

Trace Elements in Serum Samples of Patients with Multiple Sclerosis

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The aim of this study was to compare trace element concentrations in plasma samples of patients with multiple sclerosis (MS) with healthy controls to ascertain the association of these trace elements with MS. A total of 55 patients with MS 15-70 years old and 90 healthy individuals without MS were investigated. Using inductively coupled plasma atomic emission spectrometry, we determined 24 elements in blood plasma from MS patients and a control group. Compared to subjects without MS, a lower concentration level of calcium (Ca) (58.3 mg/L vs. 70.4 mg/L, $P<0.001$), potassium (K) (457.7 $\mu\text{g/L}$ vs. 546.9 $\mu\text{g/L}$, $P<0.001$), sulphur (S) (250.1 mg/L vs. 273.5 mg/L, $P<0.001$), sodium (Na) (2644.9 $\mu\text{g/L}$ vs. 2783.2 $\mu\text{g/L}$, $P<0.001$), phosphor (P) (271.3 mg/L vs. 300.5 mg/L, $P<0.001$) and zinc (Zn) (235.2 $\mu\text{g/L}$ vs. 288.1 $\mu\text{g/L}$, $P<0.001$) were found in MS patients. Lower concentration levels of Ca, K and Zn have stronger associations with MS. The results on the whole indicate the involvement of Ca, K, S, Na, P and Zn (decreased serum level) in MS that may cause or result from the disease. This warrants further study.

Keywords: Blood, Chemical elements, Multiple sclerosis, Oxidative damage, Trace elements

Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating, immune-mediated and debilitating disease that affects young adults and provokes disability and neurological symptoms (Clanet and Brassat 2000, Richards et al. 2002). The causes and mechanism of this disease are still not completely clear.

Despite the low concentration of trace elements in the body, they played an important role in various metabolic events, the development of the nervous system, myelination of the nerve fibers, and neural excitability (Wallwork and Sandstead 1993). Abnormalities in transition metals and other trace elements in biological matrices levels have been reported in several neurological diseases, including Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis and MS (Rynan et al. 1978, Schulten et al. 1983, Sayre et al. 2000, Bomboi et al. 2005, Forte et al. 2005a, Exley and House 2010, Vinceti et al. 2013, Nicoletti et al. 2011, Valera et al. 2014). Magnesium has long been known as a

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neurotoxic trace element (Nicoletti et al. 2011). Exposure to high concentrations of aluminum in workers may have negative effects on the central nervous system (Iregren et al. 2001). Copper, iron and manganese can act as pro-oxidant agents by catalyzing free radical reactions, leading to severe injuries in brain cells (Kienzl et al. 1999). Copper is needed for the enzyme reactions which are involved in the production of ATP, and for the transmission of impulse in the nerves and the muscles (Smith et al. 1989). Cadmium and mercury have been shown able to disrupt mitochondria of neuronal cells by increasing intracellular reactive oxygen species which directly inhibit neural Janus (Jak) kinase tyrosine kinase activity (Monroe and Halvorsen 2009). Zinc (Zn) is also an antioxidant which protects the cell membranes and myelin.

Information concerning the concentration of metals and trace elements in the blood of MS patients is nonetheless scarce. Whether or not trace elements are of significance in MS is unknown. From a clinical point of view it is important to clarify the role of these elements in association with MS. Our study contributes to this issue by comparing 24 trace element concentrations of patients with MS with healthy controls to ascertain the potential role of these elements as markers for this pathology.

Subjects and Methods

Between March 2010 and March 2011, 55 (8 men and 47 women) MS patients aged 15-70 who sought treatment for MS at our neurology outpatient clinics of Isfahan University of Medical Sciences, Iran, were evaluated. MS was diagnosed according to the McDonald criteria (Polman et al. 2005). Subjects had to be ambulatory with a Kurtzke Expanded Disability Status Scale (EDSS) (Kutzke 1983) of ≤ 5.5 with at least one year of natural course of the disease. The group of MS patients was compared with a control group of 95 (28 men and 67 women) healthy adults without known neurological diseases or deficits. Healthy controls were volunteers employed at the Isfahan University of Medical Sciences. None of the participants in the study have had known occupational exposure to trace elements. Subjects were excluded from the study if they had cardiac, respiratory, renal or hepatic disorders; intestinal absorption abnormalities; active infections; thyroid hormones or lithium therapy; intake of vitamin or mineral supplements; vegetarian dietary habits; consumption of any psychoactive drugs other than MS medication; artificial metallic body parts.

The study was approved by the institutional ethical committee of the Isfahan University of Medical Sciences, Iran and participants gave written, informed consent.

