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The Relationship Between Blood Lead Levels and Clinical Features Among Multiple Sclerosis Patients in Isfahan, Iran

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ABSTRACT

Lead (Pb) is one of the most likely toxicants that could be potentially a risk factor in the development of Multiple Sclerosis (MS) through changes in the immune system. The purpose of the study was to describe the clinical features of MS in general and for sub-groups stratified for gender, age, residence, disease duration, disability degree, clinical diagnosis in Isfahan, Iran and also, to elucidate the relationship between the blood Pb level and the development of MS. Blood samples of 48 patients (20 to 57 years) were selected from the department of neurology of the Kashani hospital in Isfahan, Iran and were analyzed for Pb using anodic stripping voltammetry (ASV). The clinical and demographic characteristics in our study were similar to those in other reports. The blood Pb level in 70.83% of the total population was <20 µg/l. No statistically significant difference was observed between the Pb level and some patient characteristics. These findings did not support the assumption that Pb could be a pathogenetic factor in MS. However, the exposure to a large amount of Pb can increase the risk of developing MS. Obviously; more investigation is needed to investigate the contribution of Pb to the development of MS.

Key words: Multiple sclerosis (MS), Blood, Lead (Pb), Anodic Stripping Voltammetry (ASV), Isfahan

LIST OF ABBREVIATIONS

- Multiple sclerosis (MS)
- Lead (Pb)
- Anodic Stripping Voltammetry (ASV)
- Relapsing–remitting MS (RRMS)
- Progressive–relapsing MS (PRMS)
- Secondary-progressive MS(SPMS)
- Primary-progressive MS (PPMS)
- Clinically Isolated Syndrome (CIS)
- Motor neuron disease (MND)
- Expanded Disability Status Scale (EDSS)
- Electrothermal atomic absorption spectrometry(E. A. A. S)
- Zeeman modulated atomic absorption spectrometry (Z.M.A.A.S.)
- Neutron activation analysis (N.A.A.)
- Atomic absorption spectrometry (A.A.S)
- Inductively coupled plasma mass spectrometry (*ICP-MS*)
- Graphite-furnace atomic absorption spectrometry (GFAAS)

INTRODUCTION

Multiple sclerosis (MS) is an acquired inflammatory and neurodegenerative immuno-mediated disorder of the central nervous system, characterized by inflammation, demyelination and primary or secondary axonal degeneration [1]. It clinically manifests itself with signs of multiple neurological dysfunctions (e.g. visual and sensory disturbances, bilateral Babinski signs, limb weakness, gait problems and bladder and bowel symptoms) followed by recovery or growing disability because of irreversible functional disability over time [1,3].

The categorization of the clinical course of MS has been reported to be(i) relapsing–remitting MS (RRMS), (ii) progressive–relapsing MS (PRMS), (iii) secondary-progressive MS(SPMS), (iv)primary-progressive MS (PPMS). Patients usually experience

a first neurologic event suggestive of MS which is known as Clinically Isolated Syndrome (CIS) [1,4].

The etiology of MS is still unknown, though it is likely to be caused by the interaction of changes in a range of social and environmental factors with underlying genetic differences [5,6]. Some progress has been made in trying to understand the role of heavy metals, principally lead (Pb), in neurodegenerative diseases such as multiple sclerosis, amylotrophic sclerosis, and Alzheimer's [7-9].

Lead is a non-essential and toxic metal that is naturally present in the environment. Lead enters the environment during production (including mining and smelting), use (batteries, pigments, ceramics, plastics), recycling, disposal of Pb compounds, combustion of fossil fuels, the use of mineral fertilizers, sewage sludge application, etc. [10].

There is no direct evidence showing that lead alters the immune system in a way that can increase the risk of MS. Some researchers have found that lead may stimulate the immune response by enhancing the immunogenicity of molecules of neural proteins [9]. Also, Pb, as responsible for the formation of antibodies against myelin proteins, is suspected to play a role in the pathogenesis of nervous system diseases such as MS [11]. However, the possible relation between exposure to lead and the development of neurodegenerative diseases has not been well studied [7].

Isfahan Province in Iran is now globally well known for its high prevalence of Multiple Sclerosis. Isfahan is recognized as one of the most high-risk regions for MS in Asia and Oceania [12]. The study by Isfahan MS society (IMSS) in reported a prevalence of 73.3/100,000 and an incidence rate of 9.1/100,000 in 2010 and also, an incidence rate of 9.22/100,000 in 2011 [12]. Based on the final report of IMSS, the overall age-adjusted prevalence rate was increased to 85.8/100,000 in 2014 [13], while the global median prevalence was increased from 30 (in 2008) to 33 per 100,000 (in 2013) [5]. However, the prevalence rate of MS in our province is still increasing, making the prevention and control this disease one of the most growing concerns.

