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Investigation of the Presence of Heavy Metals in the Progression of Multiple Sclerosis

Multipl Skleroz Progresyonunda Ağır Metal Varlığının Araştırılması

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Abstract

Objective: Multiple sclerosis (MS) etiology studies show that the disease is multifactorial. While genetic features are more prominent at the beginning of the disease, the existence of environmental triggers that may cause the progression of the disease is still a subject of research.

Materials and Methods: We evaluated 101 MS patients sharing the same geographical area. We performed heavy metal analysis in the blood and hair tissues of these patients. Of these patients, 67 were in the relapsing remitting MS (RRMS) group and 34 were in the progressive MS (PMS) group.

Results: Samples were analyzed using the X Series 2 model (ICP MS) instrument. In hair samples, aluminum (Al) was found to be 8.98 µg/g in the PMS group and 3.01 µg/g in the RRMS group ($P < 0.001$). Unlike Al element, magnesium (Mg), calcium (Ca), cobalt (Co), zinc (Zn) and strontium (St) elements were observed to be higher in the RRMS group. RRMS for median Ca: 931 µg/g, PMS: 400 µg/g ($P < 0.001$); RRMS for Mg: 118 µg/g, PMS: 50 µg/g ($P < 0.001$); For Co RRMS: 0.0150 µg/g, PMS: 0.0075 µg/g ($P = 0.008$); RRMS for Zn: 138 µg/g, PMS: 101 µg/g ($P = 0.008$); For St RRMS: 4.76 µg/g, PMS: 3.70 µg/g ($P = 0.008$). With these results, we thought that Al excess or Mg, Ca, Co, and Zn deficiencies might be associated with MS progression. The mercury (Hg) blood level was slightly low in PMS. This finding was not correlated with hair samples ($P = 0.047$). The relationship between blood Hg level and RRMS should be evaluated separately.

Conclusion: We could not find any study comparing PMS forms and RRMS subtypes in terms of heavy metals. In particular, it is necessary to focus on the Al element.

Keywords: Multiple sclerosis, progression, heavy metals, aluminum

Öz

Amaç: Multipl skleroz (MS) etiyoloji çalışmaları hastalığın multifaktöriyel olduğunu göstermektedir. Hastalığın başlangıcında genetik özellikler daha ön plana çıkarken, hastalığın progresyonuna neden olabilecek çevresel tetikleyicilerin varlığı hala araştırma konusudur.

Gereç ve Yöntem: Aynı coğrafi bölgeyi paylaşan 101 MS hastasını değerlendirdik. Bu hastaların kan ve saç dokularında ağır metal incelemesi yaptık. Bu hastaların 67'si tekrarlayan iyileşen MS (RRMS) grubunda, 34'ü progresif MS (PMS) grubundaydı.

Bulgular: Numuneler, X Series 2 model (ICP MS) cihazı kullanılarak analiz edildi. Saç örneklerinde alüminyum (Al) PMS grubunda 8,98 µg/g, RRMS grubunda 3,01 µg/g bulundu ($P < 0,001$). RRMS grubunda Al elementinden farklı olarak magnezyum (Mg), kalsiyum (Ca), kobalt (Co), çinko (Zn) ve stronsiyum (St) elementlerinin yüksek olduğu gözlendi. Ca medyan değeri için RRMS: 931 µg/g, PMS: 400 µg/g ($P < 0,001$); Mg için RRMS: 118 ug/g, PMS: 50 ug/g ($P < 0,001$); Co RRMS için: 0,0150 µg/g, PMS: 0,0075 µg/g ($P = 0,008$); Zn için RRMS: 138 µg/g, PMS: 101 µg/g ($P = 0,008$); St RRMS için: 4,76 µg/g, PMS: 3,70 µg/g ($P = 0,008$). Bu sonuçlarla Al fazlalığı veya Mg, Ca, Co ve Zn eksikliklerinin MS progresyonu ile ilişkili olabileceğini düşündük. PMS'de cıva (Hg) kan seviyesi hafif düşüktü. Bu bulgu saç numuneleri ile korele değildi ($P = 0,047$). Kan Hg düzeyi ile RRMS arasındaki ilişki ayrıca değerlendirilmelidir.

Sonuç: Daha önce PMS formlarıyla RRMS subtiplerini ağır metaller açısından kıyaslayan çalışma bulamadık. Özellikle Al elementine fokuslanmak gereklidir.