After overnight fasting and drug withdrawal, blood samples were taken from all subjects in accordance with the standard procedures. All samples were stored deep-frozen at -20°C until analysis. Maximum care was taken to avoid trace element contamination of the samples. All the plastic containers were

previously washed in 10% HNO₃ for 24 hrs and then repeatedly rinsed with deionized water. The efficacy of this cleansing procedure was checked for the release of the metals under study.

The concentrations of 24 trace elements were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) using a high-resolution ICP-AES Optima 5300V spectrometer (Perkin-Elmer, Norwalk, CT, USA) equipped with a AS93 plus auto-sampler. The instrument was equipped with a concentric Meinhart nebulizer connected to a Scott PFA spray chamber, platinum skimmer and interface cones and a quartz burner with a guard electrode. The nebulizer argon gas flow rate was adjusted to give a stable signal with a maximum intensity for the nuclides ⁷Li, ¹¹⁵In and ²³⁸U. All chemical analyses were performed at the Isfahan petroleum refinery laboratory.

Statistical Analysis

Since available data on the effects of trace elements on patients with MS are not sufficient for an exact statistical sample size calculation, the study was designed as a pilot study with a priori determined sample size of 50 MS patients in case group and 100 healthy individuals in a healthy control group.

Comparison between the groups was made using Mann-Whitney U tests. Age-adjusted means were calculated and compared using general linear models. Comparisons between proportions were undertaken using the chi-square or Fisher's exact test. Pearson's correlation analysis was performed for investigation of the association between the trace elements. A logistic regression analysis was used for the calculation of individual metal and trace elements effects on MS, adjusting for age and gender. Results are expressed as a mean (standard error [SEM]). All tests for statistical significance were two-tailed, and performed assuming a type I error probability of <0.05. The analyses were undertaken using SPSS for Windows (SPSS Inc., Chicago, IL, USA).

Results

Differences in distribution of characteristics among 55 MS patients and 95 healthy controls are shown in Table 1. MS patients were younger and were less likely to be men than those without MS. The mean (SD) age of MS patients was 31.6 (10.4) years and for persons without MS 45.1 (16.8). The mean (SD) EDSS was 3.0 (1.9) in the MS group, with a mean duration of disease of 5.3 (5.3) years. Twenty-three patients had a relapsing-remitting MS course, 10 patients had primary progressive and in 13 patients the disease showed secondary progression. Most MS patients (54.5%) received interferon beta. Participants also received mitoxantrone (14.5%) or immunosuppressive drugs (30.9%).

Table 1. Characteristics of Multiple Sclerosis (MS) Patients and Control Subjects without MS

Characteristic	MS (N=55)	No MS (N=90)	P-value
	Mean (SEM)	Mean (SEM)	
Age (yr.)	31.6 (2.0)	45.1 (1.6)	<0.001
EDSS	3.0 (0.25)	-	-
Duration of MS (yr.)	5.3 (0.72)	-	-
Gender, N (%)			
Men	8 (14.5)	28 (29.5)	<0.05
Women	47 (85.5)	67 (70.5)	-
Multiple sclerosis subtypes, N (%)			
Relapsing-remitting	23 (50.0)	-	-
Secondary progressive	13 (28.3)	-	-
Primary progressive	10 (21.7)	-	-
Concomitant medications, N (%)			
Interferon beta	30 (54.5)	-	-
Mitoxantrone	8 (14.5)	-	-
Immunosuppressive drug	17 (30.9)	-	-

Notes: SEM = standard error of the mean; EDSS = expanded disability status scale; CI = confidence interval; N = number; *P<0.05, **P<0.05, ***P<0.001.

Table 2 shows the concentrations of the 24 elements in blood plasma. Those with MS had slightly higher concentration levels of silver (Ag), aluminium (Al), magnesium (Mg), antimony (Sb), silicon (Si), chromium (Cr) and copper (Cu). Although the average concentration level of Ag, Al, Mg, Sb, and Cr were significantly greater in MS patients, the differences were small and not clinically relevant. The level of calcium (Ca), iron (Fe), potassium (K), sulphur (S), sodium (Na), phosphor (P) and zinc (Zn) was lower for MS patients. No significant differences were found in the concentration levels of boron (B), barium (Ba), manganese (Mn), molybdenum (Mo), lead (Pb), tin (Sn), nickel (Ni), titanium (Ti) and vanadium (V) between MS patients and healthy controls. The level of plasma Ca, K, Na, S, P and Zn is markedly decreased in MS patients whereas the level of Cu and Si increased. The level of the toxic metal cadmium (Cd) was slightly but significantly lower in MS patients compared to controls.