Therefore, our purpose was to study the clinical features of MS for the whole population and for sub-groups stratified for gender, age, residence, disease duration, disability degree, and clinical diagnosis in Isfahan, Iran. Also, this study was done to elucidate the role of the lead in the pathogenesis of MS for each stratification variable. In addition, we tested whether there were significant differences between the sub-samples and if there was a significant correlation between the variable and the metal level. National and international comparisons of Pb concentration in blood and in the air and soil environments were also reported.

MATERIALS AND METHODS

Isfahan is a large province covering an area of 107,027 km2 in the central part of Iran. The climate is dry and temperate with the average temperature of 5.7°C in winter and 27.2°C in summer. The annual rainfall is 122.7mm too. Total population is 4,165,319 , 51.3% male and 48.7% female; 84.9% live in the urban areas and 15.1% in rural districts [14].

Study population and sampling

At first, this study was carried out on 54 subjects with a new or already known diagnosis of definite, probable or possible MS. These patients, who had referred to the department of neurology at Kashani Hospital in Isfahan, Iran, participated in the study during two weeks of sampling in August 2013. From this population, 48 subjects were living inside the province and others (a few subjects) were from the surrounding Provinces and therefore, were excluded. We pursued a sample size with patients that had experienced different stages of this disease. All individuals were asked to fill a questionnaire in order to have information on the variables that could influence the blood Pb concentration. Based on similar studies investigating the effect of heavy metals on human health and the development of diseases such as the disorders of the central nervous system [6, 15-18], we chose such criteria as age groups to study exposure duration with different pollution sources in the life; among other criteria, residence was noted to know the environment near the residence; disease duration was considered to check the changes of the blood lead level during the disease; disability degree was checked to observe the effect of blood lead level of on disability; and finally, clinical diagnosis was conducted to examine the effect of blood lead level on disease type. Disability was assessed by an expanded disability status scale (EDSS). EDSS had steps from 0 (normal) to 10 (death due to MS). EDSS steps 1.0 to 4.5 refer to patients who are fully ambulatory. EDSS steps 5.0 to 9.5 are defined by the impairment to ambulation [19]. Fortunately, the selected sample size had a desirable variety of criteria, such as gender (16 males and 32 females), age (20 to 57 years), residence status (13 subjects in rural areas and 35 in urban areas), disease duration (<20 days to 12 years), disability degree (ranged from 2 to 6) and clinical diagnosis (RRMS, SPMS and CIS), that could help us to find whether these parameters could influence the changes of the blood lead level.

Their blood was considered as an index of biologically heavy metals in the body, reflecting the environmental exposure of a population; hence, it was analyzed for heavy metal of Pb as a significant factor for individual health.

Exclusion criteria were: cardiological, respiratory, kidney or liver disease, as well as intestinal absorption abnormalities, infections, assumption of thyroid hormones, intake of vitamin or mineral supplements, vegetarian dietary, and artificial metallic bodies. Also, some variables such as height, weight, marital status, dietary, education, length of stay in the area, smoking habits and occupation were not considered.

Apparatus and reagents

All electrochemical measurements of lead were performed with Metrohm (Model 797 VA computrace) equipped with static mercury dropping electrode. The three electrode system consisted of a working hanging mercury dropping electrode (HMDE), a platinum auxiliary electrode and an Ag/AgCl saturated with KCl as the reference electrode. pH values were measured with a Metrohm (Model 827 pH-lab) pH meter equipped with a combined glass electrode (Corning, Model 6.0228.010). All the reagents used were of Analytical grade. All chemicals, being of analytical reagent grade, were purchased from Merck (Darmstadt, Germany) unless otherwise stated. Deionized water of resistivity higher than 18.0 mΩ at 25 ºC was used throughout. Acetate buffer 4.6 ±0.2 contained 14.2 ml of acetic Acid plus 55.9g of KCl and 25 mL of NaOH 30% in to the 500 ml deionized water. A stock solution of 1000 ppm of lead was prepared by dissolving the salts of metal nitrates in distilled deionized water. Solutions of low concentrations was prepared by serial dilutions with de-ionized double distilled water.

