Assessment of Concentration of Lead, Cadmium, Chromium and Selenium in Blood Serum of Cancer and Diabetic Patients of Pakistan

MUHAMMAD SALMAN, RABIA REHMAN*, TARIQ MAHMUD, WAHEED-UZ-ZAMAN, UMER SHAFIQUE, JAMIL ANWAR AND SANA ZULFIQAR ALI
Institute of Chemistry, University of the Punjab, Lahore-54590, Pakistan.
grinorganic@yahoo.com*

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Summary: Heavy metals have specific role in the progression of diseases. The aim of this study was to investigate the level of toxic elements, i.e. lead, cadmium, chromium and selenium in blood serum of type 1 diabetes mellitus and cancer patients. Samples were collected from Institute of Nuclear Medicine and Oncology, Lahore (INMOL) Hospital and Jinnah Hospital, Lahore. They were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). Concentrations of toxic metals, i.e. lead, cadmium and chromium have been found relatively high in both cancer and diabetic patients where as concentration of selenium was relatively lower than the normal subjects.
***Keywords*:** Toxic elements, antioxidant, diabetes mellitus, cancer.

Introduction

The importance of trace elements regarding human health has been gradually more acknowledged. Trace elements [1-3] have dietary benefits as essential cofactors for physiological processes, but some are toxic to human health. A large number of evidences recommended that deficiency or excess of certain trace elements may be associated with danger of chronic diseases including diabetes and cancer [4].

Blood is one of the broadly used specimens for biological trace metal research because of its natural importance and ease of sampling. It provides the medium for transport of trace metals and supplies direct evidence of metabolism about the trace metal concentrations. Therefore, whole blood, plasma and serum are usually considered suitable samples for the determination of trace metal status of a person. A large number of case studies have been carried out by different scientists to assess values of trace metals and their composition in the body fluids. Because any alterations in the environment or in the human body can initiate changes in trace metal composition in the body [5].

Cancer was mainly considered to be genetically linked. However, it is now well recognized that diet has a significant effect on cancer incidences [6]. In fact, food consumption patterns could provide major insights into cancer risk and prevention despite the fact that their significance is not fully appreciated [7]. Clinical studies showed that a lot of factors leading to the development of tumor in humans have arisen from environmental factors and 65–70% of all cancers in humans are associated with the environment, including the work environment, 30–40% with nutritional habits and only 2% with consequences of genetic predispositions [8]. Cancer is a multi-factorial and complex disease. The role of trace metals in the development and inhibition of cancer has a complicated character. Several studies have focused on the relationship between trace metals and cancer in humans [9, 10].

The high levels of carcinogenic metals may be linked with a number of physiological disorders in humans. It has been reported that cadmium is a mutagen in mammalian and enhanced concentration of cadmium may result in prostate, renal and lung cancers [11–13]. Similarly, higher lead contents in dietary intake were related with the cancers of stomach, small intestine, large intestine, ovary, kidney, lung, myeloma, all lymphorms and all types of leukemia [14, 15]. Some studies on animals linked higher chromium concentration with accelerated tumor growth were also done [16, 17]. Considerable correlations have been reported between blood chromium and mortalities from cancers of breast, colon, rectum, ovary, lung, pancreas, leukemia and bladder [18, 19].

Diabetes mellitus is a disease characterized by disorder in metabolism and abnormally high blood sugar (hyperglycaemia) resulting from low levels of insulin with or without irregular resistance to the action of insulin. There are two major types of diabetes, called type 1 and type 2. In type 1 diabetes, the pancreas undergoes an autoimmune attack by the body itself, and is rendered incapable of making

*To whom all correspondence should be addressed.

insulin. In type 2 diabetes, patients can still produce insulin, but do so relatively inadequately for their body's needs. The characteristic symptoms are excessive urine productions (polyuria), unnecessary thirst and increased fluid intake (polydipsia), fuzzy vision, unexplained weight loss and exhaustion. These symptoms are likely to be absent if the blood sugar is mildly elevated. Increasing rates of type 2 diabetes in the developed and developing countries suggest that environmental factors may be involved in the etiology of type 2 diabetes mellitus [20].

People with type 2 diabetes have greater excretion of chromium and lower tissue levels of chromium than non-diabetic controls. In addition, chromium is difficult to convert into usable forms in people with diabetes. Supplemental chromium given to type 2 diabetic patients has yielded positive effects on blood glucose, insulin, lipid, and glycated haemoglobin levels [21, 22].

