Original Article

# The Role of Trace elements in Multiple Sclerosis


**Ammar A. Thamer*** **MBChB, MSc**
**Najeeb H. Mohammed*** **MBChB, DM, PhD**
**Akram M. Ibrahim**** **MBChB , FRCP**



***Summary:***

**Background:** Multiple sclerosis (MS) is a chronic, demyelinating disease of the central nervous system (CNS) affecting young adults and is considered as the leading cause of non traumatic neurological disability of young adults (1). The pathogenesis of MS is still incompletely understood. Deviation of immune responses in a genetically susceptible patient plays a central role in its pathogenesis (2). Electrophysiological, spinal tap and Radiological tools are important laboratory investigations that have added so much to the clinical diagnosis and for the classification of MS (3 and 4).

**Objectives:** The aims of the study is to estimate and evaluate the oxidative status indirectly through the serum levels of copper, zinc, zinc/copper ratio and magnesium in patients with MS, in addition to their relation with patients` degree of disability.

**Patients and Methods:** 112 patients with multiple sclerosis and 50 subjects without any neurological or psychiatric diseases as control group were recruited in this study. The cases were collected from Baghdad teaching hospital, MS center, Baghdad, Iraq at the period from May 2012 to April 2013, and studied at the toxicology center in Al-Shaheed Ghazi Al-Hareri Hospital in the Medical city. All patients and control groups were tested for serum level of copper, zinc and magnesium.

**Results:** The present study showed that serum level of copper was significantly higher in patients than in control, while the serum level of zinc, zinc/copper ratio and magnesium, were significantly lower in patients group than the control group. Also there was a positive linear correlation between the patients` disability measured by Expanded Disability Status Scale score and Cu and Mg, while a negative linear correlation between Zn and Zn/Cu ratio.

**Conclusion:** There is a disturbed oxidative status in MS patients shown by the higher increment of Cu and decrement of Zn, Mg and Zn/Cu ratio in MS patients, in addition to their relation with patients` disability than the control group, making it easy to quantify and predict MS disability objectively.



**Keywords:** Multiple sclerosis, Expanded Disability Status Scale, Copper, Zinc and Magnesium.

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## Introduction:

Multiple Sclerosis (MS): is an inflammatory disease in which the fatty myelin sheaths around the brain and spinal cord are damaged, leading to demyelination and scarring as well as a broad spectrum of signs and symptoms. Disease onset usually occurs in young adults, and it is more common in women. It has a prevalence that ranges between 2 and 150 per 100.000. MS was first described in 1868 by jean- martin Charcot (5). Till now MS is not easy to be detected, and a definite diagnosis can take several months or even years. And there is neither a single neurological or laboratory test that can definitively confirm or rule out MS, nor does any produce results in all patients (6). However, trace elements are important for the development of the nervous system, myelination of the nerve fibers, and also for the neural excitability (7). Many studies have shown variations in Cu, Zn and concentration in serum and CSF from patients with MS (8). While some studies suggested a link between exposure to trace elements and development of MS (4). It was also shown that reactive oxygen and nitrogen species play a role in demyelination, such as that occurring in the inflammatory demyelinating disorders eg. MS and Guillain-Barré syndrome. The concentrations of reactive oxygen and nitrogen species (e.g. superoxide, nitric oxide and peroxynitrite) can increase dramatically under conditions such as inflammation, and this can overwhelm the inherent antioxidant defences within lesions. Such oxidative and/or nitrative stress can damage the lipids, proteins and nucleic acids of cells and mitochondria, potentially causing cell death. Oligodendrocytes are more sensitive to oxidative and nitrative stress in vitro than are astrocytes and microglia, seemingly due to a diminished capacity for antioxidant defence, and the presence of raised risk factors, including a high iron content. Oxidative and nitrative stress might therefore results in vivo in selective oligodendrocyte death, and thereby demyelination (9). It has been reported that trace elements are important for the development of the nervous system, myelination of the nerve fibers, and also for the neural excitability (7) and many studies have shown variations in Cu, Zn and Mg concentrations in serum and CSF from patients with MS (8). While some studies suggested a link between exposure to trace elements and development of MS (4).