Sample preparation

Organic matter often interferes with voltammetric determinations and therefore, sample solutions usually have to be digested. Therefore, approximately, 2 ml blood samples were taken from each patient with special care by vein puncture using disposable syringes and needles and placed into heparinized pretreated clean polypropylene tubes. The samples were allowed to coagulate for 1 h and serum was then obtained by centrifugation at 5000 rpm for 10 min. The samples (2 ml) were then digested with nitric acid and perchloric acid. So, digested samples were made up to 4 ml. Therefore, all organic matter in the blood samples was removed for voltammetric measurement.

Voltammetric measurement

The prepared standard method in DIN 38406 Part 16 describes the determination Pb by voltammetric method. All voltammetric measurements were achieved according to this method. 0.5 ml of the digested sample was transferred into the voltammetric cell containing 10 ml of KCl-sodium acetate buffer (pH 4.6). The solution in the cell was aerated for 5 minutes by purging pure nitrogen gas. Anodic stripping was performed in the differential pulse mode after selecting pre-concentration time of 90s, a scan rate of 6 mV/s and pulse amplitude of 5 mV. The concentration of lead in the samples was determined using the standard addition method. In the standard addition method, first, a voltammogram of Pb unknown concentration was recorded. Then, a small volume of a concentrated solution containing a known quantity of the Pb concentration was added to the sample. On the basis of the assumption that the response is linear, the increase in signal of this new solution could be used to estimate the amount of Pb unknown concentration in the original solution. Reagent blanks were taken along with each batch of samples and the metal concentration observed in these blank samples was subtracted from the corresponding batch of field samples. Blank samples always showed extremely low levels of trace metals.

Statistical analysis

The distribution of each population was checked through the Kolmogorov–Smirnov(K–S) test. Rates for various subgroups were compared by the X2 or Fisher exact test. The relationship between Pb concentration in blood samples and disease duration was statistically compared using the analysis of variance (ANOVA).A p-value of $p< 0.05$ was considered significant. All analyses were performed using SPSS version 9.

RESULTS

Demographic and Clinical Results

The demographic and clinical results are given in Table 1.

Generally Table 1 showed that the overall prevalence of MS among men and women was 16/48 (33.33%) and 32/48(66.67%), respectively. Women were 2 times more likely to develop MS. The mean ±SE of the age of the patients was 33.73± 1.34 years, ranging from 20 to 57 years. The most common age group was that of 20-40years (79.17%), including 24 females (75 %) and 14 males (87.5 %). The youngest female patient was 21, and the oldest was57, the youngest man was 20, and the oldest was48. Only 6.25 % of cases were above 50. As regards the place of residence, subjects living in rural and urban areas was 13(27.08%) and 35(72.92%), respectively.

Table 1: Clinical Data on MS Patients, 16 men and 32 women*

Variables	All.	Males	Females
Age of subjects (year)			
20-30	21(43.75)	7(43.75)	14(43.75)
30-40	17(35.42)	7(43.75)	10(31.25)
40-50	7(14.58)	2(12.50)	5(15.63)
>50	3(6.25)	0	3(9.38)
Residence			
Rural	13(27.08)	6(37.50)	7(21.88)
Urban	35(72.92)	10(62.50)	25(78.13)
Disease duration (Day)[a]			
<20	8(16.67)	2(12.50)	6(18.75)
30-60	8(16.67)	3(18.75)	5(15.63)
365-730	6(12.50)	1(6.25)	5(15.63)
1095-1460	8(16.67)	6(37.50)	2(6.25)
1825-2555	7(14.58)	2(12.50)	5(15.63)
2920-3650	7(14.58)	0	7(21.88)
>4015	4(8.33)	2(12.50)	2(6.25)
Expanded Disability Status Scale (EDSS)[b]			
1	12(25.00)	3(18.75)	9(28.13)
2	25(52.08)	10(62.50)	15(46.88)
4	9(18.75)	2(12.50)	7(21.88)
6	2(4.17)	1(6.25)	1(3.13)
Clinical diagnosis			
RRMS	25(52.08)	7(43.75)	18(56.25)
SPMS	13(27.08)	6(37.50)	7(21.88)
Clinically Isolated Syndrome (CIS)	10(20.83)	3(18.75)	7(21.88)
Pb Concentration ($\mu g/l$)			
<20	34(70.83)	11(68.75)	23(71.88)
20-70	5(10.42)	2(12.50)	3(9.38)
70-100	3(6.25)	2(12.50)	1(3.13)
100-130	6(12.50)	1(6.25)	5(15.63)

- Figures in parentheses indicate percentages

*No significant differences were noted between males and females for all variables in chi-square test (P>0.05), except for Disease duration (P<0.05).

