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Risk Analysis of Specific Environmental Heavy Metals as a Trigger of CD4 & CD8 among Cases of Multiple Sclerosis:Case-control study

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ABSTRACT

Background: Environmental pollutants might be among the etiological factors for multiple sclerosis. The high rise of CD8 and CD4 is the commonly accepted pathway for the chronic inflammation of myelin sheath proteins developing the different clinical types of MS.

Objectives: To measure the level of blood lead, cadmium, mercury, CD4 and CD8 among the different clinical types of MS cases compared to controls, to find out if there is a correlation between the level of the measured heavy metals and the degree of disability among the studied cases of multiple sclerosis.

Methodology: Case-Control study was conducted at El-Hussein University Hospital, Cairo, Egypt during the period (April 2017-February 2020). The calculated sample size was 50 for cases and controls. Subjects were selected by a simple random technique.

Results: Mean blood lead of cases was higher than observed among controls (62.1±3.2 µg/L versus 19.3±1.5µg/L) with statistically significant difference p=0.000001). Regarding blood mercury: mean among cases was higher than controls (25.1 ± 0.88 µg/L versus 2.3 ± 0.2 µg/L) with a statistically significant difference between two groups by Levine's t-test (t=5.5, p=0.000001). There was a positive correlation between lead and Mercury with CD4 and CD8 among cases of Relapsing-Remitting MS and cases of Primary Progressive MS.

Conclusion: Lead and Mercury might be a risk factor for trigger CD4 and CD8 among the studied cases of MS.

Keywords: *multiple sclerosis; lead, mercury; CD4; CD8*

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INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated, inflammatory neurological disease of the central nervous system that attacks the myelinated axons and destroying them in variable degrees[1]. The clinical manifestations of multiple sclerosis are very variable and include motor, sensory, visual and cognitive symptoms, none of them being disease specific[2].Focal inflammatory plaques and axonal loss are considered as a main pathological feature of MS, while the question about the etiology of MS is still unresolved[3].

Environmental agents are believed to trigger a T-cell-mediated chronic inflammatory response to myelin proteins in individuals with a genetic predisposition, creating the characteristic lesions that cause disease[4]. Numerous immune populations are important in MS development. Among these, $CD8^+$ T cells and $CD4^+$ T cells have appeared as potential major effectors within the CNS [5].

Overexposure to lead and mercury ions is known to be neurotoxic, particularly to motor neurons. Low-to-moderate levels of lead exposure can cause functional alterations in T-lymphocytes and macrophages that lead to increased hypersensitivity and alter cytokine production, which increases the risk of inflammation-associated tissue damage [6]. Also, it has been observed that lead and cadmium toxicity contribute to a vast variety of important disease conditions such as neurological disorders, cancer, cognitive impairments, hypertension, heart disease, and diabetes [7].
The present study tried to study the possible cause of multiple sclerosis, via measuring the level of lead, cadmium, and mercury among the studied cases and controls, to find out if there a correlation between the level of the studied heavy metals and the degree of disability among the studied cases of multiple sclerosis and to determine the extent of association between the elevated CD4 and CD8 among the studied cases with an elevated level of heavy metals.

Objectives

To measure the level of lead, cadmium, and mercury among the studied cases and controls, to find out if there is a correlation between the level of the measured heavy metals and the degree of disability among the studied cases of multiple sclerosis and to determine the level of CD4 and CD8 among the studied cases with an elevated level of heavy metals.

SUBJECT AND METHODS

Type of the study:
A case-control study was conducted at El-Hussein University Hospital during the period (April 2017- Feb 2020).
Sample size estimation:
The sample size was estimated by the epi info program using the following data: Confidence level: 95%, Power of the study:80%, Ratio of controls to cases=1:1, Percentage of controls exposed=1.4 % and Percentage of cases with exposure=18%[8]. The estimated minimum sample size required is 50 cases and 50 controls.
Sampling:
Cases and controls were selected by a simple random technique by using random number tables as following: All cases of MS and relatives to patients attended Al Hussein MS clinic on Sunday and Wednesday were recorded, The duration of selection was one year, Total number of recorded cases and controls on Sunday during one year was 105 cases and 106 controls, the total number of recorded cases and controls on Wednesday during one year was 106 cases and 106 controls, by table 2 of Random numbers tables, 25 cases, and 25 controls were selected from all recorded cases and controls, by table 5 of Random numbers tables, 25 cases, and 25 controls were selected from all recorded cases and controls.
Inclusion criteria for cases: Cases with a diagnosis of definite, probable, or possible MS. Exclusion criteria for cases and controls: Cases with advanced renal, cardiac, respiratory, or liver diseases or other neurological diseases.
Methods: All cases and controls were subjected to interview medical sheet. Clinical diagnosis and disability degree of MS typewere conducted and recorded by the neurologist at MS clinicusing an expanded disability status scale (EDSS)[9]. Laboratory investigations: blood lead, cadmium, mercury, CD4, and CD8 were conducted for cases and controls.
Determination of risk level
This thesis used a risk rating exposure levelmethod to analyze the risk.
Data analysis
Data collected and presented in the tables and charts using the Epi info program. P <0.05 was accepted as a level of significance,Chi-square, mean± SD, odds ratio, t-test,F test, and correlation Coefficient were the statistical tests used in the present study.

