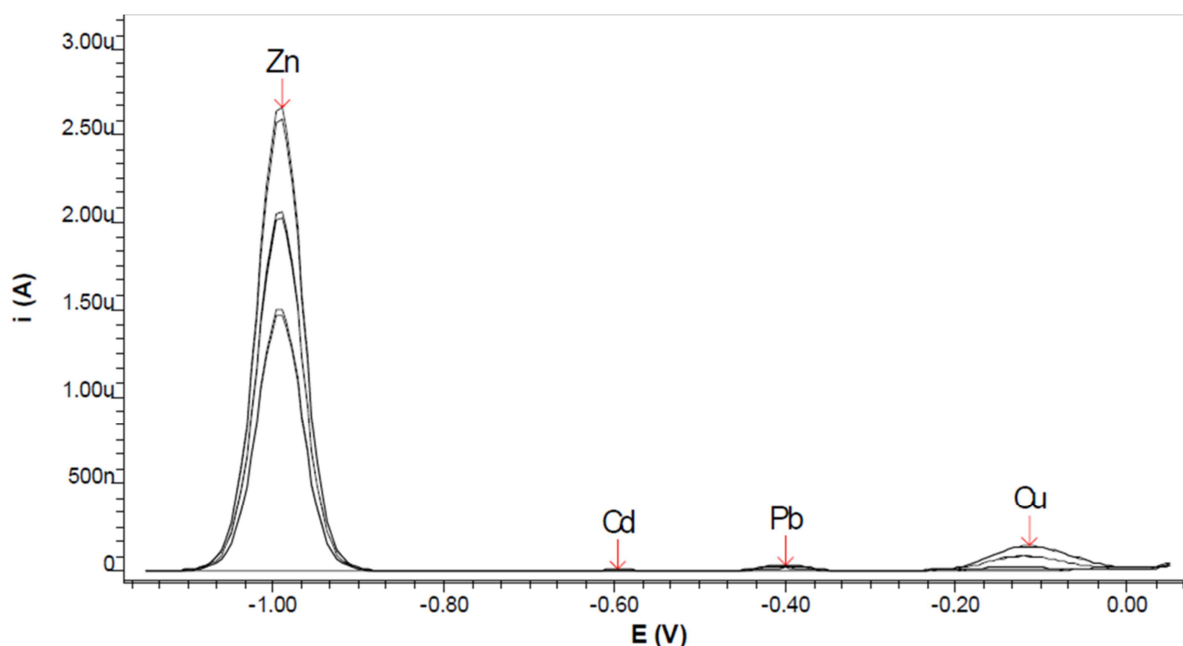

Figure 1 Polarograms obtained for samples as well as for Standard solutions (Zn^{2+}=10 mg/L, Cd^{2+}=0.1 mg/L, Pb^{2+}=0.5 mg/L, Cu^{2+}=2.5 mg/L). Condition: Sweep rate, 0.06 v/s; Pulse amplitude, 0.05 V; Deposition potential, -1.15 V vs. Ag/AgCl; deposition time, 90s; equilibration time, 10s; Scanning range, -1.15 to 10 V

Zinc, lead, cadmium, and copper detection

After samples were prepared, 1 ml of the prepared solution was poured into the polarography vessel, and 1.5 ml of ammonium acetate buffer was added; after mixing, deionized water and NaOH solution were added to the vessel, and pH was stabilized at 4.6. Analysis was carried out using polarography and the HMDE method in DP mode. Levels of zinc, lead, cadmium, and copper were determined using the standard addition method (Number of additions, 2).

Statistical analysis

In order to investigate the distribution of data, the Kolmogorov–Smirnov method was used. If the survey data followed a normal distribution the T-test was used; otherwise, the nonparametric Mann-Whitney test was used. Statistical analysis was performed using Statistics software (version 16, Stat Soft, Inc., Tulsa, OK, USA).

RESULTS

A total of 36 cases and 36 controls were recruited for this study. Comparisons of demographic factors showed no significant differences between cases and controls in gender, race, smoking and drinking status, family history of prostate cancer, education, or marriage. The distribution of age is shown in Table 3. To investigate the distribution of age, the Kolmogorov-Smirnov method was used. Results indicated that age had a normal distribution in this study, so the T-test was used to compare the means of the two groups. Outcomes showed no differences in the mean of age between the two groups.

After the equality of demographic factors in the two groups was assured, serum levels of zinc, lead, cadmium, and copper were measured. Median blood serum levels of zinc, lead, cadmium, and copper in prostate cancer patients were 85.51($\pm$5.16) µg/L, 55.46($\pm$4.51) µg/L, 26.42($\pm$4.10) µg/L, and 157.23($\pm$7.34) µg/L, respectively. Median blood serum levels of zinc, lead, cadmium and copper in the control group were 60.99($\pm$4.80) µg/L, 49.27($\pm$3.59) µg/L, 0.052($\pm$0.023) µg/L, and 70.91($\pm$5.79) µg/L, respectively (Table 3).