[a] Significant difference between males and females.(Likelihood Ratio Chi-Square = 13.198, DF = 6, P-Value = 0.040)

[b] Since the chi-squared test is generally reckoned to be unreliable if any of the expected frequencies are below 5, merging some of the smaller brands avoids expected frequencies less than 5, so with EDSS of 1, 2 and 4,this test is reliable.

The duration of disease in the total of the population was from 7 days to 12 years. The mean duration of disease was 1429.66 days (4 years). The scores of the EDSS ranged from 2 to 6 with a mean ± SE of 2.3 ± 0.18. Most patients had a RRMS course of the disease, 25(52.08%), while 13(27.08%) had a secondary progressive (SPMS) course and 10(20.83%) had a CIS course of the disease.

The blood Pb level in MS patients

The most Pb level measured in the blood samples of patients was <20 µg/l (70.83% of the total population). 10.42% samples had the Pb level of 20-70µg/l, 6.25% were within the level of 70-100µg/l and 12.50% covered the level of 100-130µg/l. Generally, regarding the patient characteristic, there were no statistically significant differences between men and women except for the variable of disease duration. As showed in table 1, the duration of disease of 4 years was mostly observed in the men. In addition, the duration of disease in 8-12 years was only observed in females. EDSS of 2 in women (46.88%) and in men (81.25%) was the highest. EDSS of 4 (25.00%) was identified only in women.

The relationship between the blood Pb level in MS patients and their clinical data

Table 2 also shows the relationship between blood Pb levels and the clinical data in the total population. To avoid the unreliability of the chi-squared test (due to frequency below 5), we had to merge some subgroups. No significant differences were noted in the levels of Pb in blood in the case of all variables except for EDSS (P<0.05). The blood samples with thePb level of <20 µg/l had the highest frequencies in EDSS score of 1(20.59%), 2(47.06%) and 4(32.35%), so it could be justified that most patients (70%) had the blood Pb level of <20 µg/l.

In spite of merging some data in the group of disease duration, the chi-square test was unreliable, so the relationship between Pb level (with the concentration of more than >20 µg/l) and disease duration was investigated by analysis of variance (ANOVA). It was found that disease duration was positively correlated (p=0.073, R-Sq = 66.23%) with blood Pb concentration (Fig. 1).

Table 2 : The relationship between blood Pb levels and their clinical data in total population

Variables	Pb (μg/l)*		
	<20	20-100	100-130
Age of subjects (year)			
20-30	15(44.12)	12(35.29)	7(20.59)
30-40	3(37.50)	3(37.50)	2(25.00)
>40	3(50.00)	3(50.00)	0(0.00)
Residence			
Rural	11(32.35)	1(12.50)	1(16.67)
Urban	23(67.65)	7(87.50)	5(83.33)
Expanded Disability Status Scale(EDSS) [a]			
1	7(20.59)	2(25.00)	3(50.00)
2	16(47.06)	6(75.00)	3(50.00)
4	11(32.35)	0(0.00)	0(0.00)
Clinical diagnosis			
RRMS	16(47.06)	4(50.00)	5(83.33)
SPMS	12(35.29)	1(12.50)	0(0.00)
CIS	6(17.65)	3(37.50)	1(16.67)

- Figures in parentheses indicate percentages

*No significant differences were noted between males and females for all variables in chi-square test (P>0.05), except for Expanded Disability Status Scale (EDSS) (P<0.05).

[a] Likelihood Ratio Chi-Square = 9.912, P-Value = 0.042

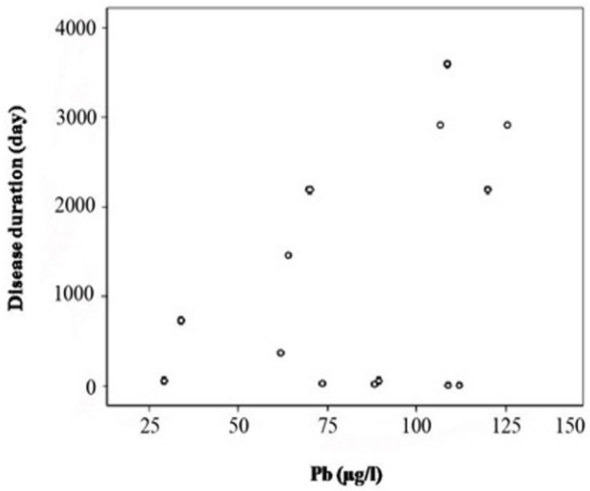

Fig. 1: The relationship between the blood Pb level of MS patients (>20 µg/l) and their disease duration