There are reports linking human exposure to cadmium with pancreatic cancer and because of the association between pancreatic cancer and type 2 diabetes, it is possible that type 2 diabetes is also associated with cadmium exposure [23]. The effects of lead poisoning in diabetes subjects have been recognized. Epidemiologic studies, mortality studies and experimental studies in animals have reported lead toxic effects at high levels of exposure and it is possible that high blood concentration of lead may contribute progression of diabetic complications in diabetic patients [24]. Selenium, an essential trace element, is involved in the complex system of defense against oxidative stress through selenium-dependent glutathione peroxidases and other selenoproteins [25]. Because of its antioxidant properties, selenium might thus prevent the development of complications in diabetic patients. The role of selenium in the regulation of free radical production has been documented. Its role in preventing glucose intolerance and the complications of diabetes mellitus has also been postulated [26]. For instance, insulin reserves are decreased with selenium deficiency causing glucose intolerance. We therefore, hypothesized that elevation of toxic metals such as cadmium, lead and deficiency of antioxidant trace element such as selenium may contribute to the origination and development of diabetes mellitus.

Main sources of metal contamination include urban industrial aerosols, solid wastes from mining activities, industrial and agricultural chemicals. Heavy metals (lead, cadmium, chromium and selenium) also enter the water table from industrial and consumer water or even from acid rain which breaks down soils and rocks, releasing heavy metals into streams, lakes and ground water. Metals are mobilized and carried into food web as a result of leaching from waste dumps, polluted soils and water. The metals increase in concentration at every level of food chain and are passed onto the next higher level. In this way, they start accumulating in the bodies of animals in larger concentration, i.e., bio-magnification [27].

The study was designed to evaluate the levels of toxic metals (cadmium, lead and chromium) and antioxidant element (selenium) in cancer and diabetic patients for establishing possible correlation between these elements and the diseases.

Results and Discussion

The average values of metal concentrations in blood serum of a normal person(i.e. controlled samples) are mentioned in Table-1 [27-29].The metal concentrations in blood serum of cancer patients (37 samples) and type 1 diabetic patients (29 samples) were analyzed and results are presented in Table-2 for cancer patients and Table-3 for diabetic patients.

Table-1: Concentration of selected metals in blood serum of normal person.

Selenium	Chromium	Lead	Cadmium
79.3 ppb	0.130 ppb	32.5 ppb	0.98 ppb

Fig. 1 is showing the comparative concentration of metals (selenium, chromium, lead, and cadmium) in blood serum of different cancer patients and Fig. 2 is showing the comparative concentration of metals (selenium, chromium, lead, and cadmium) in blood serum of different diabetic patients.

Table-2 and Table-3 revealed that in blood serum, the average value of selenium (a major antioxidant for human body) level found (64.243ppb for cancer patients and 25.753ppb for diabetic patients) was relatively lower than the controlled samples, i.e. 79.3 ppb.

In cancer patients, more than 15 samples have selenium level less than 80 ppb. Average values of other trace metals in cancer patients are 4.4088 ppb for chromium, 17.003 ppb for lead and 0.268 ppb for cadmium. Two exceptional cases contain very high concentrations for chromium, i.e. 23.93 ppb and 50.75 ppb.

In samples of blood serum of diabetic patients, more than 18 samples contain negligible

selenium level while in rest of the samples; it was relatively less than the selenium level for normal persons. The average value was 25.753 ppb. cadmium content in blood serum was also found zero in about all the cases for diabetic patients except one, which contain 2.919 ppb. The average values for chromium level was 2.564 ppb, for lead level was 24.898 ppb and for cadmium level was 0.100655 ppb (Table-3).

A. FOR CANCER PATIENTS

Table-2: Analysis data for the blood serum samples.

