Abstract

Introduction:
Multiple sclerosis (MS) is one of the most common neurologic disorders. It appears that in addition to the common risk factors, there may be other factors, such as serum level of trace elements, which affect the development or course of the disease. The aim of this study was to evaluate the serum levels of iron, zinc and copper in patients with MS and compare them with the control group.

Materials and Methods:
In this study, serum levels of iron, zinc and copper were determined in 25 patients with MS and compared with those of 25 healthy individuals matched with age and sex. Data were analyzed in SPSS, version 17 using statistical descriptive methods (mean, percentage, SD) and t-test.

Results:
In this study, there were 9 males in the case and control groups and the rest were female. The mean age of the patients was 28 ± 3.44 years for men and 24 ± 2.55 years for women. Mean serum iron levels were significantly elevated in MS patients (127.04 ± 34.67) compared to these levels in the control group (103.95 ± 33.81). Mean serum zinc levels were significantly decreased in MS patients (10.92 ± 2.114) as compared to these levels in the control group (14.05 ± 3.2). Also, mean serum copper levels were significantly decreased in MS patients (88.58 ± 19.56) compared to the levels in the control group (110.37 ± 37.1).

Conclusion:
The findings of this study show that serum levels of Iron, Zinc and Copper in patients with MS are different from those in normal population.

Keywords: Iron, Zinc, Copper, Multiple Sclerosis

Introduction
Iron, copper, and zinc are neuroactive elements that affect different parts of the body, including the central nervous system. These ions are involved in production of myelin and neurotransmitters as well as in synaptic transmissions (1, 2). They are also involved in many cellular functions like cell division, electron transfer, and ATP production (3-5). Several studies have shown that the rise and fall of these ions have a role in creating many diseases including neurodegenerative diseases like; Alzheimer’s, Parkinson’s, Friedrich ataxia (3, 6-7), or non-degenerative diseases including central nervous system cancers like; meningiomas, glial tumors (8-10).
Recently, connection of these mineral with multiple-sclerosis that is considered the most prevalent demyelizan brain diseases has drawn huge interest to itself. This disease primarily affects young adults and could be associated with frequent relapses and severe disabilities (11, 12). Despite various studies on the subject, there is still no consensus of opinion on the etiology of the disease. Genetic factors, viral infections, autoimmune disorders are just some of the theories proposed. Some of the more recent studies indicate that these minerals may have a role in causing or in the process of this disease (13). These minerals are wide ranging, and iron, copper, and zinc are among the most important ones (14). The level of effect of these minerals is not yet clear, and studies show conflicting results. For instance, Johnson emphasizes gradual increasing effect of iron and copper and decreasing effect of zinc on this disease (15). Alemente et al. found significantly low serum iron and zinc levels in these patients (13). Visconti et al. reported changes in levels of serum iron, copper, and zinc, though not significant (14). Even in some studies, the role of nutritional supplements containing minerals in the incidence of this disease has been noted. The results of a study in Belgium revealed lower levels of zinc intake than the daily recommended levels in these patients. And also, in patients with secondary progressive MS, level of dietary iron intake was lower than in other cases of MS (16). Given the above cases, limited number of studies, and lack of definitive results on the effect of these minerals on MS, and also considering that Rafsenjan is a mining rich area with such minerals as copper (17), the present study was conducted with the aim to determine serum iron, copper, and zinc levels in MS patients.

Materials and Methods
This was a descriptive-analytical, case-control study with simple non-probability sampling method, conducted on 25 MS patients attending Special Diseases Center at the Aliebn-e Abitalib (A) hospital in Rafsenjan. The reason for choosing patients from this area was the proximity to Sarcheshmeh copper mines. These mines are located within 65 Km of Rafsenjan and have the prime ores of copper, molybdenum, and gold, and minerals such as zinc and iron can also be found in the melting waste (8). Patients’ disease was confirmed by the MS committee of the Rafsanjan University of Medical Sciences using the results of the brain MRI, evoked potentials and cerebrospinal fluid analysis according to the previously approved MacDonald criteria. All patients had relapsing-remitting type of MS. Pregnant or lactating women, patients with relapse three months prior to commencement of study, and being treated with corticosteroids, and those with history of other diseases, particularly gastrointestinal and blood diseases, or those using iron compounds, nutritional supplements, and medications other than beta-interferon were excluded from the study. None of the patients had a vegetarian diet. In addition, for the purpose of comparison, 25 patients admitted to the emergency department with a preliminary diagnosis of appendicitis, with similar age, gender, and socioeconomic status to the MS patients, with no history of any other diseases and not using nutritional supplements were selected as the control group. This study was approved by Rafsenjan University Ethics Committee. After 8 hours fasting, between 7 and 9 am, patients’ blood samples were taken, centrifuged, and tested. Measurement of iron, copper, and zinc levels were performed using colorimetric auto-analyzer (Prestige) (18). Data obtained, together with demographic details in the questionnaire were recorded. For analysis of data, descriptive and inferential statistics were used including; mean comparison, and independent t-test, assuming equality of variances and normal
distribution of means, based on Levine test. The SPSS-17 software was used.