Table 2. Age-Adjusted Trace Elements Concentrations in Blood Plasma of Patients with Multiple Sclerosis (MS) and Control Subjects without MS

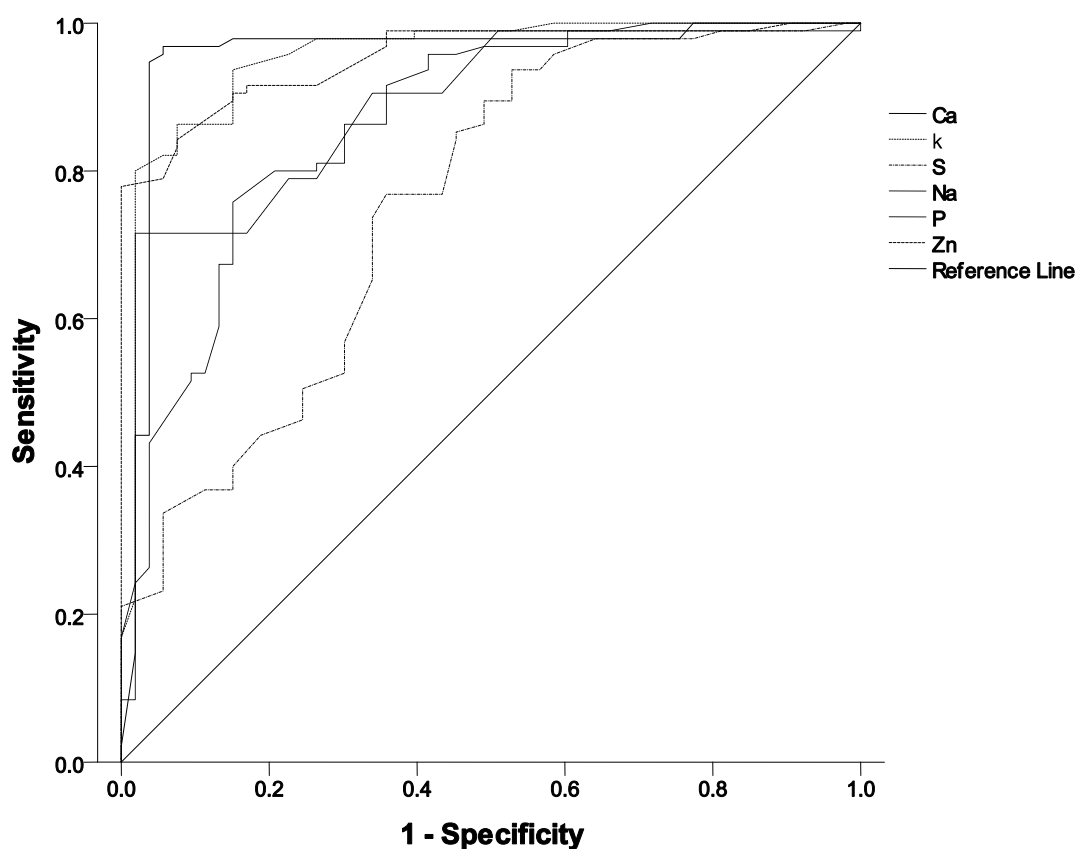
Characteristic	MS (N=55)	No MS (N=90)	P-value
	Mean (SEM)	Mean (SEM)	
Silver (Ag) ($\mu\text{g/L}$)	2.05 (0.04)	1.70 (0.03)	<0.001
Aluminium (Al) ($\mu\text{g/L}$)	3.00 (0.05)	2.75 (0.04)	<0.001
Boron (B) ($\mu\text{g/L}$)	1.15 (0.009)	1.16 (0.007)	NS
Barium (Ba) ($\mu\text{g/L}$)	1.01 (0.011)	1.01 (0.009)	NS
Calcium (Ca) (mg/L)	58.30 (0.55)	70.40 (0.42)	<0.001
Cadmium (Cd) ($\mu\text{g/L}$)	1.64 (0.01)	1.67 (0.01)	<0.01
Chromium (Cr) ($\mu\text{g/L}$)	1.17 (0.02)	1.09 (0.02)	<0.01
Copper (Cu) ($\mu\text{g/L}$)	1.67 (0.03)	1.06 (0.02)	<0.001
Iron (Fe) ($\mu\text{g/L}$)	4.13 (0.03)	4.25 (0.02)	<0.01
Potassium (K) ($\mu\text{g/L}$)	457.67 (4.96)	546.88 (3.78)	<0.001
Magnesium (Mg) (mg/L)	13.47 (0.33)	12.33 (0.25)	<0.1
Manganese (Mn) ($\mu\text{g/L}$)	0.94 (0.009)	0.96 (0.007)	NS
Molybdenum (Mo) ($\mu\text{g/L}$)	0.17 (0.06)	0.08 (0.04)	NS
Sodium (Na) ($\mu\text{g/L}$)	2644.89 (17.66)	2783.16 (13.46)	<0.001
Nickel (Ni) ($\mu\text{g/L}$)	0.016 (0.012)	0.001 (0.009)	NS
Phosphorus (P) (mg/L)	271.31 (2.61)	300.49 (1.97)	<0.001
Lead (Pb) ($\mu\text{g/L}$)	0.96 (0.02)	0.94 (0.01)	NS
Sulfur (S) (mg/L)	250.10 (2.03)	273.5 (1.55)	<0.001
Antimony (Sb) ($\mu\text{g/L}$)	2.43 (0.06)	2.09 (0.04)	<0.001
Silicon (Si) ($\mu\text{g/L}$)	177.81 (2.30)	152.51 (1.75)	<0.001
Tin (Sn) ($\mu\text{g/L}$)	46.75 (0.95)	47.03 (0.72)	NS
Titanium (Ti) ($\mu\text{g/L}$)	28.94 (0.68)	29.42 (0.52)	NS
Vanadium (V) ($\mu\text{g/L}$)	35.66 (0.81)	34.84 (0.62)	NS
Zinc (Zn) ($\mu\text{g/L}$)	235.20 (2.63)	288.11 (2.01)	<0.001