Sample No.	Concentration (ppb)			
	Selenium	Chromium	Lead	Cadmium
CS-1	0	2.156	34.23	0
CS-2	107.5	2.655	21.88	0.228
CS-3	161.7	1.175	19.05	1.020
CS-4	0	0.332	7.174	0
CS-5	0	3.544	9.558	0
CS-6	0	0.983	14.20	0
CS-7	0	3.24	32.66	0
CS-8	2.923	0.399	10.82	0
CS-9	0	1.341	14.68	0
CS-10	128.6	0.118	17.81	0.446
CS-11	0	3.020	20.37	0
CS-12	0	0	14.44	0
CS-13	0	2.751	33.47	0
CS-14	120.7	0.437	11.37	0.297
CS-15	0	0.150	8.941	0
CS-16	5.400	0.233	12.61	0
CS-17	64.84	4.727	7.92	0.634
CS-18	9.345	2.818	16.46	0.549
CS-19	77.45	0.545	19.234	0
CS-20	0	0.119	21.96	0.247
CS-21	5.929	2.464	12.46	0
CS-22	130.65	3.929	14.96	0.439
CS-23	75.71	6.457	13.64	0.056
CS-24	37.87	4.628	11.56	0.978
CS-25	120.29	5.42	20.44	0.561
CS-26	138.0	4.338	19.16	0.401
CS-27	142.8	3.429	15.37	0.612
CS-28	32.98	1.919	18.36	0
CS-29	39.42	3.521	17.42	0
CS-30	107.8	50.75	22.59	0
CS-31	120.6	8.267	20.03	0
CS-32	140.0	23.93	17.47	0.753
CS-33	117.2	7.844	15.63	0.428
CS-34	138.6	1.811	15.83	0.752
CS-35	138.7	1.083	16.39	0.657
CS-36	105.2	1.787	14.22	0.495
CS-37	106.8	0.781	14.74	0.353
Average Values (a)	64.243	4.408	17.003	0.268
Standard Deviation±	59.551	8.842	6.315	0.317
T-test	-1.538	2.943	-14.928	-13.680
P-Value	0.133	0.006	0.000	0.000

Statistical analysis of the data in Table-2 and 3 using student's *t*-test ($p < 0.05$) depicted that there was a significant difference between the levels of selenium, chromium, lead, and cadmium in the blood serum of cancer and diabetic patients as compare to controlled samples having normal levels of these metals in body.

Both diet and environment have strong influence on the distribution pattern of trace elements. Eating habits of individuals and living conditions are mostly controlling factors in this regard. The selenium level found in both cancer and diabetic patients revealed that the antioxidant (Se) has destroyed to a large extent in both diseases.

B. *FOR DIABETIC PATIENTS*

Table-3: Analysis data for the blood serum samples.

Sample No.	Concentration (ppb)			
	Selenium	Chromium	Lead	Cadmium
SS-1	28.52	1.019	15.81	0
SS-2	152.2	2.377	20.28	0
SS-3	57.88	1.147	11.67	0
SS-4	17.51	0.922	22.35	0
SS-5	98.76	0.282	9.886	0
SS-6	21.87	1.471	22.60	0
SS-7	124.3	4.180	17.90	0
SS-8	125.1	2.719	33.61	0
SS-9	120.7	0.437	11.73	0
SS-10	0	1.294	14.38	0
SS-11	0	3.142	28.82	0
SS-12	0	2.998	38.62	0
SS-13	0	3.497	44.10	0
SS-14	0	3.802	40.78	0
SS-15	0	2.479	24.39	0
SS-16	0	3.4	27.42	0
SS-17	0	3.633	36.95	0
SS-18	0	2.757	23.27	0
SS-19	0	4.505	49.14	0
SS-20	0	2.571	3.701	0
SS-21	0	6.141	53.63	0
SS-22	0	1.567	16.38	0
SS-23	0	1.547	11.84	0
SS-24	0	3.463	25.87	0
SS-25	0	2.233	27.01	0
SS-26	0	2.851	25.91	0
SS-27	0	1.305	14.14	0
SS-28	0	4.875	14.97	2.919
SS-29	0	1.739	34.87	0
Average Value.(b)	25.753	2.5638	24.897	0.101
Standard Deviation±	47.922	1.392	12.435	0.542
T-test	-6.017	9.411	-3.292	-8.736
P-Value	0.000	0.000	0.003	0.000

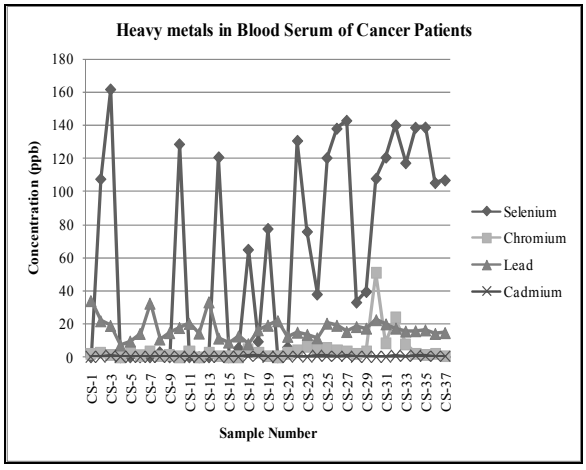

Fig 1: Comparative concentration of heavy metals in blood serum of cancer patients.