Anahtar Kelimeler: Multipl skleroz, progresyon, ağır metaller, alüminyum

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Introduction

Multiple sclerosis (MS) is an autoimmune, inflammatory, demyelinating disease of the central nervous system (CNS) (1). It is estimated that MS affects more than 2.3 million people worldwide, with a higher prevalence among women (2). The etiology of MS remains largely unknown, but the current literature suggests it may result from a complex interplay between genetic predisposition and environmental factors, one of which could be exposure to heavy metals (3). Genetic risk factors have been identified, such as variants of the *HLA-DRB1* gene (4). These environmental factors can include viral infections, vitamin D deficiency, and smoking (5). Notably, the prevalence of MS is higher in northern regions and industrialized countries (6,7).

Metals in ionic form are reactive and can interact with biological systems, thus potentially contributing to disease–gene–environment interactions (8). We found no articles that had previously investigated the effect of heavy metals on MS progression (9,10).

The heavy metals in question, including lead (Pb), mercury (Hg), and aluminum (Al), are common in the environment and can enter the human body through food, water, air, and even skin contact. Once inside the body, they can cross the blood-brain barrier and accumulate in the CNS (11). Cadmium (Cd) and arsenic (As) form oxygen radicals, while Pb and Hg contribute to the pathogenesis of MS by demyelization (12).

In addition, exposure to Al has been associated with an increased risk of developing MS (13). Al can cause oxidative stress, activate inflammatory pathways, and even directly cause damage to the myelin sheath, the protective layer around nerve fibers, which is damaged in MS (14). Similarly, in a local study, increased serum Hg levels were observed in MS patients compared to the control group (15).

We wanted to investigate the environmental effects that may cause MS progression. To understand the effect of heavy metals, one of these environmental factors, we compared a relapsing remitting MS (RRMS) group and a progressive MS (PMS) group.

While prior studies have investigated the relationship between environmental heavy metals and neurodegenerative diseases, we found no studies comparing heavy metal levels between MS subgroups in cohorts comprising solely MS patients. In our study, we analyzed hair and blood samples to measure the concentrations of heavy metals present in them.

Materials and Methods

Study Population

A total of 101 MS patients who consecutively applied to the neurology outpatient clinic of Firat University's Faculty of Medicine from January 2019 to January 2021 were included in our study. The gender, age, clinical subtypes, expanded disability status scale (EDSS) score, and disease duration were recorded for all participants (Table 1). When the two group samples were compared, the RRMS group was younger, had a shorter disease duration, and a lower EDSS score. The distribution of building age in which they had settled was not, however, significantly different. None of the patients had a history or signs of poisoning. The majority of these patients resided in the Upper Euphrates Basin. A total of 101 MS patients were selected as representatives of different neighborhoods to characterize approximately 1,200 MS patients followed in our clinic. Among them, 82 were from the province of Elazig (Abdullahpasa: 6; Aricak: 2; Alacakaya: 1; Baskil: 2; Cumhuriyet: 6; Caydacira: 4; Dogukent: 4; Harput: 3; Izzetpasa: 3; Karakocan: 1; Keban: 4; Kovancilar: 2; Kultur: 3; Maden: 1; Mustafapasa: 3; Olgunlar: 4; Palu: 2; Rizaiye: 4; Rustempasa: 3; Sanayi: 4; Sivrice: 1; Sursuru: 8; Universite: 3; Yazikonak: 4; Yenimahalle: 4) and 19 were from neighboring provinces (Bingol: 7; Tunceli: 4; Mus: 2; Others: 6). Fewer men were included in the study because they tended to have short hair, restricting the use of hair samples. All of these patients were not working in occupations that could come into contact with heavy metals.