RESULTS

As regards gender there is a higher percentage of multiple sclerosis among females compared to males (64% and 36% respectively) with ratio 2:1 and the difference was statistically significant. (X2 =1.126, P=0.045). It was observed that multiple sclerosis cases were more prevalent among age group (20-<25 years), As regards residency was revealed that multiple sclerosis cases were more prevalent among Urban area than the rural area (14% and 16% respectively) and the difference was statistically significant. (X2 =0.396, P=0.03) (Figure 1)

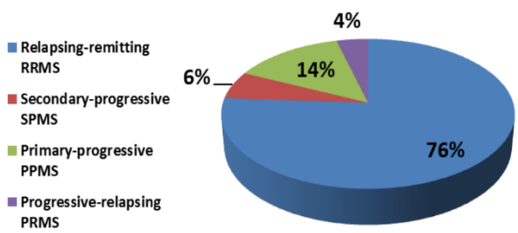

Fig. 1: shows that relapsing-remitting MS was the highest prevalent clinical type of MS among the studied cases. It represents 76%.

	Relapsing-remitting MS (N: =38) N. %	Control (N: =38) N. %	Chi 2	P value	Odds ratio
Lead	38	3	64.8	0.0*	62.3
Cadmium	100.0	7.9	NA	NA	NA
Mercury	0	0	39.7	0.0*	10.6
	0.0	0.0			
	29	2			
	76.3	5.3			

Table 1: Distribution of elevated Blood lead, Cadmium, and Mercury among cases of Relapsing-remitting multiple sclerosis.

*: significant

NA: Not Applicable

Table 1: Shows that all cases were suffering from elevated blood lead while only 7.9 % of controls were revealed elevated blood lead with a statistically significant difference (p=0.00). Regarding cadmium, all cases and controls were within normal levels. As regards, mercury 76.3% of cases revealed elevated blood mercury while 5.3% of controls revealed elevated blood mercury with a statistically significant difference (p=0.00).

	Primary-progressive MS (N: =7) N. %	Control (N.=7) N. %	Chi 2	P value	Odds ratio
Lead Cadmium Mercury	7 100 0 0.0 5 71.4	3 42.8 0 0.0 0 0.0	64.8 NA 7.7	0.0* NA 0.005*	62.3 NA 1.4

Table 2: Distribution of elevated Blood lead, Cadmium and Mercury among cases of Primary-progressive multiple sclerosis.

*: significant

NA: Not Applicable

Table 2: Shows that all cases were suffering from elevated blood lead while only **42.8** % of controls were revealed elevated blood lead with a statistically significant difference (p=0.00). Concerning cadmium, all cases and controls were within normal levels. As regards mercury **71.4** % of cases revealed elevated blood mercury while none of the controls revealed elevated blood mercury with a statistically significant difference (p=0.005).

Lead, Mercury, Cadmium & immune cells CD4 and CD 8	**Studied groups**			
	Cases **(N: =50)**	**Controls** **(N: =50)**	**t Test**	**P-value** ***: significant**
Lead (normal = 0.0-25.0 µg/dl) Mean ± SD Range	 62.1 ± 3.2 µg/L 32.9-101.3 µg/L	 19.3±1.5µg/L 14.8-43.6 µg/L	7.700	0.000001*
Mercury (normal=0.0-10.0 µg/dl) Mean ± SD Range	 25.1 ± 0.88 µg/L 0.22-29.98 µg/L	 2.3 ± 0.2 µg/L 0.3-12.8 µg/L	5.5	0.000001*
Cadmium (normal=0.0-5.0µg/dl) Mean ± SD Range	 1.99 ± 0.18 µg/L 1.81-2.17 µg/L	 1.3± 0.2µg/L 0.16-2.1 µg/L	18.1	0.000001*
CD4 (500 – 1500 cells / µl). Mean ± SD	 2513.87±21.09 cells/µl	 1233.3 ±9.1 cells/µl	1394.2	0.00000001*
CD 8 (400 – 1200 cells/ µl). Mean ± SD	 2269.1±80.8 cells/µl	 789.7±16.02 cells/µl	126.9	0.000001*