Note: Age-adjusted means were calculated using general linear models, SEM = standard error of the mean; $\mu\text{g/L}$ = micrograms/litre; mg/L = milligrams/litre; NS = non-significant.

To determine the independent predictors of the MS a forward stepwise binary logistic regression was performed to test 8 predictor variables: age, Ca, K, S, Na, P and Zn (included as continuous variables) and gender. The dependent variable was the MS (present, absent). Younger age (OR 0.89 (95% CI: 0.81, 0.98), lower Ca (OR 0.74 (95% CI: 0.62, 0.88) and Zn level (OR 0.89 (95% CI: 0.84, 0.96) significantly increased the risk of MS.

The ROC curves for the MS for Ca, K, S, Na, P and Zn are shown in Figure 1. The areas under the ROC curves were 0.956 (95% CI: 0.912, 0.999) for Ca, 0.954 (95% CI: 0.918, 0.990) for K, 0.954 (95% CI: 0.923, 0.984) for Zn, 0.862 (95% CI: 0.798, 0.926) for Na, 0.894 (95% CI: 0.843, 0.945) for P, and 0.755 (95% CI: 0.673, 0.838) for S. All elements were significant predictors for risk of MS ($P < 0.001$). Zn, K and Ca had a similar area and larger than that of Na, P and S.

Figure 1. Receiver Operating Characteristic Curves for Calcium (Ca), Potassium (K), Zinc (Zn), Phosphor (P), Sodium (Na) and Sulfur (S) for Prediction of MS



Notes: The estimates of the area under the ROC curves and their 95% confidence intervals are shown. Area under the curve (95% confidence interval): Calcium - 0.956 (0.912, 0.999); Potassium - 0.954 (0.918, 0.990); Zinc - 0.954 (0.923, 0.984); Phosphor - 0.894 (0.843, 0.945); Sodium - 0.862 (0.798, 0.926); Sulfur - 0.755 (0.673, 0.838).

These six elements were correlated with each other and the strongest Pearson correlation coefficients were found between Ca and K and the weakest ones were between S and Na (Table 3).

Plasma Ba ($r= 0.385$, $P<0.01$), Ca ($r= 0.354$, $P<0.05$), Sn ($r=0.328$, $P<0.05$), and Cd ($r=0.328$, $P<0.05$) were correlated with the disability of the MS patients as quantified on EDSS. No significant correlation was found between levels of each trace element and disease duration.

Table 3. Correlation Coefficients between Trace Elements

Variables	Ca	K	S	Na	P	Zn
Calcium (Ca) (mg/L)	1	0.729*	0.425*	0.511*	0.600*	0.702*
Potassium (K) ($\mu\text{g/L}$)		1	0.451*	0.400*	0.567*	0.683*
Sulfur (S) (mg/L)			1	0.299*	0.341*	0.551*
Sodium (Na) ($\mu\text{g/L}$)				1	0.406*	0.378*
Phosphor (P) (mg/L)					1	0.558*

Note: * $P<0.001$.