Table 3 Epidemiologic parameters and concentration of zinc, cadmium, lead and copper in blood serum (µg/L)

Characteristics	Case		Control	
	Mean($\pm$SD)	median	Mean($\pm$SD)	median
Age (year)	66.5($\pm$2.65)	67.5	64.11($\pm$3.49)	63
Zn (µg/L)	85.51($\pm$5.16)	84.31	60.99($\pm$4.80)	61.06
Pb (µg/L)	55.46($\pm$4.51)	56.89	49.27($\pm$3.59)	48.96
Cd (µg/L)	26.42($\pm$4.10)	27.45	0.052($\pm$0.023)	0.05
Cu (µg/L)	157.23($\pm$7.34)	155.42	70.91($\pm$5.79)	73.85

To investigate the distribution of serum concentrations of each metal, the Kolmogorov-Smirnov test was used. Results showed that only lead had a normal distribution ($p > 0.05$). To compare the levels of lead in these two groups, the t-test was used. The comparison of serum lead concentrations in case and control groups revealed no significant differences between the two groups ($p > 0.05$); however, the mean level of lead was higher in prostate cancer patients than in controls.

The Mann-Whitney test was used to compare serum zinc, cadmium, and copper concentrations in cases and controls. Results showed serum levels of copper and cadmium were significantly higher in patients with prostate cancer than in controls ($p < 0.005$). Serum zinc concentrations in these two groups showed no significant differences ($p > 0.005$), although the mean concentration of zinc were higher in patients than in controls.

DISCUSSION

Prostate cancer is a worldwide health problem. Western industrialized societies have significantly higher rates of prostate cancer incidence, prevalence, and mortality than all others [13,14].Population aging, lifestyle changes, and increased screening have resulted in rapid increases in both the incidence of and mortality from prostate cancer in low-incidence Asian countries [15]. Heavy metals are related to many health problems. The standard factors that impact the toxic potential of all chemicals apply to metals as well. Exposure–related factors include dose, route of exposure, duration, and frequency of exposure. Lead and cadmium are major toxic metals that are naturally toxic. Zinc and copper are essential metals that can be toxic at high doses of exposure [7].

In this study, the association between cadmium, zinc, lead, and copper levels and prostate cancer risks were investigated. This association has not previously been clearly delineated for men in Iran based on the measurement serum levels of cadmium, zinc, lead, and copper. Zinc is an essential trace element and is a normal component of many enzyme systems such as dehydrogenases, phosphatases, carboxypeptidases, and carbonic anhydrase[16]. It is more abundant in the human prostate than in other tissues. Although experimental evidence supports a role for zinc in prostate carcinogenesis, epidemiologic data is inconsistent [17]. For example, epidemiological studies of workers in electrolytic zinc and copper refining industries have not found an increased incidence of cancer associated with occupational zinc inhalation [18]. On the other hand, various studies have indicated that zinc administration will generally block cadmium carcinogenesis [19]. In experimental animals, zinc prevents cadmium-induced testicular cancer but facilitates cadmium-induced prostate tumors [7]. The current study found that serum zinc levels in prostate cancer patients did not significantly differ with those in controls ($P < 0.05$). However, mean concentrations of zinc were higher in prostate cancer patients than in controls. A recent case-control study in the US found no association between serum zinc levels and prostate cancer. Park et al. analyzed serum zinc concentrations in 392 prostate cancer cases and 783 controls matched in age, race/ethnicity, date/time of blood draw, and fasting status. The mean serum zinc concentrations between cases and controls did not differ significantly [20]. Some studies, however, found opposite results. For example, a study conducted by Ozmen in Turkey found that Zn levels were significantly lower in patients with prostate cancer than in controls. A variation in the method of zinc detection may be the reason for the differences between the results of the current study and previous studies. Cadmium (Cd) is a toxic transition metal that ranks close to lead and mercury as one of the top toxic substances. This metal is a major toxic metal, and it has been reported that exposure to cadmium is likely to cause many diseases such as nephrotoxicity, chronic pulmonary disease, neurotoxicity, and cancer [21]. In humans, occupational respiratory exposure to cadmium has been most clearly associated with lung cancer [7]. The current study found that serum cadmium levels were significantly higher in prostate cancer patients than in controls ($P > 0.05$). According to several studies, cadmium is associated with prostate cancer [19,9]. A study in Italy surveyed the relationship between cadmium exposure and prostate cancer, estimated by determining the toenail cadmium levels and prostate cancer