**Corresponding author: Ammar Ahmed Thamer, Dept. of Physiology, College of Medicine, Baghdad University.*
*ammarthamer@ mail.com*
***Iraqi Council for Medical Specialization.*

**Patients and methods:**

This is a case – control, study included 112 patients with multiple sclerosis (MS) 35 males and 77 females, ranging in age from 14 to 51 years with a mean age of (33.48 ± 8.09) years. They were recruited from the multiple sclerosis clinic at Baghdad teaching hospital, Baghdad, Iraq, collected during the period from May 2012 to April 2013. They were definite MS who were diagnosed according to the famous MacDonald criteria. A control group of fifty individuals (17 males and 33 females) with no neurological or psychiatric diseases were also enrolled in this study, their age ranged from 15 to 52 years with a mean of (33.54 ± 8.4) years. Both patients and control groups have been examined at the toxicology center in Al-Shaheed Ghazi Al-Hareri Hospital in the Medical city, and 8 ml blood samples transferred to separate sterile plastic tubes and centrifuged at 3000 rpm for 15 minutes. The separated serum was stored at -20 ºC until the time for analysis of Zinc, Cupper and Magnesium using the Atomic Absorption Spectrophotometer. A written consent from the patients was taken. Severity was calculated according to the expanded disability scale status (EDSS).

Statistics: All statistical analysis was obtained using Statistical Package for Social Sciences (SPSS) version 17.0 and Microsoft Excel (2007) softwares used for data entry and analysis. Results were expressed in simple statistical terms such as means, percentages, and standard deviations. Data from each patient and control group were compared using ANOVA tests to calculate differences between groups. Finding of p value less than 0.05 was considered significant.

**Results:**

The study included 112 patients with clinically definite multiple sclerosis with relapsing remitting (87.5%), secondary progressive (5.4%), primary progressive (2.7%) and clinically isolated syndrome (4.5%) courses attended MS clinic at Baghdad teaching hospital in the medical city complex and 50 individuals without any neurological or psychiatric diseases served as control. The patients group comprised 77(68.8%) females and 35 (31.2%) males having MS. There was a female predominance with female: male ratio = 2.2:1. The control group comprised 33 (66%) females and 17 (34%) males, there was female predominance with female: male ratio = 1.9:1the age of the patients with multiple sclerosis was ranged between 14 and 51 years. The age of subjects in the control group was between 15-52 years. The ages of the patients were classified into three groups: less than 30, 30-40 and more than 40. With a specifically more percent between (20-39) year`s subgroups (77.67 %) of total patients, which shows that this disease is mainly affecting middle age group, which is similar to nearby reports of Benamer etal (2009); Etemadifar and Abtahi (2012) (1 & 10) as well as to other countries (11 and 12).

Table (1) explores the distribution of the MS patients according to their motor disability manner by using the most popular Kurtzke, Expanded Disability Status Scale (EDSS) score, showing that the highest percent (35.71%) of patients had EDSS score of about (3- 4), and then between (2-3) (31.25%), while 18.75% with a score below 2 and the lowest percent (14.29%) for those who had the EDSS of (> 4).

**Table (1): Distribution of patients according to the Expanded Disability Status Scale (EDSS) score.**

| EDSS | Patients No. | Patients Percent (%) |
|---|---|---|
| ≤ 2 | 21 | 18.75 |
| 2- ≤3 | 35 | 31.25 |
| 3- ≤4 | 40 | 35.71 |
| > 4 | 16 | 14.29 |
| Total | 112 | 100 |

EDSS=Expanded Disability Status Scale

The mean and standard deviation of the serum levels of Copper, Zinc, Magnesium and Zinc/Copper ratio for both control and MS-patients groups are demonstrated in the table 2. There is a highly significant increases in the mean value of the serum level of Copper in MS-patients when compared with that of control (p0.0001<). While, the mean value of the serum level of zinc, magnesium and zinc/copper ratio is high significantly decreased in MS patient in comparison to that of control group (p0.0001<).