Discussion

The main finding in this study is the decreased level of Ca, K, S, Na, P and Zn and the increased level of Cu and Si in blood plasma from patients with MS. Whether these decreased trace element levels are a cause or effect of the MS it is impossible to state at present, however it seems more plausible that the disturbance in trace element levels in blood plasma may be due to the use of steroids and/or interferon beta in the course of the disease. These decreased trace element levels may also be related to the type of treatment which was prescribed. To the best of our knowledge, this is the first study comparing the 24 trace elements in the plasma of MS patients and compared with healthy volunteers. A limited number of studies have been published on the possible role of trace elements in MS (Melo et al. 2003, Mauch et al. 1995, Valberg et al. 1989, Dore-Duffy et al. 1983, Palavinskas and Schulten 1984, Palm and Hallmans 1982, LeVine et al. 1999, Rieder et al. 1983, Exley et al. 2006, Forte et al. 2005b) and the results are inconsistent. Discrepancies among studies investigating the metals and trace elements in patients with MS may be attributable to small sample sizes that resulted in insufficient statistical power to detect some relationships in the individual studies. Measurements of trace elements will depend upon the methodological factors, the definition of the MS used, and the composition of the control group by age, gender, ethnicity, and uncontrolled confounders, making comparisons between studies of limited values.

Melø et al. (2003) found an increase of Cu and a decrease of Mn levels in cerebrospinal fluid (CSF) in MS patients compared to controls, and no differences were found for Zn. Normal values for Fe, K, Na, Rb, Ca and Mg have been reported in blood or CSF (Palavinskas and Schulten 1984, Palm and Hallmans 1982, LeVine et al. 1999, Rieder et al. 1983, Exley et al. 2006). Recently, Tamburo et al. (2015) found a lower concentration of Al, rubidium and a higher concentration of uranium in scalp hair samples in MS patients compared to controls. We observed lower concentrations of plasma Zn in MS patients. Zn has an important role in the inhibition of potentially destructive immune reactions against the T lymphocytes, and in the predisposing inflammatory responses of MS. It is also an antioxidant which protects the cell membranes and myelin. Previous studies on plasma Zn levels in MS patients were contradictory with decreased (Palm and Hallmans 1982, Wong et al. 1980, Masoud and Fakharian 2007, Weinsmann et al. 1982), normal (Melo et al. 2003, Rieder et al. 1983, Halliwell 1992), and increased (Dore-Duffy et al. 1983) concentration reported. The Serum Zn level could decrease in MS patients who received corticosteroid therapy (Al-Zubadi 2012), while its levels had been reported to increase in the patients who received interferon beta (Sato et al. 1996). In the present study, plasma Cu levels increased in the MS group as compared to the control group, which was agreeable with what had been reported in the other studies (Melo et al. 2003, Wong et al. 1980). The increase in the plasma Cu concentration was found to decrease the Zn absorption. Several studies have proved the existence of competition in the intestinal

absorption and the inverse relationship between plasma Cu and Zn concentration (Al-Zubadi 2012, Sato et al. 1996). This finding contrasts with the finding of other studies (Rieder et al. 1983) which concluded that the blood concentration of Cu was normal in MS patients.

The nervous system and in particular the myelin sheath seems to be more susceptible to oxidative damage than other organs, partly due to its high metabolic activities and rather marginal antioxidant defence systems and a relatively high content of vulnerable fatty constituents (Halliwell 2006). The progressive myelin destruction that characterizes MS may be accelerated by oxidative stress. Zn has an important role in the initiation of potentially destructive immune reactions against the T lymphocytes, and in the predisposing inflammatory responses of MS. It is also an antioxidant which protects the cell membranes and myelin.

In the present study, the MS patients showed a slightly but significantly lower plasma concentration of cadmium, iron, sodium, phosphorus, sulphur and higher plasma concentration of silver, aluminium, chromium, magnesium, antimony and silicon. Are these observations just an epiphenomenon of other pathogenic mechanisms or can they actually contribute to the pathogenesis of MS? We do not have an explanation for this associations and this warrant further study.

Our study has several limitations. First, the number of subjects is relatively small. Secondly, the control group in this study was recruited from employees of the Isfahan University of Medical Sciences and may not represent healthy individuals from the community. In particular, the control group was not matched for age with MS patients, although we adjusted for age. However, after age- and gender-adjustment the differences were not statistically significant in some trace elements. As a case-control study, the present analysis is limited in its ability to elucidate causal relationships between trace element concentrations and MS.

In summary, our study indicates that the decreased levels of Ca, K, S, Na, P and Zn in the plasma of MS patients may indicate an active role of these trace elements in the course of MS. This warrants further study.

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