MSD

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# Investigation of the changes in some elements that play an active role in MS disease

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## ABSTRACT

**Objective:** In this study, we examined the role of several metals which extracted from the hair of patients who affected by multiple sclerosis (MS). The ICP-MS measurements were performed using an Agilent 7500 series. With this procedure, all elements can be simultaneously quantified.

**Material and Methods:** All studies were carried out at Kayseri City Hospital between August 9, 2021, and October 11, 2021. We measured five elements from hair in association with the clinical course in 40 male and female MS subjects, as well as 40 healthy volunteers, using 1 cm hair sections. Using a novel microwave digestion method coupled with ICP-MS, we were able to analyze large numbers of samples per day consisting of 0.5 g of hair in 5 mL of nitric acid. Following digestion, the aliquots were examined to determine the quantities of Hg, Pb, Cu, Cd, and Zn.

**Results:** The quantities recovered for all elements were in an acceptable certified value range of (97.60–99.49%). Cu, Zn, and Hg levels examined were higher in the patient group, and Cd and Pb were higher in the control group. In our patients, a positive correlation was found between age, Hg, Cd, Pb values, and the disability score of multiple sclerosis [EDSS], while there was a negative correlation between Zn and Cu values and EDSS. Additionally, female MS patients showed a higher average hair content than males for the elements studied. Both Cd and Hg levels in patients were increased compared to the controls. Lead content in hair is steadily increasing in individuals of two different age groups. Lead content in hair showed an increased trend with age in the 21-35 year and 36-60 age groups.

**Conclusion:** Our study shows some important changes and relationships with Pb. Also, it has been determined that the Pb element increases with age, and the Cd and Hg levels were noticeably different in the patient and healthy groups, and Cu and Zn values change in parallel. The study can be a guide for diagnosis and treatment of the MS.

**Keywords:** Inductively coupled plasma, Hair, MS patients

## INTRODUCTION

In recent years, tremendous research studies have been conducted to investigate different types of abused drugs and their metabolites in various biological samples, including saliva, sweat, and hair. Hair testing for the analysis of new psychoactive substances can be used as a complement to urine testing (1, 2).

Multiple reports have recognized that trace elements found in scalp hair are associated with the levels observed systemically in humans. Hair-derived elements have been used to identify metabolic disorders and environmental exposures (1-10). The standard methods typically used are laborious and difficult, especially when considering a wide variety of elements and metals. Recently, ICP-MS has been widely used to study trace amounts of substances in many tissues (11-18).

Multiple sclerosis (MS) is a debilitating neurological syndrome that presents with spontaneous CNS inflammation and excessive lymphocyte/macrophage levels, resulting in demyelination and axonal injury (19-20). Transition metal impairment and defects causing abnormal trace elements have been shown in multiple neurological complications, including Alzheimer's, Parkinson's, ALS, and MS (21–29).

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Research on the effects of metals on autoimmune diseases, such as MS, started many years ago (30-32). According to researchers, specific diets that contain certain metals, such as zinc and magnesium, have been shown to improve MS symptoms such as muscle signals. For this reason, many physicians today examine metal levels in the body. Metal shortages, such as zinc, magnesium, and calcium, can aggravate MS symptoms; however, such deficiencies can be overcome by nutrition and diet. Habits can be acquired that can help improve symptoms in the disease. Accordingly, increased metal levels such as cadmium, copper, iron, lead, and low levels of metals magnesium, zinc, and calcium have been considered characteristic and have been shown to be involved in the etiology of the disease. In the MS process, evidence of the deficiency of some metals has been shown, such as zinc, magnesium, copper, and iron. To identify the role of trace elements in MS, we obtained analytical measurements of five metals found in the scalp of subjects in Kayseri, Turkey. These subjects were remitted for MS due to relapse (RRMS) and were compared to controls.

# MATERIALS AND METHODS

**Sample collection:** In this study, hair samples were collected from 80 healthy and MS subjects in Kayseri, Turkey. Participants were informed about the study and provided their consent. They were classified into two groups based on age: 21-35 years and 36-60 years. The study was conducted at Kayseri City Hospital between August 9, 2021 and October 11, 2021. Hair was collected from individuals with natural hair color, and for males, hair was removed from several scalp locations due to their shorter hair length. For females, a minor/non-noticeable area of hair was collected. The collected samples were placed in polyethylene bags and stored at 22°C indefinitely. The hair samples were minced and washed repeatedly in water/acetone (3:1 v/v) using constant stirring, followed by rinsing with deionized water three times. The samples were then dried at 80°C and stored as mentioned above.