DISCUSSION

The primary purpose of this study was to evaluate the role of lead as a risk factor in damaging the immune system and probably, influencing the development of MS. So the study of the clinical features of MS such as gender, age, residence, disease duration, disability degree, and clinical diagnosis could clarify existence or non existence a relationship between of the increase of the blood lead level and the development of neurologic disorders. The main finding of this study showed that the highest rate of MS was in women. The survey of the Multiple Sclerosis International Federation (MSIF) also found that the median estimated female: male ratio was 2.0 worldwide [5]. In studies conducted by Gregory II *et al.* in the United States and Pugliatti *et al.* in Europe, the women to men ratio were 3.20 and around 2, respectively [1,20]. The females were more likely to self-report being diagnosed with MS thus; there were a large number of cases among females [20]. In addition, MS rate (60%) was the highest among cases who were 25- 40 years old. The findings of other researches agreed with these results [5, 21]. Relapsing-remitting MS (RRMS) was the most common disease course in patients (52%). From the geographic distribution of MS, we observed the highest total MS prevalence rates were among urban residents. This distribution was according to resident's distribution in Isfahan province. However, no statistical difference was found between the Pb level of subjects living in rural and urban areas because the populations were selected in central and suburban areas of Isfahan to assess the average exposure and exclude any specific source of the pollutant. The main sources of Pb exposure for the non-exposed general population are food, water and airborne particulate (smoke included) [16].

Also, previous researchers reported that Pb was significantly accumulated with increasing age [16, 21-23]. However, in our study, no significant difference was observed at the level of Pb among age groups, but there was a significant correlation between the blood Pb level and disease duration($p<0.05$). In fact, with the increase of MS patients disease duration, their blood Pb level (>20 µg/l) was increased too. It can be explained by the accumulation and binding of Pb to bone during time [16, 23] as Pb was used as a faulty building block in place of calcium; then we would expect blood lead levels to increase by passing time [24]. These results implied that disease duration was an important factor in increasing the accumulation of Pb.

In this study, the mean of the blood levels of lead in 70% of MS patients was <20 µg/ l and in others, it was 85.13µg/ l. Table 3 presents a brief literature review on the blood level of lead in neurological disorders such as motor neuron disease (MND) and multiple sclerosis [8, 11, 25-28].

When compared with the reference values, the mean Pb concentration in our study was <20 µg/l in 70% of MS patients and only 12.5% of patients had Pb level of 100-130 µg/l. These findings conformed to those obtained by other researchers, as shown in table 3. The difference in Pb levels in these studies was related to the area condition, different population groups and measurement method, among other things. Some reports have indicated that neurological impacts of lead occur at a level of 20- 50 µg/l in children and 180 µg/l in adults [29]. However, the lead concentration obtained from our study was lower. Also, the lead concentration in our study was in the range obtained by Talebi and Malekiha (2009) [17]. Their study was on heavy metals levels in whole blood samples obtained from those residents of Isfahan city who had referred to the Isfahan Blood Transfusion Organization during 2006-2007 (300 healthy adult people) and the mean concentrations of lead measured in the blood of the Isfahan residents was 150 µg/l by an atomic absorption spectrometer equipped with a GTA-110 graphite system. This confirmed our findings, showing that there was no significant difference in Pb level among the normal population and MS patients in Isfahan city. Also, the mean Pb concentration in most MS patients in our study was lower than the current threshold for Pb toxicity – as adopted by the US Centers for Disease Control and Prevention (CDC) since 1991 and by the World Health Organization since 1995 – is defined as the blood Pb level of <100µg/l [30,31].