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risk in forty patients newly diagnosed with prostate cancer and fifty-eight hospital controls. Results showed an excess cancer risk in subjects in the third and fourth (highest) quartiles of toenail cadmium concentration compared with subjects in the bottom quartile. These findings appear to support the hypothesis that cadmium exposure increases prostate cancer risk [22]. Lead is a heavy metal whose toxic effects have been known for more than 2000 years. The most sensitive targets for lead toxicity are the nervous system, the hematological and cardiovascular systems, and the kidneys [23]. Several studies have shown a correlation between lead exposure and overall cancer incidence [24,7]. Some recent studies have suggested an association between lead exposure and increased lung and stomach cancers [25]. Several mechanisms have been proposed for lead–induced carcinogenesis, including regenerative repair, generation of reactive oxygen species with oxidative damage to DNA, and interaction with DNA-binding proteins [26]. The current study found that serum lead levels in prostate cancer patients did not significantly differ with those in controls ($P<0.05$); however, mean concentrations of lead were higher in prostate cancer patients than in controls. A study conducted in India showed blood lead levels were significantly higher in prostate cancer patients than in controls [27]. Genetic differences may cause different results in studies. Copper is an essential heavy material. The most commonly reported adverse health effects of excess oral copper intake are gastrointestinal distress. Nausea, vomiting, and abdominal pain have been reported shortly after drinking solutions of copper sulfate or beverages stored in containers that readily release copper. Ingestion of large amounts of copper salts, most frequently copper sulfate, may produce hepatic necrosis and death. Increased serum and tumor tissue levels of copper are also observed in several cancers, although little is known about how the metal might promote disease progression at the molecular level [11]. Nayak reported that serum copper levels were increased significantly in cancer patients compared with controls [28]. Similar results have been reported by Ozmen who observed a significant increase in serum copper levels in prostate cancer patients compared with controls [29]. The present study revealed that serum copper levels in prostate cancer patients are significantly higher than controls ($P>0.05$), which is concordant with previous data. However, a study conducted by Siddiqui reported a significant decrease in blood levels of copper in prostate cancer patients when compared with controls [27].The difference in copper determination methods may be the reason for these conflicting results.

CONCLUSION

This is the first published report on determining serum concentrations of zinc, lead, cadmium, and copper in prostate cancer patients and control groups in Iran. Results of this study indicated that blood serum concentrations of copper and cadmium were significantly higher in patients with prostate cancer than in controls, serum concentrations of lead and zinc did not differ significantly in prostate cancer patients and controls, and mean levels of zinc and lead were higher in patients with prostate cancer than in controls (Figure 2). Further research, however, will be required to establish the precise roles of zinc, cadmium, lead, and copper in this important human malignancy.
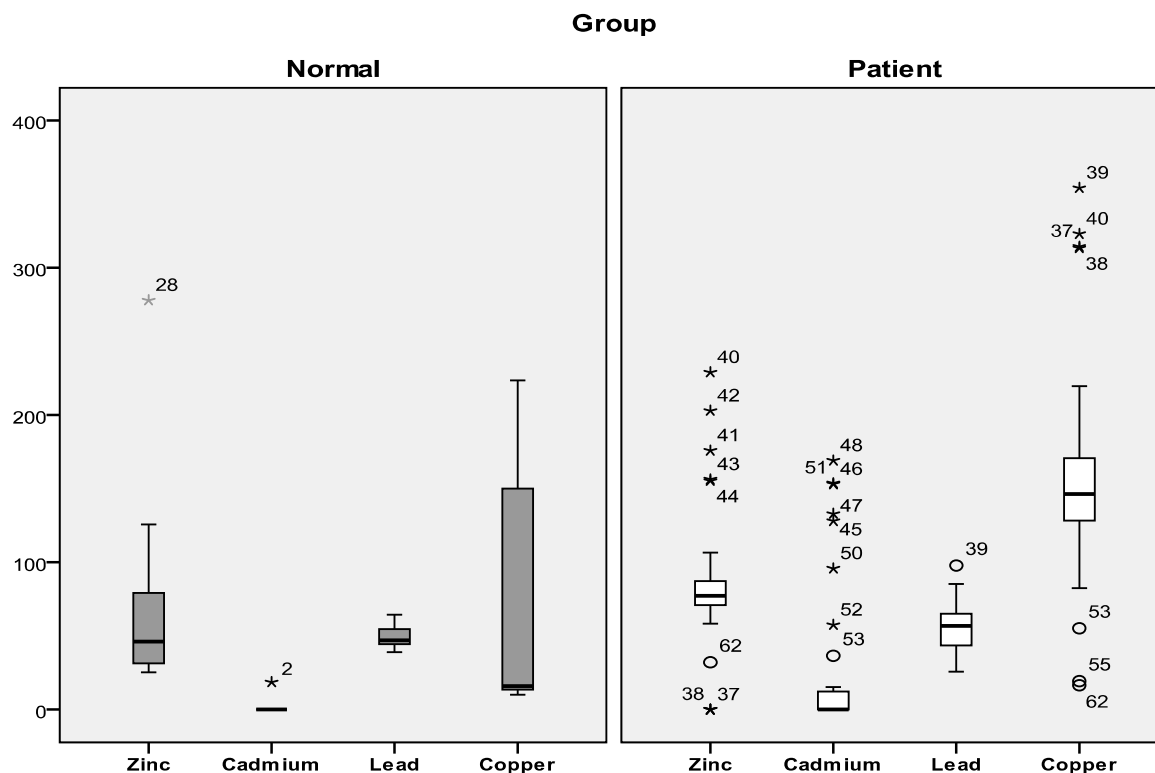

Figure 2 Serum concentrations of Zinc, Copper, Lead and Cadmium (µg/L) in prostate cancer patients and controls

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