INVESTIGATION THE AMOUNT OF COPPER, LEAD, ZINC AND CADMIUM LEVELS IN SERUM OF IRANIAN MULTIPLE SCLEROSIS PATIENTS

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ABSTRACT

Multiple sclerosis (MS) is the most common disease caused by an inflammatory demyelinating process in the central nervous system and characterized by the disseminated demyelination of nerve fibers in the brain and spinal cord. MS is a leading cause of disability in young adults and is accompanied by considerable socioeconomic consequences. It is believed that MS is triggered by as-yet-unidentified environmental factor(s) in a person who is genetically predisposed to respond. It seems that environmental exposure to heavy metals maybe associated with a higher incidence of multiple sclerosis. In this work, a possible relationship between serum zinc, cadmium, lead, and copper levels and the development of multiple sclerosis was found. The serum levels of zinc, cadmium, lead, and copper were measured in 50 Iranian MS patients and 50 healthy persons as the control group. Sample preparation serum concentrations of zinc, cadmium, lead, and copper were determined by polarography. Significantly higher serum Cu, Zn, and Cd levels were found in MS patients than in the controls ($p < 0.05$). There was no significant difference between the serum Pb levels of patients and controls ($p > 0.05$). Serum levels of zinc, cadmium, and copper were significantly higher in MS patients than in the controls ($p < 0.05$).

Keyword: Heavy metal; Nervous disease; Assessment; Neurologic disease; Differential pulse polarography

INTRODUCTION

Multiple sclerosis (MS) is an unpredictable, often disabling disease of the central nervous system characterized by disseminated demyelination of nerve fibers of the brain and spinal cord. It is a leading cause of disability in young adults. Because MS has only a modest negative effect on longevity but the potential for considerable disability over many years, the socioeconomic consequences are considerable. MS is an autoimmune disease of the central nervous system (Melo et al., 2003). Although genetic and immunological factors have been strongly associated with MS pathogenesis, the ultimate etiology of MS remains uncertain (Attar et al., 2012). Apparently, both genetics and environmental factors are important in its pathogenesis (Chisholmborns et al., 2008). Recently, environmental factors such as pathogens and chemicals (toxic elements) have been suggested as playing a significant role in MS pathogenesis. Moreover, exposure to heavy metals has been associated with a higher incidence of the disease (Attar et al., 2012). The use of metal has been critical to the progress of human civilization. It would be difficult to imagine an advanced society without the extensive utilization of metallic compounds (Aliyev et al., 2011). Metals are unique among pollutant toxicants (Attar et al., 2012). Many of them have become essential to various biological processes. Nonetheless, even essential metals will become toxic with increasing exposure (Massadeh et al., 2010). The precise chemical basis of metal toxicology is inadequately understood, but a uniform mechanism for all toxic metals is implausible because of a great variation in their chemical properties and toxic endpoints. Chemically, metals in their ionic form can be very reactive and can interact with biological systems in a large variety of ways (Klaassen, 2007). Blood, urine, and hair provide the most accessible tissue for quantifying metal exposure. Concentration of heavy metals in blood and urine usually reflective of more recent exposure and correlate with acute adverse effects (Wanchu et al., 2002). Cadmium is one of the most toxic heavy

metals and a serious environmental and occupational contaminant which may represent a serious health hazard to man and animals. Cd toxicity in humans and experimental animals has been widely studied, and the reports have shown that Cd is involved in the development of lung, kidney, urinary bladder, and prostate cancers as well as in renal injuries, hepatic and reproductive dysfunction and bone damage (Bulat et al., 2008). There is only limited data from animals and humans indicating that cadmium can be neurotoxic (Klaassen, 2007).

Lead is a very toxic metal, and many people were exposed to it for years (Daftsis and Zachariadis, 2007). It is able to adversely affect hemebiosynthesis and the gastrointestinal, nervous and cardiovascular systems. Lead is naturally present in all the Earth's elements (e.g., plants, rocks, air, soils, water) (Forte et al., 2011).

Zinc has been used since ancient times in alloys and medicines. It is an essential metal to life, and the deficiency of zinc results in severe health consequences. However, zinc toxicity is relatively uncommon and occurs only at very high exposure levels (Zaksas et al., 2010). Zinc has dual effects in the brain. Excess zinc may trigger neuronal death that is independent or synergistic with the toxic effect of β-amyloid. In addition, excess zinc released by oxidants can act as a potent neurotoxin (Klaassen, 2007).

