

Evaluation of Trace Elements in Pancreatic Cancer Patients in Iran

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Abstract

Background: Pancreatic cancer is a major worldwide health problem. Little is known about the etiology of pancreatic cancer, which is an important cause of cancer mortality in developed countries. This study evaluates the importance of amounts of trace elements in pancreatic cancer etiology and diagnostics.

Methods: Atomic absorption spectrometry was used to estimate zinc, selenium, copper, cadmium and lead concentrations in 80 patients with pancreatic cancer admitted to various hospitals in Tehran Province over an 18-month period and in 100 control subjects.

Results: There were significantly lower levels ($P < 0.001$) of zinc in patients' sera ($63.12 \pm 26.45 \mu\text{g/dl}$) compared with controls ($107.05 \pm 30.23 \mu\text{g/dl}$). The mean concentration of cadmium in patients ($3.10 \pm 1.05 \mu\text{g/l}$) was higher than in healthy subjects ($1.52 \pm 0.88 \mu\text{g/l}$; $P < 0.0001$). In addition, there were significant variations in blood cadmium concentrations due to tobacco smoking in both groups ($P < 0.001$). No significant differences in levels of selenium, copper and lead were observed between the two groups ($P > 0.05$).

Conclusion: In this study and by analyzing data from recent major reported series, we have found that cadmium is a plausible pancreatic carcinogen. This study also suggests a significant relationship between zinc metabolism and pancreatic cancer.

Keywords: Pancreatic cancer, Trace elements, Atomic absorption spectrometry, Iran

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Introduction

Cancer of the pancreas is an important cause of cancer mortality in developed countries and accounts for more than 28000 deaths in the United States per year. It produces few specific symptoms in its early stages and is usually detected at an

advanced and incurable stage.^{1,2} All types of pancreatic cancer (PC) begin when abnormal cells grow out of control within the pancreas. Exocrine tumors comprise 95% of all PC because they begin in the exocrine cells that produce enzymes to aid in digestion. Accounting for less than

5% of all pancreatic tumors are endocrine tumors, also called neuroendocrine or islet cell tumors.

Pancreatic cancer usually occurs in the sixth, seventh and eighth decades of life and rarely in adults less than 40 years of age.^{3,4} In Western countries, the incidence of PC peaks between 60-80 years of age.⁵ This cancer is still the disease of elderly people in Iran, but patients are comparatively younger at the time of diagnosis than in Western countries. This may suggest that some genetic and/or environmental factors could contribute at least in part to the development of PC in Iran. In many areas of Iran, particularly in rural regions, consanguineous marriage is still common. This may cause aggregation of genetic defects that lead to PC.

In addition, in Iran where there is rapid industrial development, the amount of toxic metals that accumulate in the human body from a polluted environment is expected to increase.

Trace elements play an important role in human health and disease. It has become evident that there is an intimate relationship between trace elements and cancer. Some trace elements are known carcinogens; others appear to provide protection against cancer. The immune system (immunoglobulins, cell mediated immunity, phagocytosis complement, lysosomes, interferon, metabolic function, hormones, metabolic and respiratory alkalosis) is the natural mechanism which defends against cancer. Trace elements zinc (Zn), selenium (Se) and copper (Cu) augment this natural mechanism. They are important cofactors for several enzymes that play a role in maintaining DNA integrity. The high levels of cadmium (Cd) and lead (Pb) may be linked with a number of physiological disorders in humans. There is a growing body of scientific research that suggests Cd and Pb contribute to carcinogenesis by increasing oxidative stress.⁶ Oxidative stress damages DNA and can lead to mutations which promote cancer. These heavy metals also disrupt the process of apoptosis (programmed cell death).⁷ Apoptosis is vital for safe removal of sick/unhealthy cells, including those that may become cancerous.

Most Cd in the body is bound to metallothioneins, low molecular weight proteins that function in the homeostasis of essential metals, such as Zn.^{8,9} The Cd-metallothionein complex is distributed to various tissues and organs and is ultimately reabsorbed in kidney tubuli.¹⁰ Because the body has no mechanism for the excretion of Cd, it accumulates in tissues. In humans, the largest amount of Cd is deposited in the kidneys and liver, followed by the pancreas and lungs.