**Table (2): Comparison of the serum level of different trace elements studied between patients and control groups.**

| Parameters | Control N=50 mean±SD | Patients N=112 mean±SD |
|---|---|---|
| Serum Cu (µg/dl) | 117.28±15.49 | 152.13±22.89** |
| Serum Zn (µg/dl) | 103.24±24.41 | 77.17±9.03** |
| Serum Mg (mg/dl) | 2.13±1.05 | 1.27±0.14** |
| Serum Zn/Cu ratio | 0.89±0.23 | 0.52±0.11** |

** significant difference with control , p < 0.001

By measuring serum level of magnesium, the current study shows a highly significant decrease in the mean values of MS patients group (2) when compared with those of control group (1) (p0.0001<) (table 2).

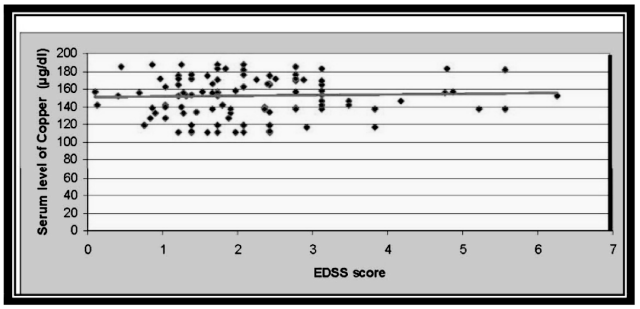
In table (3), 77 patients (68.75%) found to have abnormal visual signs and symptoms and 35 patients (31.25%) haven't any visual manifestations at the time of examination, there are statistically significant increases in the mean value of the serum level of copper in the two MS subgroups with and without visual involvement than those of control group. While the mean values of serum level of zinc, magnesium and Zn/Cu ratio are significantly decreased in the MS subgroups with and without visual manifestations. Whilst, there is no statistical differences in the mean values of these biochemical parameters within the two MS subgroups themselves.

**Table (3): Comparison of serum levels of copper, zinc, magnesium and Zn/Cu ratio between controls and MS patients with and without visual symptoms.**

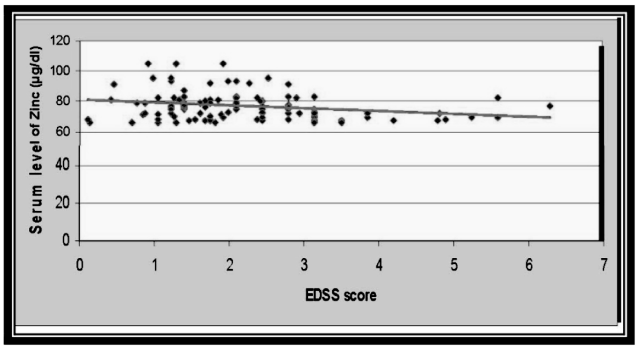
| Parameters | Control N=50 mean±SD | Visual symptoms | |
|---|---|---|---|
| | | Present N=77 mean±SD | Absent N=35 Mean±SD |
| S. Cu (µg/dl) | 117.28+15.49 | 151.6+21.29** | 153.29+26.38** |
| S. Zn (µg/dl) | 103.24+24.41 | 76.61+9.1** | 78.41+8.88** |
| S. Mg (mg/dl) | 2.13+1.05 | 1.28+0.14** | 1.25+0.14** |
| Zn/Cu ratio | 0.89+0.23 | 0.53+0.13** | 0.52+0.1** |