Heavy Metals Analysis

To conduct the sample analysis, a 3-cc blood sample was collected in 1 purple capped complete blood count tube and 1 gram hair samples were taken from each patient, stored at -20 °C, and then sent to Istanbul University Forensic Toxicology Department in cold chain by cargo. The dyed hair samples were cut into small pieces with clean scissors and placed into a dry Falcon® tube in 0.20–0.25-gram samples. For washing, Triton X100 was used to wash the samples twice with 1:200 v/v, three times with ultra-pure water, and twice with acetone. The washed samples were dried at 75 °C. After drying, the samples were again weighed and the results were recorded. After the hair samples were placed in a microwave vessel, 7 ml of 65% HNO3 + 2 ml of hydrogen peroxide were added. The samples were then exposed to wet burning using a CEM Mars5 system digestion oven. After acid digestion, the sample was taken into a 50 ml Falcon® tube and completed to 50 ml with distilled water. Next, 100 µl of 2 ppm In + Ga was

Table 1. Socio-demographic characteristics of the patients

	RRMS n = 67	PMS n = 34	*P*
Gender			
Male	14 (20.9%)	7 (20.6%)	
Female	53 (79.1%)	27 (79.4%)	0.971[a]
Age	31 (24–40)	50 (40–55)	<0.001[b]
EDSS	1 (1.0–1.5)	6 (4.3–7.0)	<0.001[b]
Disease duration (year)	2 (1–6)	12 (8–20)	<0.001[b]

[a]Chi-square test, [b]Mann–Whitney U test, RRMS: Relapsing remitting multiple sclerosis, PMS: Progressive multiple sclerosis

added to 1 ml of the hair sample, taken from the sample that was finalized to 50 ml, and was completed to 10 ml with 2% HNO3 for use in the inductively coupled plasma mass spectrometry (ICP-MS) device. Lithium (Li), magnesium (Mg), manganese (Mn), copper (Cu), zinc (Zn), selenium (Se), strontium (St), nickel (Ni), As, Cd, cobalt (Co), Hg, platinum (Pt), thallium (Tl), Pb, and uranium (U) levels were measured from the blood samples and Li, boron (B), Mg, phosphorus, calcium (Ca), vanadium, chromium, manganese, Co, Cu, Zn, Se, St, zirconium, molybdenum, iodine, barium, beryllium, Al, nickel, As, Cd, antimony, Pt, Hg, Tl, Pb and bismuth levels were measured from the hair samples. The dyed hair samples were placed in a dry Falcon® tube as 0.25-gram samples. For washing, Triton X100 was used and the samples were washed twice with 1:200 v/v, three times with ultrapure water, and twice with acetone. The washed samples were dried at 75 °C. After drying, the samples were again weighed and the results were recorded. After all of the samples had been placed in the microwave vessel, 7 ml of 65% HNO3 + 2 ml hydrogen peroxide were added. Then the samples were wet burned for 15 minutes using the *CEM Mars5 system. After acid digestion, the sample was placed into a Falcon tube and finalized to 50 ml with distilled water. Then, 100 µl of IS (2 ppm In + Ga) was added to 1 ml of the hair samples (taken from the sample that was prepared up to 50 ml) and was completed to 10 ml with 2% HNO3; at this point, the sample could be used in the ICP-MS device.

The study was initiated after obtaining permission from Firat University's Neurology Department and approval from the Firat University Clinical Ethics Committee (decision number: 13; project number: TF 18.58). Consent was obtained from all patients before the procedure.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS 25.0) software was used for statistical analysis. Descriptive statistics of the data included the median, first, and third quartiles, and minimum and maximum frequencies. The Mann–Whitney U test was employed for quantitative variables, while the chi-square test was used for the analysis of qualitative data.

Results

In the hair samples, Al content was found to be 8.98 µg/g in the PMS group and 3.01 µg/g in the RRMS group ($P < 0.001$). Unlike Al, Mg, Ca, Co, Zn, and St elements were observed in high quantities in the RRMS group. For Ca, the median value in the RRMS group was 931 µg/g, and in the PMS group, 400 µg/g ($P < 0.001$); for Mg, the RRMS group result was 118 µg/g, and for PMS, 50 µg/g ($P < 0.001$); for Co, the RRMS group result was 0.0150 µg/g, and for the PMS group, 0.0075 µg/g ($P = 0.008$); for Zn, the value in the RRMS group was 138 µg/g, and in the PMS group, 101 µg/g ($P = 0.008$); for St, the RRMS result was 4.76 µg/g, and in the PMS group, 3.70 µg/g ($P = 0.008$) (Table 2). The reason for the high element levels observed in the RRMS group can be explained by the fact that this patient group was younger and more mobile based on the group's low EDSS score. This may have been caused by auxiliary substances (especially Mg stearate) used in oral preparations prescribed in the early stages of the disease. The Mg, Ca, Zn, Co, and St levels in hair samples were found to be statistically higher in the RRMS than in the PMS group.