Table 3: Mean ± S.D. of blood Lead, Mercury, Cadmium, and immune cells CD4 and CD 8 among the studied groups.

Table 3: shows that the mean blood lead of cases was higher than observed among controls (62.1±3.2 µg/L versus 19.3±1.5µg/L) with statistically significant difference p=0.000001). Regarding blood mercury: mean among cases was higher than controls (25.1 ± 0.88 µg/L versus 2.3 ± 0.2 µg/L) with a statistical significant difference between two groups by Levine's t-test (t=5.5, p=0.000001). As regards the blood cadmium: mean among cases was higher than control (1.99 ± 0.18 µg/L versus 1.3± 0.2µg/L) with statistically significant difference (p=0.000001). As regards the blood CD4: mean among cases was higher than control (2513.87±21.09 cells/µl versus 1233.3 ±9.1 cells/µl) with a statistical significant difference between (p=0.00000001*) and regarding the blood, CD8 mean among cases was higher than control (2269.1±80.8 cells/µl versus 789.7±16.02 cells/µl) with statistically significant difference (p=0.000001).

Clinical types of MS	**Lead and Mercury**	**Correlation Coefficient (r)**	
		CD4	**CD8**
Relapsing-remitting RRMS (N: =38)	Lead (Range=32.9-79 µg/dL)	r = 0.850 p=0.000*	r = 0.576 p=0.000*
	Mercury (Range=0.22-19 µg/dL)	r = 0.692 p=0.000*	r = 0.330 p=0.043*
Primary-progressive PPMS (N: =7)	Lead (Range=71-99 µg/dL)	r = 0.736 p=0.059	r = 0.773 p=0.042*
	Mercury (Range=10.25-15.6 µg/dL)	r = 0.878 p=0.009*	r = 0.955 p=0.001*

Table 4: Correlation coefficient between levels of lead and mercury and the levels of CD4 and CD8 among the relapsing-remitting multiple sclerosis and Primary-progressive multiple sclerosis.

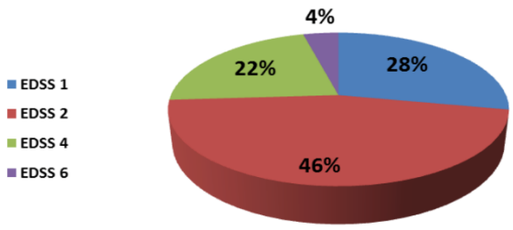

Fig. 2: Shows that EDSS 2 (Expanded Disability Status Scale) was the more prevalent disability type among studied cases followed with EDSS 1, EDSS 4 (46%, 28%, and 22% respectively).

Different groups of Expanded Disability Status Scale (EDSS)	Lead, Cadmium and Mercury	CD4	CD 8
EDSS 1 (N: =14)	Lead	r =0.981 p=0.000*	r =0.982 p=0.000*
	Mercury	r =0.980 p=0.000*	r =0.988 p=0.000*
EDSS 2 (N: =23)	Lead	r = 0.278 p=0.199	r =-0.457 p=0.028*
	Mercury	r =0.429 p=0.041*	r =-0.264 p=0.224
EDSS 4 (N: =11)	Lead	r =0.716 p=0.020*	r = 0.844 p=0.002*
	Mercury	r = 0.840 p=0.002*	r =0.977 p=0.000*

Table 5: Correlation Coefficient between levels of lead, cadmium and mercury and the levels of CD4 and CD8 among the different scales of Expanded Disability Status