Copper has been used for many centuries. It is an essential element widely distributed in nature (Cabrera et al., 2008). Copper exposure in industry occurs primarily through inhaled particulates in mining or metal fumes in smelting operations, welding, or related activities (Klaassen, 2007). According to studies, copper can be a cause of the pathogenesis of MS (Ghazavi et al., 2012). A relationship between trace metals (e.g. zinc, cadmium, chromium) and the pathogenesis of MS was proposed (Bellantonio et al., 2013). Animal studies have suggested that exposure to lead could aggravate neurological diseases like MS by enhancing the immunogenicity of nervous system proteins (Turabelidze et al., 2008). The current study purposed to assess the relationship between studied heavy metals (Zn, Cd, Pb, and Cu) and multiple sclerosis. 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.

MATERIALS AND METHODS

Reagents and solutions: All solutions were prepared from analytical reagent grade materials in deionized water. Water was purified using 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 50 diagnosed multiple sclerosis patients and 50 controls who were similar in age, gender, race, smoking, and drinking status at the time of blood sampling.

Blood sampling and preparation: Three ml samples of blood were collected from subjects in the morning. Collected blood samples were left at the ambient temperature for 30 minutes to coagulate and then centrifuged at 3000 rpm for 5 minutes. Next, serums were separated by a sampler. 1 ml of serum was added to the digestion vessel and then 7 ml of nitric acid was added. The vessels were put into Microwave Digestion (Microwave Digestion and extraction MDS-10). 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 polarographic determinations of zinc, lead, cadmium, and copper amounts are shown in Table.1.

The polarographic measurements were performed using a Metrohm 797 VA Computrance (Herisau, Switzerland). The three-electrode configuration consisted of a mercury drop electrode (HMDE) hanging from a multi-mode electrode (Metrohm) as the working electrode, an Ag/AgCl reference electrode with a 3 M KCL filling solution, and a platinum wire as the auxiliary electrode. For all measurements, the Differential Pulse (DP) mode was used. Table 2 shows the optimized typical parameters in DPP detection.

Preparation of Standard Solution: One mg L^{-1} mixed standard of zinc, cadmium, lead, and copper was prepared for the Polarographic analysis from stock solution of 1000 mg L^{-1}. Polarogram of the standard solutions are shown in Fig. 1.

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. The mixture was stirred, and deionized water and NaOH solution were added to the vessel. The pH was stabilized at 4.6. Analysis was

carried out in DP mode using polarography and the HMDE method. 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 AND DISCUSSION

A total of 50 cases and 50 controls were recruited. Comparisons of demographic factors showed no significant differences between cases and controls in sex, race, smoking and drinking status, education, and 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 means in the two groups. No differences in mean of age were found between the two groups.

After the equality of demographic factors in both groups was assured, serum levels of zinc, lead, cadmium, and copper were measured. Median blood serum levels of zinc, lead, cadmium, and copper in participants suffering from multiple sclerosis were 72.9($\pm$3.76)µg/L, 34.9($\pm$2.85) µg/L, 0.4($\pm$0.29) µg/L, and 188.2($\pm$8.24) µg/L, respectively. Median blood serum levels of zinc, lead, cadmium, and copper in the control group were 45.8($\pm$4.28) µg/L, 31.3($\pm$5.17) µg/L, 0.000 µg/L, and 103.1($\pm$4.45) µg/L, respectively (Table 2).

To investigate the distribution of the serum concentration of each metal, the Kolmogorov-Smirnov test was used. The results showed that lead and copper had a normal distribution ($p > 0.05$).To compare levels of lead and copper 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 multiple sclerosis than in controls. Serum levels of copper were significantly higher in patients with multiple sclerosis than in the controls ($p < 0.05$).The Mann-Whitney test was used to compare serum zinc and cadmium concentrations in cases and controls, and the values were found to be significantly higher in patients with multiple sclerosis than in the controls ($p < 0.005$).

DISCUSSION

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, and its distribution varies across the world. Epidemiological studies implicated two broad categories of influence: genetics and environmental factors.Traditional epidemiological studies have also been used to examine the influence of environment on MS risk. Heavy metals are related to many health problems. A relationship between trace metals (e.g., zinc, cadmium, chromium) and the pathogenesis of MS has been proposed.In our study in Zanjan, Iran, the serum levels of zinc (Zn), cadmium (Cd), lead (Pb), and copper (Cu) were measured in 50 Iranian patients with MS as the case group and 50 healthy persons as the control group. Participants were matched in sex and age by polarography.

A few studies have investigated the effects of heavy metals on MS, and the protecting role of zinc against MS has been identified in previous studies. However, some other studies have shown that elevated levels of zinc may cause this disease.A 1982 study conducted by Mr. Palm showed that zinc concentrations were lower in MS patients' serum than in that of healthy subjects. 50 patients and 50 healthy persons who were similar in age and sex participated in that study. The current study also investigated 50 patients and 50 healthy volunteers who were similar to each other in age and gender. Differences in results between the two studies may be due to differences in the participants' nutrition, special genetics of persons in different areas, or differences in the subjects' metabolism. The lower concentration of zinc may also be due to its incomplete absorption (Palm and Hallmans, 1982).