When the blood samples were examined, Hg levels were evaluated as follows: RRMS, 0.59 µg/g, and PMS, 0.39 µg/g ($P = 0.047$) (Table 3). Additional data are needed to examine the relationship of Hg with RRMS.

These elements, when evaluated against control groups and large series, may indicate Hg as an early trigger of MS. We did not detect any correlation between the blood and hair samples collected for this study.

The difference in Al levels between the groups aroused the suspicion that Al may cause MS progression.

No significant statistical difference was observed in the sex-based evaluations of blood and hair samples in MS patients included in this study (Tables 4, 5). When the hair analyses of all the patients were examined in terms of having been dyed, the presence of B was found to be statistically higher in those who had not dyed their hair (Table 6). Based on this finding, hair dye was not concluded as a factor that would affect the outcome of our study.

Discussion

MS is a multifactorial disease. Our aim in this study was to review possible triggers during the course of the disease. The research aimed to evaluate the relationship between heavy metal levels and MS subgroups. While previous articles compared MS patients and control groups, in this study, blood and hair samples were audited between subgroups. When heavy metal levels were examined in the hair samples according to MS type, Al levels were found to be statistically higher in the PMS than in the RRMS group. This finding indicates a hypothetical background concerning the role of Al in MS progression that has not previously been addressed. If this can be supported by additional pathogenesis studies, it will enable us to reconsider the role of Al in MS patients.

The analysis revealed notable differences in the concentrations of specific metals between the two groups. For instance, the median concentration of Mg was significantly higher in the RRMS (118 µg/g) than PMS (50 µg/g) group, with a P value of 0.020 indicating a statistically significant difference. This may suggest a potential role of Mg in the pathophysiology of RRMS. However, the use of Mg stearate as an adjuvant in drugs frequently used for patients with RRMS reduces the meaning of this statistical finding. Some studies have suggested that Mg could have a neuroprotective effect, possibly by maintaining the integrity of the blood–brain barrier or by modulating the immune response (16).

Similarly, Al levels showed a significant difference between the two groups, with a median value of 3.01 µg/g for the RRMS and 8.98 µg/g for the PMS group ($P < 0.001$). This indicated a significantly higher concentration of Al in PMS patients. Al is a known neurotoxin and its elevated levels in PMS patients could potentially be linked to the progressive nature of the disease (17). However, further studies are required to confirm this association and better understand the underlying mechanisms.

Interestingly, the levels of Ca showed a contrasting trend, with a significantly higher median concentration in the RRMS (931 µg/g) than in the PMS (400 µg/g) group ($P < 0.001$). This could potentially indicate a disruption in Ca homeostasis in patients with RRMS, which has been associated with neurodegeneration and the pathogenesis of MS (18).

Other metals such as Co and Zn also showed significant differences, with higher concentrations indicated in patients

with RRMS. These findings suggest potential differences in the metabolic and physiological processes of RRMS and PMS, as reflected by the variations in heavy metal concentrations in hair samples. Further studies are needed to understand the underlying mechanisms and to explore the potential of these metals as biomarkers or therapeutic targets in MS.

Al has been implicated in several neurological disorders, including Alzheimer's disease and Parkinson's disease (19,20). It has been suggested that Al might induce neurotoxicity through processes like oxidative stress, inflammation in the CNS, and disruption of the blood–brain barrier (21). Indeed, Al triggers the NLRP3 inflammasome pathway, causing microglial activation (22). It is plausible that elevated Al levels could intensify this inflammatory response, thus potentially contributing to the progression of PMS.

Al exposure can also lead to dysregulation of Ca homeostasis, a crucial process for neuronal health and function (23,24). Given that MS is characterized by demyelination and neurodegeneration, the potential role of Al in inducing neuronal cell death could be significant in the disease's progression. Al's ability to modulate the immune response is well-documented (25). It can alter the function of T-cells and B-cells, both of which are known to play critical roles in the pathogenesis of MS.

While these findings point towards a possible correlation between elevated Al levels and PMS, it is important to note that further research is needed to establish a causal relationship and elucidate the precise mechanisms involved.