**Sample preparation:** Approximately 0.2 grams of hair from each sample was digested using acid-treated PTFE tubes in equal volumes of nitric acid (65% v/v) and H2O2 (30% v/v). Mineral digestion was performed in a closed microwave (Berghoff 4) at different scanning ranges of temperature and time. After cooling to room temperature, the samples were quantitatively transferred into 25-mL flasks, diluted with deionized water, and stored at 4°C. Certified standards were digested under the same conditions as a control. Thirty microliters of each sample were administered to tubes using an automated sample, and experiments were performed in triplicate (n = 3). Elements were analyzed using ICP-MS (Agilent 7500). Each sample was analyzed in triplicate, and the ICP-MS was calibrated using a classical external method. The operating conditions were as follows: RF power, 1,550 W; plasma gas flow rate, 14 L/min; auxiliary gas flow rate, 0.89 L/min; carrier gas flow rate, 0.91 L/min; helium collision gas flow rate, 4.5 mL/min; spray chamber temperature, 270°C; sample depth, 4.27 mm; sample introduction flow rate, 0.93 mL/min; nebulizer pump, 0.1 rps; and extract lens 1 voltage, 1.5 V.

**Table 1.** ICP-MS operating conditions for analysis

| Parameter | Value |
|---|---|
| Radio frequency power | 1550 W |
| Sample depth | 7.9 mm |
| Torch-H | -0.4 mm |
| Torch-V | 1 mm |
| Carrier gas | 0.91 L/min |
| Makeup gas | 0.1 L/min |
| Auxiliary gas flow rate | 0.89 L/min |
| Plasma gas flow rate | 15 L/min |
| Nebulizer pump | 0.12 rps |
| Spray chamber temperature | 2 ,70°C |

During the analysis, a solution containing internal standard elements at a concentration of 200 ppb (including Be, Sc, Rh, and Bi), which represent the entire periodic table, was added to the instrument to minimize variations. Standards of Li, Yb, and Cs were used to calibrate the MS for optimal sensitivity, resolution, and mass detection of the elements being analyzed. To minimize memory effects, a 20-second wash was performed between subsequent samples, and a blank was analyzed after every fourth sample to correct for any background analytes. Correlation coefficients ranged from 0.9985 to 1.0000 and were recorded every five samples.

**Table 2.** Analysis of certified standards

| | Concentration (μg $g^{-1}$) | | |
|---|---|---|---|
| Element | Certified | Found | Recovery (%) |
| Cd | 17.97 ± 0.53 | 18.00 ± 0.20 | 99.83 |
| Cu | 0.062 ± 0.014 | 0.064 ± 0.010 | 96.87 |
| Hg | 0.031 ± 0.007 | 0.035±0.004 | 88.57 |
| Pb | 12.6±1.6 | 13.6±1.20 | 92.64 |
| Zn | 420±12 | 482±5.40 | 87.13 |

A total of 5 elements, including Cd, Cu, Fe, Hg, Pb, and Zn, were analyzed from each sample. The detection and quantification limits were estimated as previously described and reported as LOD and LOQ, respectively (Table 1).

Comparisons of the five elements in control subgroups and the MS group were performed and analyzed for statistical differences. Clinical stages were analyzed using the Mann-Whitney U test. Correlation/Pearson analyses were performed based on the distribution characteristics of the groups. A p-value of less than 0.05 was considered statistically significant.

# RESULTS

Cu, Zn, Hg from MS subjects were higher in the patient group compared to controls, while Cd and Pb were lower in the patient group.

In our patients, a positive correlation was found between age, Hg, Cd, and Pb values, and the EDSS, while there was a negative correlation between Zn and Cu values and EDSS.

In this study, we evaluated the relationship between the accumulation of 5 different elements in the hair tissue and MS. We found that some elements were quite different from the healthy control groups. Considering the groups examined, we believe that the findings have merit (**Table 3-7**).

