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ORIGINAL PAPER

Evaluation of heavy metal blood concentrations in patients with essential tremor: A preliminary study*

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Abstract

Essential Tremor (ET) is one the most common mobility disorders, affecting 1% of the population. Both genetic and environmental factors may trigger the pathophysiology that eventually causes ET. Pb blood levels in ET patients have been studied. However, there are no clear data in the literature evaluating the blood levels of other heavy metals (aluminum, chromium, manganese, nickel, copper, zinc, cadmium, antimony, tin) in ET patients. We aimed to investigate the relationship between heavy metal blood levels and tremor characteristics of ET patients. A total of 110 ET patients and 146 healthy controls were included. The control group was subdivided into control-1 (52 patients' household members) and control-2 (94 unrelated healthy individuals). The average age of patients was 52.10±17.00 and that of the control group was 46.00±15.85. All patients underwent a detailed neurological examination, and scores on the Basic Tremor Evaluation Scale and Fahn-Tolosa-Marin Tremor Scales were recorded for the patient group. Peripheral blood samples were collected from all participants and heavy metal levels were examined and analyzed. The Al, Cd, Cr and Sb blood levels were statistically significantly higher in ET patients than in the control group (for Al: $\chi 2$ =8.684, p=0.013, for Cd: F=7.883, p<0.001, for Cr: $\chi 2$ =8.175, p=0.017, for Sb: $\chi 2$ =9.075, p=0.011). The duration of the disease was found to be positively correlated with Al blood levels (r=0.227, p=0.017). Our results revealed that ET is associated with elevated blood levels of Al, Cd, Cr and Sb. Investigating the etiological role of heavy metals will enable the establishment of novel therapeutic approaches to prevent or cure ET.

Keywords: essential tremor, heavy metal, etiology, toxicity, spectrometry

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INTRODUCTION

Essential Tremor (ET) is a chronic neurological disorder characterized by the involvement of the upper extremities and/or other body parts, with distinct postural and kinetic tremors (Louis et al. 2001). ET is one of the most common movement disorders, showing a bimodal increase in incidence with peaks in young adults and those aged 60 and above (Bhidayasiri et al. 2005, Louis et al. 2010). Genetic and environmental factors play a significant role in the etiology of the disease. Studies have explored the association of ET with environmental factors such as heavy metals, organic solvents (Louis et al. 2004), pesticides (Salemi et al. 1998, Louis et al. 2006) and β-carboline alkaloids (Louis et al. 2005), identifying these factors as predisposing elements.

Heavy metals are naturally occurring elements with high atomic weights and at least five times greater density than water (Mora et al. 1992, Tchounwou et al. 2012). Industrial, agricultural, medical and technological applications have led to their widespread distribution in the environment, impacting human health and the ecosystem negatively (He et al. 2005). It is known that exposure to heavy metals can result in the accumulation of these elements in the basal ganglia of the brain, leading to various movement disorders (Chen et al. 2016). This accumulation has been associated with neurodegenerative diseases such as Wilson's disease, Parkinson's disease, Alzheimer's disease and Huntington's disease (Rivera-Mancía et al. 2010, Bandmann et al. 2015). Acute exposure to heavy metals can cause encephalopathy, confusion, mood changes, headaches, seizures, coma, and postural tremor (Caito et al. 2015). Research has been conducted on the detection of lead levels in the blood of ET patients (Louis et al. 2003, Dogu et al. 2007). However, when the literature is reviewed, there are no clear data on the impact of other heavy metals (aluminum, chromium, manganese, nickel, copper, zinc, cadmium, tin, antimony, mercury) to ET.

This study aims to identify the relationship between the levels of heavy metals in their blood and the tremor characteristics of patients diagnosed with ET. Additionally, the goal is to uncover the potential role of these heavy metals in the etiology of the disease.

MATERIALS AND METHODS

Selection of participants

For this study, 110 voluntary patients, who presented to the Department of Neurology Movement Disorders Clinic at Tekirdag Namik Kemal University Training and Research Hospital between December 2019 and March 2022, and 146 voluntary healthy controls were selected. The control group was divided into two groups. Control-1 consisted of primary household mem-

bers of the patients (spouse or healthy individuals sharing the same household in the case of unmarried patients) and unrelated healthy individuals formed control-2. Inclusion criteria were age between 18-80, a definitive diagnosis of ET for patients and voluntary participation in the study. Exclusion criteria were being under 18 or over 80 years of age, known exposure to drugs causing tremor, the presence of toxicity and systemic diseases, such as demyelinating diseases, cerebrovascular diseases, peripheral neuropathy, neurological diseases, e.g. parkinsonism, dystonia, and spinocerebellar ataxia, and refusal to participate in the study.