Experimental

Reagents Required

All reagents used were of AnalR grade and

were used as such. Anti-coagulant i.e. EDTA solution and GFS multi-element standard were used. De-ionized water was used for dilution and standard solution preparations.

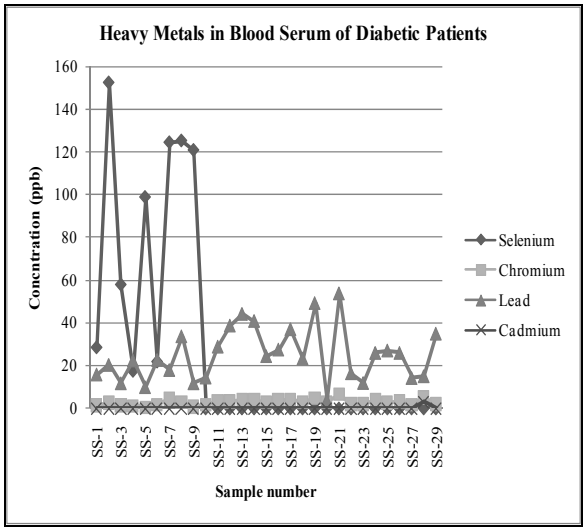

Fig. 2: Comparative concentration of heavy metals in blood serum of diabetic patients.

Instrument

Inductively Coupled Plasma Optical Emission Spectrometer Perkin Elmer Optima (ICP-OES) 2100 DV was used for determination of concentration of metal ions. Centrifuging machine (K2042, Centurion Scientific Ltd.) was used for separating blood serum.

Sampling Procedure

The samples of blood of cancer patients were collected from general ward and leukemia ward of Institute of Nuclear Medicine and Oncology, Lahore (INMOL) Hospital (Pakistan). A few outdoor patients from the same hospital were also selected (1-11-2009 to 14-11-2009). The samples of total 37 patients (male/female) were collected including patients with throat, blood, and breast cancer. Samples of blood of diabetic patients were collected from Medical unit-3 of Jinnah Hospital (Lahore, Pakistan) from outdoor diabetic patients also (from 16-11-2009 to 23-11-2009). Twenty nine samples were collected. Samples of blood from fifteen normal people having no disease were also collected from Institute of Chemistry, University of the Punjab, Lahore, during same days. The samples of blood from each subject was collected in voiles containing anti-coagulant i.e. EDTA solution.

Sample Preparation of Blood Serum

Same procedure was adopted for preparing blood serum of normal persons and patients. Blood samples were prepared by separating the serum from blood cells. Serum was separated in a centrifuging machine, after centrifuging at 4000 rpm for 5 minutes. After centrifuging the serum was separated in the upper layer while cells were separated in lower layer. Thus 1 mL of serum was then taken out by syringe and diluted up to 5mL in voiles and preserved in freezer. Serum samples were marked as CS-1 to CS-37 for cancer patients and SS-1 to SS-29 for diabetic patients.

Preparation of Standard Solutions

Standard solutions of 50, 100, 150, 200 and 250 ppb were prepared for calibration by diluting the GFS multi-element standard (100 mg/L).

Analysis of Sample Solution

The solution was shaken well to get homogeneity of concentration throughout the solution to perform analysis. ICP-OES was turned on and calibrated for selenium (196.026 nm), lead (220.352 nm), chromium (267.716 nm), and cadmium (228.802 nm). Blank solution was used to set the zero emission. Emissions of all the standards were noted and plotted against the concentrations of standard in order to have the calibration line. The emissions of all the samples (blood samples of cancer, diabetic and normal persons) were also noted and the concentrations were found from the respective calibration line for each element.

Statistical Analysis

Student 't' test was applied for data analysis using SPSS 12 software. 95% confidence interval was applied. P value of <0.05 was considered to be statistically significant.

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