Clinical types of multiple sclerosis	Heavy metals	Mean ± SD	Exposure severity	Risk Rating Level
Relapsing-remitting RRMS N: = 38	**Lead (Normal=0-25 µg/dL)**	**57.1 ± 16.76**	At or greater than normal	**High**
	Mercury (Normal= 0-10 µg/dL)	**17.78 ± 5.60**	Between 50-100% of normal	**Moderate**
	Cadmium (Normal= 0-5 µg/dL)	**2.04 ± 0.29**	Less than 50% of normal	**Low**
Secondary-progressive SPMS N: = 3	**Lead (Normal=0-25 µg/dL)**	**66.66 ± 5.77**	At or greater than normal	**High**
	Mercury (Normal= 0-10 µg/dL)	**19.36 ± 0.56**	Between 50-100% of normal	**Moderate**
	Cadmium (Normal= 0-5 µg/dL)	**1.97 ± 0.17**	Less than 50% of normal	**Low**
Primary-progressive PPMS N: = 7	**Lead (Normal=0-25 µg/dL)**	**81.28 ± 9.56**	At or greater than normal	**High**
	Mercury (Normal= 0-10 µg/dL)	**12.73 ± 1.83**	Between 50-100% of normal	**Moderate**
	Cadmium (Normal= 0-5 µg/dL)	**1.93 ± 0.022**	Less than 50% of normal	**Low**
Progressive-relapsing PRMS N: = 2	**Lead (Normal=0-25 µg/dL)**	**100.65 ± 0.92**	At or greater than normal	**High**
	Mercury (Normal= 0-10 µg/dL)	**18.39 ± 2.25**	Between 50-100% of normal	**Moderate**
	Cadmium (Normal= 0-5 µg/dL)	**2.065 ± 0.148**	Less than 50% of normal	**Low**

Table 6: Estimating the risk Rating Exposure Level of lead, cadmium, and mercury among the clinical types of multiple sclerosis.

Table 6: shows that the risk rating level of exposure to lead, mercury and cadmium was high (greater than normal 100% of normal), moderate (between 50-100% of normal) and low (less than 50% of normal) respectively among the studied clinical types of multiple sclerosis[10].

DISCUSSION

As regards the distribution of elevated Blood lead, Cadmium and Mercury among relapsing-remitting multiple sclerosis **Table (1);** the present study found that all cases revealed elevated blood lead representing 100% while only 3 controls revealed elevated blood lead representing 7.9% with a statistically significant difference between two groups (p=0.00) and (odds ratio 62.3). This is higher than found by Mohammad Dehghanifiroozabadi[11] who stated that the risk of MS increased 1.17 times per one µg/dL increment of blood lead level

As regards**the** distribution of elevated Blood lead, Cadmium, and Mercury among cases of Primary-progressive multiple sclerosis **Table (2);** the present study revealed that all cases were suffering from elevated blood lead while only 42.8% of controls were revealed elevated blood lead with statistically significant difference (p=0.00). In regards to cadmium, all cases and controls were within normal levels. As regards mercury 71.4% of cases revealed elevated blood mercury while none of the controls revealed elevated blood mercury with a statistically significant difference (p=0.005). Zahra Razavi[12]had found that35.29% of multiple sclerosis cases with blood lead <20 µg/dL and 12.50% of multiple sclerosis cases with blood lead 20 -100 µg/dL.This might be said that lead is not a contributing factor tothe pathogenesis of MS.

As regards the mean values of lead, mercury, cadmium and immunological cells CD4, CD8 among MS cases it was found a statistically significant different between all cases of multiple sclerosisand controls **table(3)**;This inline with Zahra Razavi[12] who found that blood lead level <20 µg/L in 47.06% of clinical type relapsing-remitting multiple sclerosis and 35.29% of clinical type secondary progressive multiple sclerosis and 17.65% of clinical type clinically isolated multiple sclerosis also 20-100 µg/L in 50.00% of clinical type relapsing-remitting multiple sclerosis and 12.50 % of clinical type secondary progressive multiple sclerosis and 37.65% of clinical type clinically isolated multiple sclerosis and 100-100 µg/L in 83.00% of clinical type relapsing-remitting multiple sclerosis and 0.0 % of clinical type secondary progressive multiple sclerosis and 16.76% of clinical type clinically isolated multiple sclerosis during his study the relationship between blood Lead Levels and clinical features among multiple sclerosis patients in Isfahan, Iran

As regards the distribution of multiple sclerosis cases according to Expanded Disability Status Scale (EDSS) Figure (6); the present study revealed that EDSS 2 (Expanded Disability Status Scale2) was the more prevalent disability types among the studied cases followed with EDSS1 and EDSS 4(46%, 28%, 22% respectively). This is in line with Zahra Razavi[12] who had found that EDSS 2 (Expanded Disability Status Scale) was the more prevalent disability type among the studied cases followed by EDSS1, EDSS 4 and EDSS6(52.00%, 25.08%, 18.75%, 4.17% respectively).