The low number of PMS types in our study (because patients who lived in the same environment had to be selected) and the lack of soil, frequently consumed foods, and water analyses of the conditions in which these patients lived limited the study. If

Table 2. Comparison of heavy metal levels in hair samples according to MS type

	RRMS	PMS	*P**
Lithium	0.0113 (0.0000–0.0190)	0.0100 (0.0000–0.1925)	0.951
Beryllium	0.0000 (0.0000–0.0051)	0.0000 (0.0000–0.0083)	0.330
Boron	0.4237 (0.0000–2.2100)	1.6487 (0.0750–2.9907)	0.201
Magnesium	118 (27–239)	50 (28–1179)	**0.020**
Aluminum	3.01 (1.87–5.11)	8.98 (3.17–12.60)	**<0.001**
Phosphorus	127 (93–154)	135 (94–166)	0.551
Calcium	931 (425–1839)	400 (279–621)	**<0.001**
Vanadium	0.0180 (0.0060–0.0425)	0.0168 (0.0050–0.0407)	0.928
Chromium	0.4520 (0.3037–0.4900)	0.4400 (0.3256–0.4721)	0.153
Manganese	0.2533 (0.1150–0.4000)	0.3405 (0.1300–0.5316)	0.120
Cobalt	0.0150 (0.0066–0.0260)	0.0075 (0.0000–0.0150)	**0.008**
Nickel	0.1780 (0.08000.3061)	0.1801 (0.0760–0.3061)	0.960
Copper	11.3 (8.4–15.0)	9.6 (6.8–12.4)	0.083
Zinc	138 (90–210)	101 (69–143)	**0.008**
Arsenic	0.0516 (0.0000–0.0800)	0.0275 (0.0000–0.0980)	0.682
Selenium	0.4140 (0.2316–0.5525)	0.4070 (0.2160–0.5977)	0.977
Strontium	4.76 (2.10–6.98)	3.60 (1.21–4.77)	**0.008**
Zirconium	0.013 (0.007–0.033)	0.017 (0.012–0.028)	0.275
Molybdenum	0.040 (0.021–0.056)	0.053 (0.017–0.075)	0.121
Cadmium	0.0062 (0.000–0.0130)	0.0076 (0.0030–0.0150)	0.346
Antimony	0.0293 (0.0150–0.0450)	0.0300 (0.0128–0.0440)	0.946
Iodine	0.5680 (0.2162–0.8716)	0.4318 (0.2151–0.7450)	0.911
Barium	1.13 (0.50–1.56)	0.87 (0.55–1.19)	0.145
Tungsten	0.0010 (0.0000–0.0020)	0.0016 (0.0000–0.0046)	0.245
Platinum	0.0000 (0.0000–0.0000)	0.0000 (0.0000–0.0000)	0.958
Mercury	0.062 (0.032–0.106)	0.034 (0.011–0.089)	0.061
Thallium	0.0000 (0.0000–0.0030)	0.0000 (0.0000–0.0004)	0.067
Lead	0.175 (0.066–0.409)	0.204 (0.085–0.513)	0.358
Bismuth	0.0080 (0.0025–0.0150)	0.0075 (0.0000–0.0137)	0.688

*Mann–Whitney U test, MS: Multiple sclerosis, RRMS: Relapsing remitting multiple sclerosis, PMS: Progressive multiple sclerosis

Table 3. Comparison of heavy metal levels in the blood by type of MS			
	RRMS n = 66	**PMS n = 34**	***P****
Lithium	1.51 (0.04–11.00)	2.30 (1.22–11.93)	0.171
Magnesium	13835 (11072–17000)	13985 (11347–17430)	0.799
Manganese	17.38 (15.50–24.62)	18.15 (14.70–26.17)	0.749
Cobalt	0.78 (0.48–1.22)	1.06 (0.44–1.55)	0.359
Nickel	10.67 (6.71–14.26)	10.87 (4.96–15.00)	0.841
Copper	1057 (881–1232)	1035 (933–1307)	0.724
Zinc	4639 (3779–5682)	4958 (4070–5929)	0.348
Arsenic	11.66 (9.53–14.41)	11.62 (9.23–15.20)	0.741
Selenium	186 (148–232)	195 (140–223)	0.830
Strontium	15.7 (4.5–20.4)	20.0 (5.1–24.8)	0.303
Cadmium	0.500 (0.354–0.832)	0.568 (0.381–0.777)	0.668
Platinum	0.0000 (0.0000–0.0033)	0.0000 (0.0000–0.0113)	0.493
Mercury	0.59 (0.36–1.76)	0.39 (0.26–1.27)	**0.047**
Thallium	0.0260 (0.0158–0.0398)	0.0360 (0.0177–0.0508)	0.326
Lead	17.3 (13.4–25.4)	19.1 (12.9–26.5)	0.861
Uranium	0.0000 (0.0000–0.0150)	0.0000 (0.0000–0.0180)	0.980