Cu median values differ according to the groups (p<0.001). While the median value was 0.052 in the control group, it was 0.087 in the MS group. Zn median values differ according to the groups (p<0.001). While the median value was 358.69 in the control group, it was 414.485 in the MS group. Cd median values differ according to the groups (p<0.001). While the median value was 18,604 in the control group, it was 13,718 in the MS group. Hg median values differ according to the groups (p<0.001). While the median value was 0.052 in the control group, it was 0.084 in the MS group. The median Pb values differ according to the groups (p=0.018). While the median value was 12,934 in the control group, it was 12,437 in the MS group (Table 8).

**Table 3:** MS patients and healthy controls

| | Gender | | |
|---|---|---|---|
| | female | male | Total |
| Case (MS) | 32 | 8 | 40 |
| Control (Healty) | 28 | 12 | 40 |

**Table 4:** Smoking status of the patient and control group smoking status

| | | | cigaret | | Total |
|---|---|---|---|---|---|
| | | | Yes | No | |
| Case_cont | Case | Count | 24 | 16 | 40 |
| | | % within case_cont | 60.0% | 40.0% | 100.0% |
| | | % within cigaret | 49.0% | 51.6% | 50.0% |
| | | % of Total | 30.0% | 20.0% | 50.0% |
| | Control | Count | 25 | 15 | 40 |
| | | % within case_cont | 62.5% | 37.5% | 100.0% |
| | | % within cigaret | 51.0% | 48.4% | 50.0% |
| | | % of Total | 31.3% | 18.8% | 50.0% |
| Total | | Count | 49 | 31 | 80 |
| | | % within case_cont | 61.3% | 38.8% | 100.0% |
| | | % within cigaret | 100.0% | 100.0% | 100.0% |
| | | % of Total | 61.3% | 38.8% | 100.0% |

**Table 5:** MS Disease Duration

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| sickness_duration | 40 | 2.0 | 25.0 | 12.38 | 5.23 |
| Valid N (listwise) | 40 | | | | |

**Table 6:** EDSS score in MS Patients

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| edss_skor | 40 | 0.0 | 5.0 | 1.70 | 1.26 |
| Valid N (listwise) | 40 | | | | |

**Table 7:** Examination of the standard deviations of the changes of the examined elements in the case and control groups

| | Case_cont | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| **Cu** | Case | 40 | 0.087 | 0.009 | 0.001 |
| | Control | 40 | 0.0518 | 0.01 | 0.002 |
| **Zn** | Case | 40 | 405.94 | 64.69 | 10.230 |
| | Control | 40 | 336.37 | 49.50 | 7.827 |
| **Cd** | Case | 40 | 13.97 | 2.07 | 0.326 |
| | Control | 40 | 19.60 | 2.49 | 0.394 |
| **Hg** | Case | 40 | 0.081 | 0.039 | 0.006 |
| | Control | 40 | 0.050 | 0.015 | 0.002 |
| **Pb** | Case | 40 | 12.46 | 1.44 | 0.227 |
| | Control | 40 | 13.72 | 2.05 | 0.324 |

**Table 8.** Comparison of trace elements by groups

| | Control | | MS | | P* |
|---|---|---|---|---|---|
| | Mean ± s. deviation | median (min-max) | Mean ± s. deviation | median (min-max) | |
| Cu | 0,052 ± 0,01 | 0,052 (0,033 - 0,07) | 0,087 ± 0,009 | 0,087 (0,069 - 0,099) | <0,001 |
| Zn | 336,367 ± 49,502 | 358,69 (204,23 - 394,62) | 405,948 ± 64,693 | 414,485 (21,64 - 472,94) | <0,001 |
| Cd | 19,599 ± 2,491 | 18,604 (16,126 - 26,78) | 13,967 ± 2,066 | 13,718 (10,684 - 17,977) | <0,001 |
| Hg | 0,05 ± 0,015 | 0,052 (0,022 - 0,078) | 0,081 ± 0,04 | 0,084 (0,011 - 0,28) | <0,001 |
| Pb | 13,724 ± 2,051 | 12,934 (11,342 - 19,942) | 12,456 ± 1,436 | 12,437 (9,355 - 15,377) | 0,018 |

*Mann Whitney U testi

# DISCUSSION

Previous reports have shown that environmental factors may be involved in MS disease. Besides, occupational factors such as exposure to organic solvents, Zn used in production facilities, ecosystems in arable soils to a contaminant or the presence of Zn can be listed (32-36). Studies on the effects of zinc on MS patients began many years ago. Zinc, destructive immune reactions against T lymphocytes as well as Zinc with the preparatory inflammatory responses of MS antioxidant can protect cell membranes and myelin. Besides, it reduces the activity of glutamate dehydrogenase (GDH).