(**) significant difference with control, $p < 0.001$,

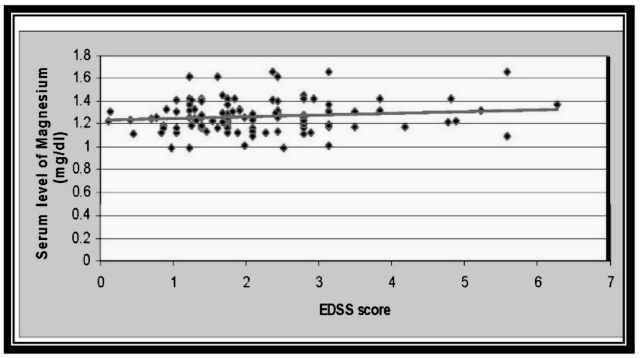
In correlations, this study reveals that there is a positive correlation between the EDSS score and the mean value of the serum copper, i.e., as the EDSS score increases, the mean value of the serum Copper increases too (figure 1). Whereas, it is a negative correlation between the EDSS score and the mean value of the serum level of Zinc (figure 2), and between the mean value of the ratio of Zn/Cu with the increase in the disability of the examined MS patients. While, a positive correlation between the mean values of the serum level of magnesium with the progress of the disease>s disability (EDSS) score is reported (figure 3).




**(r=0.043----------P=0.651)**
**Figure (1): Correlation between serum copper and the EDSS score.**




**(r=-0.236----------P=0.012)**
**Figure (2): Correlation between serum zinc and the EDSS score.**




**(r=0.122----------P=0.199)**
**Figure (3): Correlation between serum magnesium and the EDSS score.**

**Discussion:**

Although some studies suggested a link between the exposure to trace elements and development of MS disease, clear information on their role in the etiology of MS is still lacking (4). One of the postulated links reported is that, trace elements (zinc, copper, magnesium, etc) act as co-factors enabling the liver to detoxify natural toxins in different foods. Poor detoxification means that these toxins will circulate freely in the body, and some inevitably find their way into the brain, where these toxins bind with the fatty myelin sheaths. Immune system starts to attack it with consequent inflammation and destruction of the myelin sheath (13). In addition, the trace elements may affect antioxidant defense system as essential trace elements (copper, zinc and magnesium) are important parts of antioxidant enzymes as superoxide dismutase and important parts of transport protein with antioxidant properties as ceruloplasmin (14 & 15). Nutritional deficiencies and/or accumulations of key minerals and toxic heavy metals have been cited as important contributing factors that can adversely impact the health of patients with MS (16). Numerous studies of patients with multiple sclerosis have shown increased free radical activity or deficiencies of important antioxidant enzymes or both as compared with healthy controls (15).

The present study found a highly significant decrement in the serum level of zinc in the MS patients. This result is in agreement to the study of Forte etal, (2005); Masoud and Fakharian, (2007) and Al-Zubaidi, (2012) (4, 17 & 7). This decrease in Zn level could be due to its important role in the facilitation of T-cell proliferation and chemotaxis, inhibition of potentially destructive immune reactions against T lymphocytes, influences T-cell response to certain interleukins, its ability to modify cytokine production by means of the matrix metallo-protinases, and its antioxidant protecting role of the cell membranes, in addition to its stabilizing the association of the myelin basic protein with brain myelin membranes (13 & 17), while its deficiency was seen to cause lymphopaenia and reduction in the immune capacity among the affected humans (7). In contrast, Shaheen etal, (2006) found an increase in the serum level of Zn, and this could be due to their selection of the MS patients, as no one of their patients was in a stage of

activity, they were either in remission in RR-MS or a stage of plateau in the SP-MS or PP-MS forms. Their explanation is that Zn level is significantly elevated in between attacks and dramatically decreased during a clinically documented exacerbation of MS, and it has been suggested that mechanisms which govern cellular availability, compartmentalization of Zn, or the binding of Zn to cell surface membranes may be altered in patients with MS, according to disease activity (15). Moreover, the supposed effect of corticosteroid treatment in the active stages of MS, might disturbs the Zn and Cu homeostasis in their blood (17 & 7).

