

**Comparison of serum Concentration of Se, Pb, Mg, Cu, Zn, between MS patients and healthy controls**

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Abstract

Introduction: Multiple Sclerosis (MS) is defined as one of the inflammatory autoimmune disorders and is common. Its exact etiology is unclear. There are some evidences on the role of environmental factors in susceptible genetics. The aim of this study is to evaluate the possible role of Selenium, Zinc, Copper, Lead and Magnesium metals in Multiple Sclerosis patients.

Methods: In the present analytical cross-sectional study, 56 individuals including 26 patients and 30 healthy controls were enrolled in the evaluation. The serum level of Se, Zn, Cu, Pb were quantified in graphite furnace conditions and flame conditions by utilizing an atomic absorption Perkin Elmer spectrophotometer 3030. The serum levels of Mg were measured by auto analyzer 1500 BT. The mean level of minerals (Zn, Pb, Cu, Mg, Se) in serum samples were compared in both cases and controls. The mean level of minerals (Zn, Pb, Cu, Mg, Se) in serum samples were compared in both cases and controls by using independent-samples t-test for normal distribution and Mann-Whitney U test as a non-parametric test. All statistical analyses were carried out using SPSS 11.0.

Results: As well as the Zn, Cu, and Se, there was no significant difference between MS patients and healthy individuals in Pb concentrations (p-value = 0.11, 0.14, 0.32, 0.20 respectively) but the level of Mg was significantly different (p= 0.001).

Conclusion: All serum concentrations of Zn, Pb, Se, Cu in both groups were in normal ranges and there was no difference in MS patients compared with the healthy group who were matched in genetics. Blood level of Mg was significantly lower in MS patients. But it should be noted that even with the low level of serum magnesium in MS patients, this value is still in the normal range.

Keywords: Multiple sclerosis, Zinc, Lead, Copper, Magnesium, Selenium

1. Introduction

Multiple Sclerosis (MS) is defined as one of the inflammatory autoimmune disorders with the existence of progressive focal inflammatory myelin (demyelinating) and axonal loss, which affects the central nervous system (1). More than 60,000 patients were registered in the Iranian MS Society (IMSS) by the end of 2014 (2, 3).

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According to these data from the Iranian MS Society (IMSS), the number of new cases in MS patients has increased from 0.68 to its peak of 5.68 per 100,000 residents over the past few years in Tehran, Iran (3). Furthermore, it is one of the most progressive disabilities in young adults, especially in women with genetic predisposition worldwide. It is clear that MS incidence and prevalence are high in Iran especially in big cities. It is necessary to research the etiology. Despite the fact that several studies have stated the exact mechanisms of progressive demyelination in MS, it has not been clearly revealed whether there is some evidence about the role of environmental factors in susceptible genetics. It has been stated that infections such as Epstein–Barr virus (EBV) may contribute to the risk of MS (4). Moreover, the effect of noninfectious factors such as Vitamin D and sun exposure, smoking and diet are under research (5-9). Experts have suggested that the dietary food program with high protein and high fat may increase the risk of MS (10-13). Apart from the mentioned risk factors, several studies have indicated that trace metals may have a role in the progression of neurodegenerative disorders (14-17). The aim of this study was to ascertain if the serum concentrations of Zn, Cu, Se, Mg and Pb are different between multiple sclerosis patients and healthy controls.

2. Material and Methods

2.1. Research design

In the present cross sectional study, 56 individuals were enrolled. Twenty six new patients who were diagnosed with relapsing-remitting multiple sclerosis (RRMS) were enrolled in this study. They were referred to the Neurology Department of Ghaem Teaching Hospital in Mashhad, Iran (2013-2014). Thirty healthy controls were also recruited into our study. In order to eliminate the genetic differences, the individuals in the control group were chosen from the first degree relevant (family member) of patients, but environmental factors (such as their address) were different. All patients were evaluated during a stable period of their illness: 1) They should not have had any other previous major neurological disease or any type of diseases which might have interaction with copper (Cu), magnesium (Mg), lead (Pb), zinc (Zn) and selenium (Se) in the previous year, 2) They should not have received any type of mineral supplements, 3) They should not have received any type of corticosteroids in the previous month and also no drugs, 4) None of the women was pregnant.