Results
In this study, 36% of participants were male and the rest female. Men’s age ranged from 25 to 38 years and women’s from 19 to 45 in both groups. In the patients’ group, men’s mean age was 28±3.44 and women’s mean age was 24±2.55, and in the control group, men’s mean age was 28 ±4.85 and women’s mean age was 24 ±2.31. In both groups, 6 participants were under 20 years of age, 11 between 20 and 29, 5 between 30 and 39 years, and the rest were 40 years of age and older. In the patients’ group, serum iron level was significantly lower than serum copper and zinc levels (table 1). No significant relationship was found between copper to zinc ratio in the two groups. Also, no significant relationship was found between age, gender, and serum iron, copper, and zinc levels in the patients’ groups, either. Mean serum iron, copper, and zinc levels in participants with Extended Disability Status Scale (EDSS) under 3 were; 120.66±45.12, 11.34±2.86, and 106.66±42.64, respectively, and in participants with EDSS over 3 were; 101.18±75.72, 117.77±55.19, and 12.16±2.59, respectively. No significant relationship was observed between EDSS and serum iron, copper, and zinc levels.

![Table 1 - Patients and control groups serum iron, copper, and zinc levels](image)

<table>
<thead>
<tr>
<th>Test</th>
<th>Control group</th>
<th>Patients group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Standard deviation</td>
<td>Mean Standard deviation</td>
</tr>
<tr>
<td>P≤0.05</td>
<td>33.81 103.95</td>
<td>34.67 127.04</td>
</tr>
<tr>
<td>P≤0.05</td>
<td>3.2 14.05</td>
<td>2.114 10.92</td>
</tr>
<tr>
<td>P≤0.05</td>
<td>37.10 110.37</td>
<td>19.56 88.58</td>
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Discussion
The role of minerals in the incidence or process of the disease has long been studied. In the present study, in the patients group, serum iron, copper, and zinc levels were significantly higher than in the control group. Forte’ in a study on 60 patients showed that serum iron level in these patients was higher compared to the control group (19). Johnson also found gradual rise in iron in patients with MS (15), but Viscont in a study on 12 patients with MS for the first time observed that serum iron level did not change in the acute phase, nor did it change in the six months follow-up (41). Asphagus et al. showed that iron level was not different in patients group compared to control group (20). Abukrish showed that there was no difference in serum iron level between patients and control groups, but serum transferrin level was higher, which indicated iron dysfunction (21). Levin et al. study on iron level and ferritin in the cerebrospinal fluid in patients with MS suggested an increase in ferritin level (22). Also, Oxley showed that iron level excreted in the urine of patients with MS was greater than that in the control group (23). Some studies on nutrition show that reducing dietary iron is associated with severity of the disease (13). How iron affects initiation or trend of MS still needs to be further investigated, but the most likely scenario is that iron imbalance can cause inflammation in the brain tissue, eventually leading to MS (21). In the present study, serum zinc level in the patients group was significantly lower than in the control group. The results of Alemente et al. study on serum zinc level in MS patients was in agreement with that found in this study (13). Johnson, based on his clinical observations, also believes that gradual reduction in zinc is involved in the incidence of MS (15). Despite all the
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above results, Guita et al. found no difference in zinc levels between patients and control groups even though the patients dietary zinc intake was lower (16). Mellow et al., in studying cerebrospinal fluid, did not find any difference in zinc levels between the two groups, either (24). It seems zinc mostly affects the brain by helping function of the brain enzymes, whose fluctuations cause brain dysfunction and brain symptoms (11, 25-26). In this study, serum zinc level in the patients group was significantly lower compared to the control group. Guitar et al. did not find any significant difference in the serum zinc levels in German patients compared to the control group. However, they found that dietary copper was significantly lower than the standard daily recommendation in examining diet of these patients (16). Diane et al. found that erythrocyte copper level in MS patients was in the normal range, but their copper level significantly increased when treated with corticosteroids (27). Mellow et al. studying cerebrospinal fluid in MS patients found that the level of this ion was significantly higher than normal (24). Johnson found elevated serum level of copper in MS patients and believed that gradual rise in copper is a stimulating factor in the onset of the disease (15). Even comorbidities of this disease and Wilson’s disease that is due to copper deposition in various tissues including brain tissues has been reported (28). It appears the effect of copper is due to cytochrome oxidase enzymes, and its imbalance affects enzymatic functioning, causing clinical symptoms (11). Generally, the results of this study indicate a significant difference in serum iron, copper, and zinc levels in MS patients compared to the control group. Although the findings of this study are insufficient for drawing a generalized conclusion about the effect of minerals on the trend of this disease, it strengthens the hypothesis of the effect of minerals on MS. Given the above findings, it is recommended that further studies in other regions and with other minerals be carried out.

Acknowledgements

The authors wish to extend their gratitude to all the participating patients.

References:

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