The highly increased serum Cu level in MS patients group is similar to what had been reported by Forte etal, (2005) and Al-Zubaidi, (2012) (4 & 7), while differ from Shaheen etal, (2006) and Masoud and Fakharian, (2007) (15 & 17) studies who found a decrement in the Cu serum level concentration, and it may be explained also by the influence of corticosteroid treatment on the Zn-Cu homeostasis, however, the increase in serum Cu concentration was found to decrease the Zn absorption (and vice versa) as several reports have proved the existence of a competition in the intestinal absorption and the inverse relationship between serum Cu and the Zn concentration (15 & 7).

In addition, there will be a subsequent deficiency of Cu/Zn superoxide dismutase (CuZnSOD) which can protect against random free radicals` damage both in the cytoplasm and in blood plasma as an antioxidant, yielding an increment in the oxidative stress (18). However, brain is able to concentrate physiological metals e.g. Cu and Zn up to toxic levels determining a possible production of oxidative species (19) while, it is still unclear if aberrant metal interactions are a primary or secondary factor, or a consequence of the neurodegeneration (4), still, Cu chelators have been seen to attenuate experimental autoimmune encephalomyelitis, and this fact would suggest a crucial role of Cu and reactive oxygen species in initiation and progression of the disease (20).

With regards to Mg, a highly significant decrement in the blood of MS patients is observed in comparison with its level in control group. This is in agreement with other studies of Forte etal, (2005) and Masoud and Fakharian, (2007). As this essential element may affect the maintenance and function of nerve cells as well as the proliferation and synthesis of lymphocytes. Moreover, Mg interacts with other minerals such as aluminum, calcium and zinc in biological systems, supporting the assumption that modification of this element may be a co-factor in the development of the disease (21 & 22).

Regarding EDSS score, the positive correlations between the serum level of Cu and Mg with the progression of the score, and the negative correlation with the Zn serum level, might be due to the more accumulation of Cu with an increased demand for Zn, resulting in a deficiency of the cellular antioxidant CuZnSOD enzyme, which in turn leads to increased levels of superoxide and nitric oxide and their production of peroxinitrite, an extremely powerful free radical that eventually leads to an increase in the oxidative stress which was implicated as mediator of the myelin and axonal damage in MS (23 & 17). While Visconti etal, (2005) found no significant changes of Cu, Zn or Mg with his follow up study of MS patients (13), however, the increment of Mg with disability progression in the present study might reflects some changes in its metabolism and homeostasis with the use of drugs in the course of disease (17), however, some researchers found oral Mg therapy effective in improving the range of motion and the measures of angles at resting position in lower limbs in patients with MS (14). These results support the hypothesis that the picture of chemical elements in blood may be a potential discriminating factor for MS (4), and that oxidative damage induced by the redox activity of a target protein, that interacts with free radicals and metal ions, has been found as a typical hallmark in the majority of neurodegenerative disorders. This hallmark could in principle be either the primary cause or the consequence of disease progression (24).

**Conclusion:**

This study shows the existence of a clear imbalance in oxidant-antioxidant status, towards an increasing oxidative state in MS patients. Physical disability of MS patients can also be quantified by biochemical studies and serum Copper, Zinc, Magnesium and Zinc/Copper ratio levels can be used as biological markers of patients` disability. These changes in the oxidative state and trace elements` metabolism in MS, may be the cause behind the disease, a stage during its pathogenesis, or as a results of it, in all states they seems to have a potential role in this disease and need further studies to clarify it.

**Authors Contributions:**

Dr. Ammar Thamer: Preparation, Performing and doing the tests of the research.

DR. Najeeb Mohammad: Supervision & support, as it is derived from PhD thesis.

Dr. Akram Ibrahim: Supervision & provide patients for this research.

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