2.2. Sampling and collection

According to relevant formula ($n = z^2 p (1-p) / d^2$) sample size in case and control group determined about 30. Ten milliliters (10 ml) of fasting blood samples were collected from eligible cases and controls and the plasma samples were separated and transferred to a research laboratory in the Mashhad University of Medical Sciences. The serum levels of Se, Zn, Cu, and Pb were quantified in graphite furnace conditions and flame conditions by utilizing an atomic absorption Perkin Elmer spectrophotometer 3030 (made in West Germany 1982). The serum level of Mg was measured by autoanalyzer 1500 BT made in Italy 2013.

2.3. Statistical analysis

The mean level of minerals (Zn, Pb, Cu, Mg, Se) in serum samples were compared in both cases and controls, by using independent-sample t-tests for normal distribution and Mann-Whitney U test as a non-parametric test. All statistical analyses were carried out using SPSS 11.0 (SPSS Inc., Chicago, Illinois, USA). The level of statistical significance was set at $p < 0.05$.

2.4. Ethics of research

Patients gave written informed consent; and the ethics committee of Mashhad university of Medical Sciences evaluated and approved the study protocol before we conduct the research

3. Results

The population of the current study comprises 26 new Relapsing-Remitting Multiple Sclerosis (RRMS) patients including 22 women (average age 30.00 ± 5.34 year) and 4 men (average age 26.25 ± 1.25 year). The control group also consists of 16 women with an average age of 29.87 ± 7.65 year and 14 men with an average age of 32.78 ± 13.09 year. The Kolmogorov-Smirnov test was used to test mineral distribution normality. Zinc, selenium, and copper have normal distributions. So, the t-test for independent means was utilized to compare the means between MS patients and controls. In all MS patients, the serum levels of Zn, Se and Cu were similar to the values obtained for the control subjects (p -value = 0.113, 0.146, and 0.326 respectively). There was no significant difference in both groups. Based on the significance of the Kolmogorov-Smirnov test for Pb and Mg, the nonparametric Mann-Whitney test was applied in the analysis of the difference between the case and study group. In Zn, Cu and Se, there was no significant difference between MS patients and healthy individuals in Pb concentrations ($p = 0.202$). On the other hand, the level of Mg in MS patients tends to be significantly lower than in control subjects ($p = 0.001$). In the

gender subgroups analysis, the statistics also show no significant difference in the serum concentration of Cu, Mg, Pb, Zn and Se in women.

4. Discussion

The results of our study generally revealed no significant difference in the serum concentrations of Se, Cu, Pb, and Zn. We also found that the level of Mg in the patients group with MS was significantly less when compared with healthy control individuals. For more than 3 decades, several researchers have worked on the relationship between trace elements or metals, and multiple sclerosis which consider the importance of the possible effect of trace elements and metal on MS etiology and treatment approaches. In spite of all, there is serious dispute among scientists and clinicians. The difference in methods and experimental measurements, and lack of vast and detailed studies with a large enough number of participants, are the most prevalent obstacles to reach a definite conclusion.

4.1. Selenium

Many studies have demonstrated the effect of oxidative disturbance in MS patients (18-20). Antioxidants such as selenium, vitamin E, and vitamin C have a protective impact on harmful free radicals. Selenium is highly popular for its key role in glutathione peroxidase enzyme and its anti-inflammatory effect. Its deficiency may lead to cell death and may have some effects on induction of MS (21). In the study of Socha et al., 101 relapsing-remitting MS patients dietary intake questionnaires were compared to their selenium concentration and GSH-Px activity. The results showed that the selenium concentration and GSH-Px activity in patients with MS were significantly lower when compared with healthy controls. Furthermore, a number of dietary habits have a direct correlation on blood Se concentration (22). Even if some studies agree with the above belief, others were unable to show the significant difference of selenium level in MS patients and healthy controls (23). Wikström et al. could not find the significant difference in selenium level in whole blood and plasma, although the selenium was rather high and low in low risk and high-risk MS patients respectively (23). Korpela et al. also reported no statistical difference in serum selenium concentration in patients with multiple sclerosis (MS) and healthy twins, as a control group (24).