*Mann–Whitney U test, MS: Multiple sclerosis, RRMS: Relapsing remitting multiple sclerosis, PMS: Progressive multiple sclerosis

Table 4. Heavy metal levels in hair samples by gender			
	Male	**Female**	***P***
Lithium	0.011 ± 0.008	0.04 ± 0.28	0.841
Beryllium	0.004 ± 0.006	0.003 ± 0.004	0.615
Boron	1.57 ± 1.65	1.36 ± 1.46	0.762
Magnesium	115.9 ± 123.2	123.56 ± 113.96	0.819
Aluminum	5.43 ± 5.29	6.65 ± 8.18	0.688
Phosphorus	122.61 ± 51.05	130.4 ± 49.8	0.527
Calcium	1248.9 ± 1634.2	938.11 ± 807.42	0.574
Vanadium	0.04 ± 0.07	0.03 ± 0.04	0.741
Chromium	0.36 ± 0.22	0.42 ± 0.20	0.343
Manganese	0.28 ± 0.28	0.31 ± 0.23	0.449
Cobalt	0.01 ± 0.01	0.03 ± 0.12	0.471
Nickel	0.29 ± 0.39	0.23 ± 0.25	0.297
Copper	11.66 ± 4.52	10.69 ± 4.55	0.387
Zinc	189.53 ± 170.40	153.94 ± 179.30	0.169
Arsenic	0.05 ± 0.10	0.07 ± 0.09	0.132
Selenium	0.34 ± 0.20	0.47 ± 0.37	0.137
Strontium	4.40 ± 3.90	4.71 ± 3.86	0.523
Zirconium	0.027 ± 0.026	0.05 ± 0.29	0.352
Molybdenum	0.04 ± 0.02	0.04 ± 0.02	0.841
Cadmium	0.008 ± 0.008	0.009 ± 0.011	0.814
Antimony	0.031 ± 0.020	0.030 ± 0.019	0.869
Iodine	0.518 ± 0.380	0.535 ± 0.369	0.819
Barium	1.37 ± 1.60	16.32 ± 96.34	0.639
Tungsten	0.003 ± 0.007	0.001 ± 0.002	0.738
Platinum	0.0001 ± 0.0003	0.0003 ± 0.0009	0.394
Mercury	0.11 ± 0.13	0.07 ± 0.09	0.176
Thallium	0.0009 ± 0.0016	0.001 ± 0.003	0.621
Lead	0.374 ± 0.452	0.355 ± 0.490	0.600
Bismuth	0.017 ± 0.026	0.014 ± 0.026	0.848

Table 5. Heavy metal levels in blood samples by gender			
	Male	**Female**	***P***
Lithium	8.49 ± 7.40	4.64 ± 6.09	0.117
Magnesium	11760.48 ± 5578.91	14036.62 ± 5889.89	0.102
Manganese	26.21 ± 24.89	21.73 ± 19.13	0.983
Cobalt	1.13 ± 0.80	0.99 ± 0.76	0.300
Nickel	24.33 ± 53.51	13.80 ± 16.51	0.585
Copper	1092.01 ± 229.59	1094.04 ± 289.12	0.976
Zinc	4549.52 ± 1079.86	4971.21 ± 1220.93	0.228
Arsenic	11.50 ± 4.18	12.22 ± 3.84	0.457
Selenium	178.45 ± 48.00	195.02 ± 54.00	0.205
Strontium	16.72 ± 8.73	15.37 ± 9.32	0.451
Cadmium	0.66 ± 0.55	0.699 ± 0.611	0.953
Platinum	0.009 ± 0.030	0.007 ± 0.020	0.745
Mercury	0.991 ± 0.988	0.89 ± 0.77	0.986
Thallium	0.037 ± 0.025	0.03 ± 0.01	0.262
Lead	27.58 ± 34.41	20.95 ± 13.55	0.943
Uranium	0.021 ± 0.042	0.01 ± 0.04	0.977