Demographic, medical history and clinical tests

All participants were provided with information about the study, including its purpose, duration, and expectations. After explaining the details, informed consent forms were obtained from those who agreed to participate in the study. Detailed neurological examinations were conducted by the same neurologist for the patient group, tremor characteristics were determined by applying the Fahn-Tolosa-Marin Tremor Clinical Rating Scale (FTM) and Tremor Research Group Essential Tremor Rating Assessment Performance Subscale (TETRAS). TETRAS and FTM have international validity and are currently used in the clinical evaluation and follow-up of essential tremor (Fahn et al. 1988, Elble et al. 2012). The patients' existing laboratory and neuroimaging tests (Cerebral Computed Tomography, Cranial Magnetic Resonance Imaging) were recorded. Socio-demographic characteristics and medical histories of all participants were also collected.

Determination of heavy metal blood concentrations

Peripheral blood samples were collected from all participants via venipuncture into ethylenediaminetetraacetic acid (EDTA) tubes. Peripheral blood plasma were obtained by centrifugation at 3000 rpm (revolutions per minute) and stored frozen at temperatures below -80°C. The levels of elements in peripheral blood samples were measured at Istanbul University--Cerrahpasa, Faculty of Medicine, Department of Biophysics, using an ICP-OES (Inductively Coupled Plasma Atomic Emission Spectroscopy) device iCAP 6000-Thermo. The concentrations of 10 heavy metals, including aluminum, chromium, manganese, nickel, copper, zinc, cadmium, tin, antimony and lead were determined and recorded in $\mu g\ L^{-1}$ units.

Statistical analyses

Statistical analyses were performed using an IBM SPSS Statistics 24 software package. Frequency tables and descriptive statistics were utilized for the interpretation of findings. Parametric methods were applied for measurement values that followed a normal distribution. Accordingly, the Independent Sample-*t* test (*t*-table value) was used for the comparison of measurement values between two independent groups, and the ANOVA test (*F*-table value) was used for the comparison of three or more indepen-

dent groups. For measurement values that did not follow a normal distribution, non-parametric methods were employed. In line with non-parametric methods, the Mann-Whitney *U* test (*Z*-table value) was used for the comparison of measurement values between two independent groups and the Kruskal-Wallis *H* test (χ2-table value) was used for the comparison of three or more independent groups. The relationship between two quantitative variables with a normal distribution was examined using the Pearson correlation coefficient, and in cases where at least one variable did not exhibit a normal distribution, the Spearman correlation coefficient was used. The relationship between two categorical variables was explored using the Pearson-χ2 cross-tables. The results were presented as a mean ± standard deviation for quantitative variables and as a median (minimum-maximum) or frequency (percentage) for categorical variables. The significance level was set at $p<0.05$.

RESULTS

A total of 110 patients and 146 healthy controls were included in the study between December 2019 and March 2022 at the Department of Neurology Movement Disorders Clinic at Tekirdag Namik Kemal University Training and Research Hospital. The patient group consisted of 50 (45.5%) males and 60 (54.5%) females, Control-1 group consisted of 31 (59.6%) males and 21 (40.4%) females, Control-2 group consisted of 41 (43.6%) males and 53 (56.4%) females. The clinical characteristics of the patient group are presented in Table 1, while a comparison of demographic data between

Table 1

Clinical characteristics of the patient group

Specification	**Patient group**
Mean age	52.10±17.00
Age at diagnosis	38.55±11.12
Duration of disease	13.46±7.83
Tremor distribution	
Upper extremity	80(%72)
Upper extremity+ head	23(%20)
Upper extremity+ lower extremity	2(%1.8)
Upper extremity+ head+ jaw	2(%1.8)
Upper extremity+ lower extremity +Head	1(%0.9)
Medication	
Propranolol	52(%47)
Primidone	13(%11)
Propranolol+ Primidone	7(%6)
Gabapentin	2(%1.8)
Clonazepam	1(%0.9)
Other	2(%1.8)
No treatment	33(%31.5)
TETRAS	7.51±2.16
FTM	27.72±9.23

Abbreviations: FTM – Fahn-Tolosa-Marin Clinical Rating Scale for Tremor, TETRAS – the Essential Tremor Rating Assessment Scale.

Table 2

Comparison of demographic data between patient and control groups

Variable	Patient group (n=110)		Control 1 group (n=52)		Control 2 group (n=94)		Statistical analysis* P value
	n	(%)	n	(%)	n	(%)	
Age							
<30	17	15.5	7	13.5	19	20.2	
30-44	17	15.5	14	26.9	25	26.6	χ^2=7.534
45-59	31	28.2	15	28.8	23	24.5	p=0.274
≥60	45	40.8	16	30.8	27	28.7	
Gender							
Female	60	54.5	21	40.4	53	56.4	χ^2=3.810
Male	50	45.5	31	59.6	41	43.6	p=0.149
Marital status							
Single	25	22.7	6	11.5	26	27.7	χ^2=5.051
Married	85	77.3	46	88.5	68	72.3	p=0.080
Number of children							
No	24	21.8	8	15.4	36	38.3	
1 child	13	11.8	10	19.2	15	16.0	χ^2=18.356
2 children	47	42.8	24	46.2	36	38.3	***p*=0.005**
≥3 children	26	23.6	10	19.2	7	7.4	
Education level							
Primary school or below	43	39.1	20	38.5	25	26.6	
Middle school	15	13.6	5	9.6	2	2.1	χ^2=25.203
High school	17	15.5	12	23.1	11	11.7	***p*<0.001**
Collage and above	35	31.8	15	28.8	56	59.6	
Smoking							
Yes	35	31.8	12	23.1	30	31.9	χ^2=5.539
No	62	56.4	29	55.8	56	59.6	p=0.236
Quit	13	11.8	11	21.1	8	8.5	
Alcohol							
Yes	32	29.1	11	21.2	35	37.2	χ^2=4.259
No	78	70.9	41	78.8	59	62.8	p=0.119