4.2. Copper and Zinc

Copper (Cu) as a part of the synthesis of myelin has a basic role in many neurodegenerative diseases (25). However, ceruloplasmin as a copper-transporting protein has high molecular weight, thus, it is unable to penetrate the blood brain barrier (26). So, in order to find the effect of copper in MS as a demyelinating process, the concentration of copper was measured in a different body fluid such as CSF and serum (23, 27-30). Some studies claim that its deficiency may cause myelopathy (31) while others believe its toxicity as a trigger for MS (32). On the contrary, some other studies have also stated that there is no significant difference in Cu contents of body fluid specimens in both patients with MS and healthy controls (23). Zinc (Zn) is also assumed as one of the indirect immune system regulators, and its deficiency may lead to abnormal immune system function. It is also believed that the Zn and Cu are involved in many metabolic mechanisms and interactions, and the high level of zinc can proceed the low level of Cu and iron deficiency (31). Due to many developments in molecular techniques, some studies have suggested that investigating the interactions between Cu, Zn, and many enzymes, might be more useful in finding the effects of these elements in demyelinating disease of central nervous system (32-37). In a study in Iran, the serum zinc and copper levels from 35 MS patients and their matched control group, were measured using ELISA method. Contrary to what was found in this study, their analysis revealed significant reduction in serum levels of copper and zinc in patients with MS (27). In another Iranian study, to find the role of copper and zinc in the occurrence of MS, the serum level of copper and zinc in 56 MS patients were compared with their levels in a 39 healthy control group in a cross-sectional study in Kerman. Their study results reveal that there is no significant difference in serum levels between both case and control group (31). Although many biochemical studies on Cu and Zn related enzymes and cofactors were carried out and also many investigations have been conducted on the role of Cu and Zn in MS patients, there are still many controversies about the difference in its clinical levels in blood serum or even CSF, due to different methodology and also a lack of an adequate number of samples (23, 27, 28, 32, 38).

4.3. Lead and Magnesium

Although our data show no difference in serum Pb concentration in both case and control group, some evidence suggested that the gradual accumulation of lead may play a role in MS (38, 39). In the present study, the serum level of magnesium was significantly lower than that in healthy individuals. Although the role of magnesium was approximately clear in neuromuscular transmission (40), its probable effect on neurodegenerative disease is still vague. There are few studies on magnesium concentration in patients with MS (38). Masoud et al. in their study on 35 MS patients, revealed that the serum level of magnesium was significantly lower than their matched control

group (27). Our analysis was also in line with the result of Masoud et al. based on magnesium level. Kapaki in his study, reported that the level of some elements such as magnesium (Mg) concentrations in serum and cerebrospinal fluid (CSF) in patients suffering from various neurological diseases such as multiple sclerosis, amyotrophic lateral sclerosis (ALS) and peripheral nervous system disease, were slightly lower than healthy controls. In this study, no significant difference was detected in multiple sclerosis patients (28). Another previous study quantifies the magnesium (Mg) concentrations in samples taken from their brains and some other organs, of 4 patients with definite multiple sclerosis (MS) and 5 controls. The average Mg content in the CNS tissues in MS patients showed a significantly lower value than in control cases (41). From another point of view, there are some possible theories about the correlation between vitamin D and multiple sclerosis. It has been proposed that vitamin D has possible protective effects on the incidence of MS (9) and the immunoregulatory effects of vitamin D have been confirmed (42-44). It has also been confirmed that parathyroid hormone (PTH) has a role in the metabolism of vitamin D (45) and subsequently the amount of parathyroid hormone secretion is controlled by serum calcium. These processes are completely well documented but the relationship between PTH and magnesium is still being debated. It is supposed that although the low level of magnesium can induce the secretion of PTH, the very low concentration of Mg may depress the parathyroid hormone secretion which results in hypocalcemia in harsh hypomagnesemia (46). Therefore, the significantly lower level of magnesium might have an indirect effect on the induction of multiple sclerosis. But in our current study, although the level of Mg in serum was significantly lower in multiple sclerosis patients than in healthy controls, the concentration of magnesium is still in the normal range. It is suggested that a large enough population study with accurate measurements of vitamin D, PTH, serum calcium in patients with severe hypomagnesemia should be used in the future study.

4.4. Study limitation

The ratio of “female population to male“ in the case group, was more than the same ratio in the control group, which may be due to sex impurity in “Multiple Sclerosis”.

5. Conclusions

It can be concluded that all serum concentrations of Zn, Pb, Se, Cu in both groups were in normal ranges and there were no differences in multiple sclerosis patients when compared with the healthy group who were matched in genetics factors. On the other hand, it was observed that the blood level of Mg is significantly lower in our patients with multiple sclerosis. But it should be noted that even the low level of serum magnesium in MS patients is still in the normal range. Moreover, this study has helped in knowing that these environmental factors (Cu, Se, Zn, Pb, Mg) don't have a significant role in multiple sclerosis. Furthermore, the results of this research show that more investigation in this field is not economically feasible, and more study is needed about the role of magnesium and other MS etiology.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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