Ghazavi et al. conducted a study in Tehran in 2012 in which the zinc and copper concentrations in the serum of 60 patients with MS and 60 healthy subjects were compared. None of the participants had any other neurological disease, and they were all similar in terms of age, gender, and social class (Ghazavi et al., 2012). The results indicated that zinc levels were lower in the serum of MS patients than in that of healthy individuals, and copper levels were higher in MS patients' serums than in that of healthy subjects. The zinc and copper levels in MS patients were higher than those in healthy individuals in a study we conducted in Zanjan, Iran. Our results concerning zinc differed from those of Mr. Ghazavi, possibly because of differences in the nutrition, genetics, or metabolism of the study participants. The accuracy of the study may have been increased by patients and healthy subjects being similar in terms of social class and selected from the same region, because people in the same area live under similar climatic conditions and may have the same type of nutrition. Moreover, people are genetically more similar to those who live in the same area than to those in other areas. A study conducted by Palm in 1982

concluded that copper concentrations were lower in MS patients than in healthy individuals. Like the current study, 50 patients and 50 healthy volunteers who were similar in terms of age and gender participated in Palm study (Palm and Hallmans, 1982). The difference in results may be due to differences in nutrition, genetics, metabolism, and weather conditions. Incomplete absorption of metals may also be a reason for low zinc concentrations. In 1983, a study was conducted by Dore-Duffy et al. where zinc concentrations in the plasma of patients were compared with those of a control group. The researcher found that zinc plasma levels were higher in patients than in the control group. This result is similar to our study results (Dore-Duffy et al., 1983).

In another study in Norway in 2003 by Melo et al., zinc concentrations in the CSF of MS patients and healthy subjects were investigated, and no significant differences were found (Melo et al., 2003). Contrarily, the current study measured zinc concentrations in its participants' serums and concluded that metal concentrations in the serum and CSF of people are different. Polarography was used in our study in Zanjan. In the current study, however, the two methods of atomic absorption spectrometry and high-resolution inductively coupled plasma-mass spectrometry were used, and the concentration results obtained with both methods are mentioned. Using two methods to measure concentrations increases the accuracy of the study. The average copper concentration in serum measured using atomic absorption spectrometry methods and spectrometry high-resolution inductively coupled plasma-mass were 0.8 ± 11.5 and 1.1 ± 10.9 micrograms per liter, respectively, in MS patients and 0.6 ± 9.10 and 0.5 ± 8.7 mg l, respectively, in healthy subjects.

Lead was also investigated as a metal in this study. A study was conducted in 1976 that compared the concentrations of lead in the blood serum of 22 MS patients and 22 healthy controls, and its results indicated no difference between lead levels (Birmingham Research Unit, 1976). The current study also showed no differences between serum lead levels of MS patients and healthy controls. The number of participants in the current study (50 patients, 50 controls) is higher than that of previous studies, and thus the accuracy of the current study is increased.No previous study has examined the relationship between cadmium and MS. In this study, the first to investigate cadmium levels in the blood of MS patients in Iran, a significant difference between the amount of cadmium in the blood of MS patients and that of healthy subjects was found. The average cadmium concentration in the serum of patients was $0.4(\pm 0.29)$ μg/L, while that of healthy persons was lower than detectable limits. More research in this area is necessary considering the role of cadmium in causing MS. As mentioned before, the differences between the findings of this study and others may be due to genetic differences, differences in nutrition, or differences in metabolism and heavy metals absorption. The amount of heavy metals in different climatic conditions may differ, and this is factors into the different values of these metals in the body.

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

Concentration Max	Determination limit	Element
50 mg/L	1 μg/L	Zn
50 mg/L	0.1 μg/L	Cd
50 mg/L	0.1 μg/L	Pb
50 mg/L	1 μg/L	Cu

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

Table.3.Epidemiologic parameters and concentration of Zinc, Cadmium, Lead and Copper in blood serum (µg/L)