The toxicity of Pb results from its avidity for the sulfhydryl group of proteins and various enzymes, which leads to jeopardy of their function. Lead binds hemoglobin in red blood cells and slowly accumulates in the soft tissues and skeleton.

The advent of atomic absorption spectrometry (AAS) techniques has provided more accurate determinations of low levels of trace elements in human body fluids. In this study, we have assessed serum Zn, Se, Cu, Cd and Pb levels in PC patients compared to subjects from the same region in Iran, using AAS. We have also examined the contributions of age, sex and smoking status to overall risk for PC. Although the sample size in this study is small, it is our hope that these initial data will act as a springboard for larger, more in-depth studies that will analyze the relationship between these elements and PC in a more detailed fashion.

Materials and Methods

Patients and controls

Between February 2010 and August 2011, 80 newly diagnosed patients with adenocarcinoma of the pancreas (exocrine PC) from various hospitals of Tehran Province were recruited to participate in this study. PC patients (group I) consisted of 34 women and 46 men, whose ages ranged from 37 to 80 years. No patients with chronic pancreatitis were included in this study. Group II (controls) were composed of 100 healthy subjects, 45 females and 55 males. The age of these volunteers ranged from 35 to 79 years.

We used an interviewer administered questionnaire, which included questions about age, lifetime occupational and smoking histories.

Table 1. Characteristics of the study population [n (%)].

Characteristics	Group I	Group II	P-value
Age (years)			
<60	37 (46.25)	49 (49)	0.42
≥60	43 (53.75)	51 (51)	
Sex			
Male	46 (57.5)	55 (55)	0.52
Female	34 (42.5)	45 (45)	
Smoking			
Yes	24 (30)	25 (25)	0.40
No	56 (70)	75 (75)	
Occupation			
Housewife	17 (21.25)	28 (28)	0.30
Industrial	30 (37.5)	33 (33)	
Professional	33 (41.25)	39 (39)	

There were no significant differences between the PC patients and the control subjects in terms of age, sex, smoking status or occupation, as shown in Table 1. The donors belonged to a middle socio-economic status with urban dietary habits. Information was also collected about family history of PC. The purposes of the study had been previously explained to all volunteers. A written informed consent was obtained from the participants in this study. The present Study is approved by IRB (Institutional Review Board).

To avoid effects of concurrent infections on Zn, Se, Cu, Cd and Pb concentrations, individuals who had an infection as recently as one month before the study were excluded.

Sample preparation and analytical methods

Blood samples were collected in the early morning, into plastic tubes that contained lithium heparin (Vacurette, Geiner Labortechnik, Kremsmünster, Austria). A portion of the blood was used for the measurement of Cd and Pb concentrations; the remainder was centrifuged at 3000 g at room temperature to determine serum Zn, Se and Cu levels. The samples were stored at 20 °C until trace element analysis.¹¹

Special care was taken to avoid any contamination with metals during the blood sampling, storage and analysis. All laboratory ware including pipette tips and autosampler cups were cleaned thoroughly with detergent and tap

water, rinsed with distilled water, soaked in dilute nitric acid then rinsed thoroughly with deionized distilled water. All chemicals used were of analytical grade for spectroscopy (Merck, Germany).

A Varian model AA-220 atomic absorption spectrometer, equipped with a deuterium lamp for background correction was used for all experiments. Adapted temperature program, appropriate sample dilution and other analytical features of the method have been described elsewhere.^{12,13}

Statistics

Statistical evaluation was carried out by using the SPSS 11.5 version for Windows. Summary statistics (n, mean, standard deviation) were calculated. Values were statistically compared using one-way analysis of variance (ANOVA) taking into account sex, age and smoking as a grouping variable. All results were expressed as mean±SD and statistical significance was defined as $P < 0.05$.

Results

The results obtained from determination of Zn, Se, Cu, Cd and Pb levels in two groups, according to sex, age and smoking status are given in Table 2.