Table 6. Hair heavy metal values according to the hair dye status of the patients			
Hair dye	**No**	**Yes**	***P***
Lithium	0.04 ± 0.28	0.008 ± 0.007	0.086
Beryllium	0.003 ± 0.005	0.001 ± 0.002	0.086
Boron	1.53 ± 1.52	0.92 ± 1.30	**0.044**
Magnesium	130.43 ± 121.91	88.12 ± 77.80	0.309
Aluminum	6.12 ± 6.91	7.49 ± 10.26	0.952
Phosphorus	127.48 ± 42.64	133.95 ± 73.36	0.607
Calcium	1036.75 ± 1096.20	869.92 ± 743.05	0.938
Vanadium	0.038 ± 0.060	0.025 ± 0.020	0.756
Chromium	0.43 ± 0.22	0.34 ± 0.17	0.277
Manganese	0.307 ± 0.236	0.30 ± 0.27	0.819
Cobalt	0.034 ± 0.123	0.01 ± 0.02	0.205
Nickel	0.26 ± 0.30	0.17 ± 0.18	0.156
Copper	10.66 ± 4.76	11.81 ± 3.49	0.312
Zinc	163.03 ± 195.52	154.94 ± 68.12	0.281
Arsenic	0.06 ± 0.10	0.08 ± 0.07	0.081
Selenium	0.41 ± 0.24	0.57 ± 0.60	0.451
Strontium	4.73 ± 4.13	4.31 ± 2.47	0.823
Zirconium	0.05 ± 0.29	0.01 ± 0.01	0.666
Molybdenum	0.04 ± 0.02	0.03 ± 0.02	0.359
Cadmium	0.009 ± 0.011	0.008 ± 0.008	0.767
Antimony	0.031 ± 0.019	0.02 ± 0.02	0.282
Iodine	0.54 ± 0.36	0.49 ± 0.41	0.464
Barium	10.22 ± 79.95	25.01 ± 107.42	0.959
Tungsten	0.002 ± 0.004	0.0019 ± 0.0017	0.441
Platinum	0.0002 ± 0.0009	0.0002 ± 0.0006	0.518
Mercury	0.075 ± 0.082	0.110 ± 0.162	0.839
Thallium	0.0014 ± 0.003	0.001 ± 0.002	0.947
Lead	0.36 ± 0.50	0.34 ± 0.34	0.518
Bismuth	0.016 ± 0.028	0.0098 ± 0.0157	0.224

detailed alongside other analyses, our outcomes can be used more effectively.

The additives present in the drugs used by patients with MS included in our study were examined. Titanium dioxide (E171) and iron oxide (E172) were frequently found. However, these elements were not included in our study. Excipients in the drugs used by the patients included in the study did not affect the results, except for Mg.

Conclusion

Our analysis of heavy metal concentrations in the hair samples of patients with different types of MS revealed a significantly higher median concentration of Al in patients with PMS compared to those with RRMS. Given Al's established neurotoxic effects and its involvement in neurological disorders, this finding suggests a potential role of Al in the progression of PMS.

Al's neurotoxicity has been linked to oxidative stress, inflammation, disruption of the blood–brain barrier, activation of microglia, and dysregulation of Ca homeostasis, all of which are potentially relevant to the pathogenesis and progression of MS. Furthermore, Al's immunomodulatory properties, particularly its influence on the function of T and B-cells, could be significant, considering the immunological component of MS.

While this study provides a compelling correlation between elevated Al levels and PMS, it is essential to proceed with caution when interpreting these results. Therefore, further research, including prospective cohort studies and experimental investigations, are warranted to confirm these results and to elucidate the precise role of Al in MS. This will not only contribute to our understanding of MS pathogenesis but may also open up new avenues for therapeutic intervention.

Our findings should also prompt a re-evaluation of Al exposure sources, including diet, personal care products, and medical treatments, among MS patients. It is crucial that we develop a comprehensive understanding of the impact of Al on human health, particularly in the context of chronic neurodegenerative diseases like MS.

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Ethics

Ethics Committee Approval: The study was initiated after obtaining permission from Firat University's Neurology Department and approval from the Firat University Clinical Ethics Committee (decision number: 13; project number: TF 18.58).

Informed Consent: Consent was obtained from all patients before the procedure.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.Z.Y., M.G., Design: M.Z.Y., M.G., Data Collection or Processing: M.Z.Y., Analysis or Interpretation: M.Z.Y., M.G., Literature Search: M.Z.Y., M.G., Writing: M.Z.Y., M.G.

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