The vertebral column is an area of the central nerve containing metal ions such as zinc and copper. Many studies show that low Zinc levels are very important in MS patients. Zinc deficiency causes thymus deterioration, decreased thymulin activity, decreased T cell altered proliferation and Th1/Th2 balance versus Th2 answer (37). Thus, it seems that zinc is in the diet of patients. May be useful in eliminating and reducing muscle with MS contractions and effectively reduce muscle symptoms sickness and fatigue in patients.

Literature has shown effects of impaired copper metabolism on the pathogenesis of degenerative nerve diseases. (38). Zinc is an abundant metal found in almost all organs, tissues, and fluids in the human body. This micronutrient is essential and is important in multiple reaction processes, including zinc metalloenzymes, the macronutrient metabolism, gene expression/replication.

Zinc also acts as a component of metallothionine, which is an important feature of sulfur-based metal clusters in proteins. In addition, it is attached by cysteine thiolate together with histidine and methionine, which make up keratin in the hair.

It is a catalytic redox-active metal that is important in copper elements such as zinc, active antioxidant enzymes and free radical formation. Studies conducted in various animal species, copper and zinc deficiency can reduce myelination and deficiency of elements such as copper and zinc is important in the onset and rapid progression of neurological diseases (39).

Our data show that the median hair Zn and Cu levels in MS subjects was reduced significantly compared to the controls. The positive association for Cu and Zn in these subjects suggests a mutual association between these two elements.

In addition, female MS patients showed a higher average hair content than males for the elements studied. These data suggest a potential role for MS pathology and the impairment of many different types of elements and changes in their metabolism.

High Hg may suggest metabolic or genetic factors are involved in the disease. Mercury, a known toxic element, can be found in hair and has been associated with the consumption of seafood (40).

In our study, both Cd and Hg levels increased in MS group compared to the controls, while the mean value of MS patients compared to healthy individuals living in the same area did not show similar increases. Also, there was a positive increase in Cd and Hg levels in the MS group, which was not found in the control group.

Lead content in hair was shown to steadily increase in individuals of two different age groups. Lead content in hair tended to increase with age in the 21-35 year and 36-60 age groups. MS Interestingly, autoimmune-mediated stripping and disruption of the myelin sheath that protects the nerve fibers has been shown in MS. The myelin structures are predominantly vulnerable to free radical insult, and this has been linked to the distribution of metals in the body (41).

A group of researchers from Iran examined the relationship between MS distribution and soil heavy metals in their study to understand the reasons for the high MS rate in their country. The researchers divided the city of Isfahan into 5 different regions, namely the center, north, south, east and west, based on the feeding regions. They examined soil samples from feeding areas with high MS incidence and prevalence. They analyzed the samples for the absorbable forms of cobalt, lead, cadmium, copper, iron and lead, cadmium, and cobalt. They used linear regression to examine the relationship between MS prevalence and soil heavy metal in the center of Isfahan city. When the researchers looked at their study results, they saw a strikingly strong association between exposure to absorbable lead and cadmium in soil and the risk of MS. They stated that more studies are needed to verify this relationship and to understand its mechanisms (42).

In our study, the rate of lead that increases with age may be due to the difference in natural habitats of individuals when they were young and middle-aged. In our study, studies such as disease duration, EDSS score, smoking rates, and their effects on the disease in MS patients are given in **Table 3 - Table 7**.

# CONCLUSION

Considering the important role of metals in the course of MS disease and of course in the diagnosis, it can be said that physicians should conduct research on the patient's social environment and exposure to heavy and toxic metals, and provide some recommendations to patients during diagnosis and treatment. Also, our study shows some important changes and relationships with Pb. It has been determined that the Pb element increases with age, Cd and Hg levels are noticeably different in the patient and the healthy group, and Cu and Zn values change in parallel. The study can be a guide in diagnosis and treatment.

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**Author Contributions: SK;** designed of the study, data collection and analysis. **SK;** submission of the manuscript and revisions

E**thical approval:** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and/or with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study. Written consent was obtained from each patient to use their hospital data.

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