In this case-control study, we observed a significant difference in both Zn and Cd levels

between the patient ($P<0.001$) and control ($P<0.0001$) groups. Zinc level was 63.12 ± 26.45 $\mu\text{g}/\text{dl}$ in PC patients, which was statistically lower than controls (107.05 ± 30.23 $\mu\text{g}/\text{dl}$). The mean concentration of Cd in patients (3.10 ± 1.05 $\mu\text{g}/\text{l}$) was higher than in healthy subjects (1.52 ± 0.88 $\mu\text{g}/\text{l}$). There was a nonsignificant difference in Se, Cu and Pb levels observed between both groups.

Taking into consideration the sex of the subjects, we observed a significant decrease in serum Zn levels in female PC patients compared to males ($P<0.01$). In addition, when the subjects were divided into two age groups, there appeared to be a significant increase ($P<0.05$) in blood Cd levels of patients older than 60 years.

As shown in Table 2, there were significant variations in blood Cd concentrations due to tobacco smoking in patients and healthy subjects. Among all subjects, we observed a general tendency for blood Cd to increase significantly with tobacco consumption ($P<0.001$). In addition, the Pb concentration in the control group increased with smoking ($P<0.0001$).

Discussion

Worldwide, there are more than 10 million new cancer cases each year and cancer is the cause of approximately 12% of all deaths.¹⁴ A large number of epidemiologic studies have been undertaken to identify potential risk factors for cancer, amongst which the association with trace elements has received considerable attention.

In this study, we compared the levels of each of the trace elements noted above between PC patients and healthy controls. There was a significant increase ($P<0.0001$) in blood Cd in PC patients compared with controls. Our findings agreed with earlier studies that suggested significantly higher Cd levels in cases compared to controls.¹⁵⁻¹⁷ The strongest suspicion of an association between Cd exposure and PC has been reported in Louisiana.^{18,19} Whereas, Zn levels were found to be statistically lower in patients compared to controls. This result agreed with results reported elsewhere.²⁰⁻²²

The pancreas is a secretory tissue with unusual

Zn requirements. Thus it must tightly regulate Zn metabolism through the integration of Zn import, sequestration and export mechanisms. Recent findings indicate that this tissue utilizes Zn for basic cellular processes but also requires Zn for unique cellular needs. In addition, abundant Zn is transported into the secretory pathway and a large amount is subsequently secreted in a tightly regulated manner for unique biological processes. There is a growing body of information implicating Zn dysregulation in the pathogenesis of PC.^{23,24}

Zinc participates in the regulation of cell proliferation in several ways; it is essential to enzyme systems that influence cell division and proliferation. Recent studies have shown that zinc availability is also important for tumor growth and progression because zinc is a critical component for many enzymes, which are involved in hypoxia, angiogenesis, cancer cell proliferation and metastasis of cancer.^{25,26} High Zn concentrations are toxic to cells; therefore, cells have evolved a complex system to maintain the balance of Zn uptake, intracellular storage and efflux.^{27,28} Two solute-linked carrier (SLC) gene families have been identified in Zn transport, SLC30, which encodes for Zn transporter (ZnT) proteins, and SLC39, which encodes for ZIP proteins.²⁸⁻³⁰ They appear to have opposing roles in cellular Zn homeostasis. ZnT transporters reduce intracellular Zn availability by promoting Zn efflux from cells or into intracellular vesicles, whereas ZIP transporters increase intracellular Zn availability by promoting extracellular Zn uptake and vesicular Zn release into the cytoplasm.

It has been observed that ZIP4 was substantially overexpressed in 94% of clinical pancreatic adenocarcinoma specimens compared with surrounding normal tissues. The localization of ZIP4 to the cell membrane of pancreatic β -cells suggests that ZIP4 participates in Zn import into the cells. The increased expression of ZIP4 is strongly associated with the pathology of PC by facilitating increased intracellular Zn accumulation^{23,31} and proliferation of PC cells.²⁴ Thus, decreased Zn concentrations in the serum

Table 2. Concentrations of trace elements according to sex, age and smoking status.