The present study assumes that cases of MS might be developed because of the pollutant effects of Lead and Mercury which trigger the elevation of CD8 and CD4 leading to the chronic inflammation of myelin sheath. This might be explained by studies in experimental allergic encephalomyelitis (EAE), histopathological studies of MS lesions, and immunologic markers in serum and cerebrospinal fluid of MS patients suggest that MS is an immune-mediated disease and environmental toxin might induce an immune response in genetically susceptible persons Prineas [13], Also, Frohman [14]stated that antigen-presenting cells (APCs) provide relevant antigens to CD4+ T helper cells in the periphery, which lead to their activation and the subsequent generation of autoreactive pro-inflammatory T helper (Th) 1 and 17.

The presenting study claims that lead and mercury might lead to induction of increasing the level of CD4 and CD8, this is supported with the findings of the present study which found a statistically significant difference between cases and controls with high odds ratio as regards lead and mercury among the different clinical types of MS (Table 1 and 2) and highly statistically significant difference between cases and controls as regards CD4 and CD8 (Table 3). Also, table 4 shows a positive correlation between the level of lead, mercury, and CD4, CD8 with a statistical significance difference. It was noted in the present study that these observations were found among cases of relapsing-remitting MS and primary progressive MS. Also, the same findings were observed among EDSS1, EDSS2, and EDSS4. This inline with Zahra Razavi[12] who found that Lead exposure is one of the environmental factors considered to play a role in the etiopathogenesis of MS.

The present study found that elevation of blood lead and mercury trigger the elevation of CD4 and CD8 (**Table 4**). This inline with Chibowska [15] who concluded that Exposure to lead may result in microgliosis and astrogliosis by triggering a signaling cascade and the production of proinflammatory cytokines. On the other hand, Zahra Razavi[12] reported that Lead produces MS through its ability to be attached to myelin proteins and act as a hapten. He added that; some researchers consider that lead is responsible for the formation of antibodies against myelin proteins and thereby can play a role in the pathogenesis of neurological diseases, particularly in MS.

As regards mercury, it was demonstrated that; acute Methyl mercury poisoning leads to a stimulation of the immune system, especially of cytotoxic CD8+ T cells, whereas CD4+ T cell number and activation remain unaltered. Of note, certain functionally and phenotypically distinct subpopulations of thymic-derived CD4+ T regulatory cells can control and limit potentially harmful immune responses Kleffner[16].

However, Höftberger[17] reported that; Studies strongly suggest that infiltrating CD8 T lymphocytes in the CNS of patients with MS selectively enter this organ. Unfortunately, the antigen specificity of these infiltrating CD8 T lymphocytes remains still unknown. Under basal physiological conditions, CNS cells including neurons, oligodendrocytes, and astrocytes express low levels of MHC class I molecules, which are recognized by CD8 T lymphocytes. However, up-regulated MHC class I is observed on resident CNS cells in MS lesions even in the initial phases of the disease). This in accordance with Kebir[18] who stated that; the presence of CD8 T lymphocytes in MS lesions positively correlates with the extent of axonal damage. Several studies reported enrichment of IL-17-producing CD8 T lymphocytes (i.e., Tc17) in MS lesions. Recent data support that both CD4 and CD8 T lymphocytes work in concert to cause the autoimmune attack observed in EAE. Additional investigations on the interplay between CD4 and CD8 T lymphocytes during different phases of MS and EAE could shed light on the complex and heterogeneous immune mechanisms involved in the disease pathobiology[19]. It has been suggested that CD8 T lymphocytes can kill myelin-specific CD4 T lymphocytes in a HLA-E restricted manner, Correale and Villa [20]

The present study found that lead was high risk, mercury was a moderate risk and cadmium was low risk among cases of multiple sclerosis in estimating risk rating among cases of multiple sclerosis Table (6); This agrees with present study that found lead is high in all EDSS and is correlated with an increase of CD4 and CD8 in different groups of EDSS. These findings encourage the need for more environmental studies that measure the level of lead and mercury in air, water, and food products to make a match between biological monitoring and environmental monitoring.

CONCLUSION

Lead and Mercury might be a risk factor for trigger CD4 and CD8 among the studied cases of MS.

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