Table 3 : Literature review on the blood level of lead in neurological disorders (µg/l)

Disease	Country	Subject	Measurement method	Pb Level
Multiple sclerosis (MS)	[a] England	39	E.A.A.S.	68.31(37.26- 99.36)
	[b] Canada	59	E.A.A.S.	0.180 (0.090-0.320)
			Z.M.A.A.S.	194 (80-200)
			N.A.A.	-
	[c] England	21	AAS	187 (108-266)
	[d] Italy	60	ICP-MS	24.3 (11.6-37)
Motor neuron disease (MND)	[e] Italy	15	GFAAS	127.1 (59.3-194.9)
	[f] Germany	9	E. A. A. S	86.5 (47.4-125.6)

[a] Data from McGrother *et al.*,1999 [8], [b] Ward *et al*.,1979 [25] , [c] RCGP–BRU,1976[26], [d] Forte *et al*., 2005[11], [e] Vinceti *et al.,* 1997 [27], [f] Stober *et al*., 1983[28].
-Not reported/or determined

However, one the most important reason for the relatively high level of Pb in blood sample of Isfahan people was that the high concentrations of Pb in the air of Isfahan closely were correlated with the high concentrations of these metals in soil such that the average concentration of Pb in the soils of Isfahan was 37.4 (17- 107) mg/kg dry soil in 2006 [32] and 297 mg/kg in 2008-2010 [33], which was less than the United States Environmental Protection Agency (US EPA) guideline (400 mg/kg) [34] and in range of Iran soil standard in pH>7 like alkaline soil of Isfahan (75-300 mg/kg) [35]. Also, the average concentration of Pb in the air of Isfahan was 297.5 ng/m3 [32], is less than that of the World Health Organization (WHO) (500 ng/m3) [36] and more than US EPA guideline (150 ng/m3) [37]. The widespread presence of pollution sources in Isfahan, such as the Irankouh lead and zinc mine located in the southwest of Isfahan (Pb level of 101.87mg/kg) [38], Isfahan Mobarakeh Steel Company and Isfahan Steel Company (Pb concentration of 1.3 to 4.85 mg/kg in 2001), agriculture run off, and automobiles, yields a vast amount of the contaminant materials, especially Pb[32,39-40].

However, while there are a potential risk as a result of the levels of Pb exposure and the possible effects on neurodevelopment [16,27], in our study no statistically significant difference was found between the Pb level of subjects and some patient characteristics including age, gender, residence, clinical diagnosis. So these findings did not provide evidence to support the hypothesis that lead may cause the MS disease, although its limited size prevents the generalization of the results. Turabelidze *et al.,* McGrother *et al.* and Birmingham Research Unit of the Royal College of General Practitioners (RCGP–BRU), in their studies on multiple sclerosis prevalence and possible Pb exposure, did not confirm the possibility that abnormal tissue lead levels would be related to multiple sclerosis in the patients. [8,41,26] However, in some areas, an unusually high prevalence of neurodegenerative diseases such as MS and MND was found to be associated with high levels of Pb[27, 42, 28]. In addition, in the study by Fort *et al.* a significant reduction in blood mean concentration of the Pb level in MS patients was observed [11].

However, we cannot ignore the possibility that a short exposure to a large amount of lead during the development of the nervous system might interfere with myelin synthesis such that it may, in turn, start a series of irreversible changes which, in time, produce the symptoms of multiple sclerosis. In fact, the etiological significance of this relationship is not clear, but it is proven that there are the significant relationship between the high level of lead and incidence of it, both singly or synergistically with

other trace element, the same progress is being concerning to other elements [26].

CONCULUSIONS

The mean concentration of lead in 70% of the blood sample used in our study population was lower than 20 µg/l and only about 12% patients had Pb level of 100-130 µg/l. We found no direct evidence for lead intoxication as a cause of MS using the relationship between the blood lead level and the clinical features of MS patients but its limited size did not allow us to generalize our results. However, the exposure to heavy metals such as Pb is a potential candidate because of the proven neurotoxicity, but it is still not well known if the lead exposure can affect human health risk for the development of MS. The clinical and demographic characteristics in our study were similar to those in other reports. Therefore, more accurate studies with larger samples are required to further support these results. Also, the findings of this report highlighted the need for other studies that can identify the major reasons at high risk for MS.

ETHICAL ISSUES

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all volunteers for being included in the study.

CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

Razavi Z designed of the study, coordinated research activities, contributed in conceptualization, preparing and results interpretation of the article. Jokar M participated in the technical parts and helped to the design of the study. Allafchian A.R. carried out technical analysis of data and participated in the final revision of the manuscript. Hossinpour Z and Berenjani L participated in the sequence alignment and helped to draft the manuscript. Shayegan Nejad V participated in preparing the sample from MS clinic in Kashani hospital in Isfahan and conceived of the study. All authors read and approved the final manuscript.

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