		Se	Cu	Zn	Cd	Pb
		Serum (µg/dl)	Serum (µg/dl)	Serum (µg/dl)	Blood (µg/l)	Blood (µg/l)
Total	Cases	9.25±2.14	87.14±19.57	63.12±26.45*	3.10±1.05**	157.16±51.40
	Controls	10.06±2.59	91.17±20.11	107.05±30.23*	1.52±0.88**	145.21±46.13
Males	Cases	8.02±2.54	86.33±19.10	67.47±25.10***	3.19±0.97	148.33±53.08
	Controls	8.97±2.68	90.78±20.14	109.11±29.33	1.50±0.89	146.82±46.09
Females	Cases	10.91±2.43	88.24±19.73	57.23±29.41***	2.98±1.10	169.11±50.31
	Controls	11.39±2.60	91.65±19.97	104.53±32.08	1.54±0.87	143.24±46.20
Age						
<60 years	Cases	9.10±1.99	89.05±19.44	63.97±25.88	2.51±1.08****	160.55±49.97
	Controls	9.92±2.15	90.54±21.05	109.21±29.78	1.54±0.85	144.80±46.18
≥60 years	Cases	9.38±2.27	85.50±19.67	62.39±28.12	3.61±0.97****	154.24±52.10
	Controls	10.19±2.29	91.78±19.89	104.97±31.56	1.50±0.90	145.60±46.11
Smoking	Cases	9.15±1.89	86.63±19.33	55.22±27.13	4.06±1.08*	167.36±54.38
	Controls	9.97±2.44	92.88±20.57	98.23±30.12	2.11±0.93*	166.24±45.14**
Non-smoking	Cases	9.29±2.03	87.36±20.02	66.51±26.25	2.69±1.04*	152.79±50.07
	Controls	10.09±2.50	90.60±19.94	109.99±30.25	1.32±0.87*	138.2±46.23**

Values represent the mean±SD for patients (n=80) and controls (n=100). Asterisks denote the significance of differences between groups (* $P<0.001$; ** $P<0.0001$; *** $P<0.01$; **** $P<0.05$).

of PC patients can be linked to increased expression of ZIP4.

Currently, there is a marginal increase in exposure to Cd in daily life. Cadmium is a known human carcinogen. This toxic element accumulates in the body over time because there are no specific mechanisms for its removal. The half-life of this metal in the body ranges from 10 to 30 years, with an average of 15 years.³²

Cadmium can induce the activation of several oncogenic and tumor suppressor proteins known to be overexpressed in human PC, such as ras proteins and the p53 protein.³³⁻³⁵ Cadmium also induces expression of the c-fos oncogene,³⁶ which is increased in many PCs and inhibits the function of the p53 tumor suppressor protein.^{37,38} Finally, Cd can enhance the initiation of carcinogenesis induced by other carcinogens, such as dimethyl-nitrosamine and hepatitis B, and inhibit DNA repair.³⁹⁻⁴² Cadmium is one of the most potent agents known to induce transdifferentiation of the pancreas.⁴³ Transdifferentiation or metaplasia is a change from one differentiated cell type to another. Because the process of metaplasia involves cellular dedifferentiation, proliferation, and ultimately redifferentiation, agents that induce metaplasia (such as Cd) may place cells at

increased risk for neoplasia.^{44,45}

In summary, Cd can cause the transdifferentiation of pancreatic cells, increase the synthesis of pancreatic DNA and regulate the expression of oncogenes that are implicated in pancreatic carcinogenesis. Thus, Cd is a plausible pancreatic carcinogen.

Cigarette smoking is a significant source of Cd. One cigarette contains 1–2 µg of Cd⁴⁶ and inhaled Cd is absorbed much more efficiently than ingested Cd.⁴⁷ Measurement of Cd in the blood of the PC and control groups has shown significantly higher levels in smokers than non-smokers.

An insignificant difference in Se, Cu and Pb levels was observed between the two groups in the current study, although some of findings have shown decreased bodily levels of Se¹⁷ or increased Pb and Cu concentrations in PC patients compared to controls.^{17,20} According to the study published in the Gut journal 17, people whose diets include high amounts of the mineral selenium may have a lower risk of pancreatic cancer.

While our findings need to be replicated in independent studies, they suggest the role of trace elements in PC pathogenesis and justify further research.

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