Characteristics	Case		Control	
	Mean(±SD)	median	Mean(±SD)	median
Age (year)	32(±3.35)	33	32(±2.65)	31
Zn (µg/L)	72.9(±3.76)	73.3	45.8(±4.28)	42.7
Pb (µg/L)	34.9(±2.85)	34	31.3(±5.17)	33.3
Cd (µg/L)	0.4(±0.29)	0.4	0.000	0.000
Cu (µg/L)	188.2(±8.24)	185.2	103.1(±4.45)	103.9
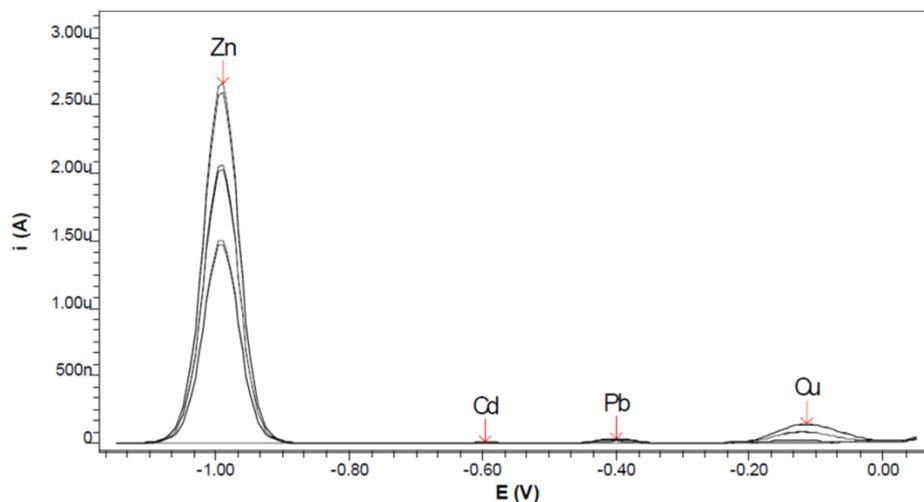
Figure.1.Polarograms obtained for samples as well as for Standard solutions (Zn^{2+}=10 mg/L, Cd^{2+}=0.1 mg/L, Pb^{2+}=0.5 mg/L, Cu^{2+}=2.5 mg/L). Condition: Sweep rate, 0.06 v/s; Pulse amplitude, 0.05 V; Deposition potential, -1.15 V vs. Ag/AgCl; deposition time, 90s; equilibration time, 10s; Scanning range, -1.15 to 10 V
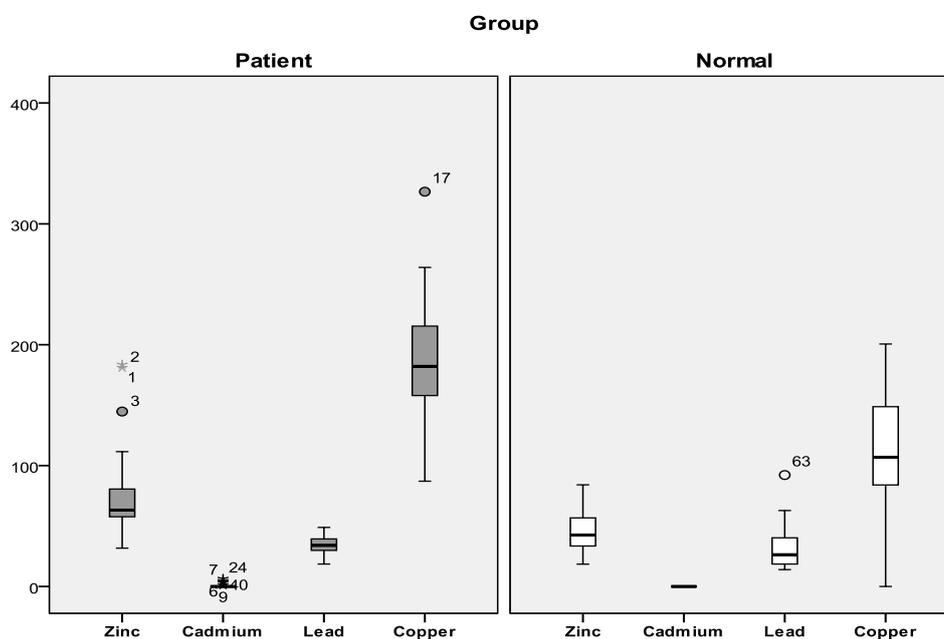

Figure.2.Serum concentrations of Zinc, Copper, Lead and Cadmium (µg/L) in multiple sclerosis patients and controls

CONCLUSION

Few studies have investigated the effects of heavy metals on multiple sclerosis. Results of this study indicated that blood serum concentrations of zinc, cadmium, and copper were significantly higher in patients with multiple sclerosis than in the controls; serum concentrations of lead did not differ significantly in multiple sclerosis patients and controls; however, mean levels of lead were higher in patients with multiple sclerosis than in the

controls. Further research will be required to establish the precise role of zinc, cadmium, lead, and copper in multiple sclerosis.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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