* The relationships were examined using "Pearson's χ^2" cross-tabulation tables.

patient and control groups is given in Table 2. The parameters revealed by examining the personal history, family history and occupational relationships between the patient and control groups are presented in Table 3.

Statistical analysis revealed significant differences between the patient and control groups in terms of Al and Sb blood levels (μg L^{-1}) for Al: χ2=8.684, p=0.013, for Sb: χ2=9.075, p=0.011). Bonferroni-corrected pairwise comparisons were conducted to identify the source of the significant difference, and it was found that the patient group differed significantly from both control-1 and control-2 groups. The Al and Sb blood levels (μg L^{-1}) of the patient group were significantly higher than those of control-1 and

Table 3

Parameters revealed by examining the personal history, family history and occupational relationships between the patient and control groups

Variable	Patient group (n=110)		Control 1 group (n=52)		Control 2 group (n=94)		Statistical analysis P value[1]
	n	(%)	n	(%)	n	(%)	
Medical history							
Hypertension	37	21.5	24	29.3	19	22.9	
Diabetes mellitus	19	11.1	9	11.0	10	12.1	
Hyperlipidemia	21	12.3	8	9.8	6	7.2	
Hyperthyroidism	3	1.8	-	-	3	3.6	
Hypothyroidism	14	8.2	4	4.9	9	10.8	
Anxiety disorder	23	13.4	9	11.0	5	6.0	
Asthma	3	1.8	4	4.9	4	4.8	χ^2=13.613
COPD	3	1.8	1	1.2	1	1.2	p=0.754
Cancer	3	1.8	1	1.2	1	1.2	
Other	45	26.3	22	26.7	25	30.2	
Family history							
No	57	51.8	30	57.7	85	90.4	χ^2=34.675
Yes	53	48.2	22	42.3	9	9.6	***p*<0.001**
Family history feature							
1st-degree relative	46	86.8	21	95.5	8	88.9	χ^2=1.221
2nd-degree relative	7	13.2	1	4.5	1	11.1	p=0.543
Occupation[2]							
Contact group	13	11.8	9	17.3	7	7.4	χ^2=3.287
Non-contact group	97	88.2	43	82.7	87	92.6	p=0.193

Abbreviations: COPD – Chronic Obstructive Pulmonary Disease;z

[1] The relationships between two categorical variables were examined using Pearson-χ2 cross-tabulation tables.

[2] Contact group – industrial worker, farmer, worker, non-contact group – defined as housewife, civil servant, healthcare worker, self-employed, teacher, student, retired, unemployed

control-2 groups. A statistically significant difference was found in Cr blood levels (μg L^{-1}) between patient and control groups (χ2=8.175, p=0.017). Bonferroni-corrected pairwise comparisons identified a significant difference between the patient group and the control-1 group, with the patient group having significantly higher Cr blood levels (μg L-1) than control-1 group. A statistically significant difference was observed in Cd blood levels (μg L^{-1}) between the patient and control groups (F=7.883, p<0.001). Considering the homogeneity of variances, the Tukey pairwise comparisons revealed a significant difference between the patient group and control-2 group, with the patient group having significantly higher Cd blood levels (μg L^{-1}) than control-2 group. A statistically significant difference was found in Zn blood levels (μg L^{-1}) between the patient and control groups (χ2=13.002, p=0.002). Bonferroni-corrected pairwise comparisons identified a significant difference between the patient group and control-2 group, with control-2 group having significantly higher Zn blood levels (μg L^{-1}) than both the patient and control-1 groups. The comparison of heavy metal blood levels between the pa-

Table 4

Comparison of heavy metal blood levels (μg L^{-1}) between patient and control groups

Variable	Patient group (n=110)		Control 1 group (n=52)		Control 2 group (n=94)		Statistical analysis[1] P value
	mean ± SD	median [IQR]	mean ± SD	median [IQR]	mean ± SD	median [IQR]	
Al	0.20±0.11	0.17 [0.2]	0.16±0.10	0.15 [0.2]	0.16±0.10	0.15 [0.2]	χ^2=8.684 **p=0.013 [1-2,3]***
Cd	0.36±0.23	0.32 [0.3]	0.30±0.19	0.27 [0.2]	0.26±0.14	0.25 [0.7]	F=7.883 **p<0,001 [1-3]***
Pb	1.49±1.07	1.21 [1.5]	1.44±1.09	1.20 [1.6]	1.47±1.13	1.37 [1.6]	χ^2=0.281 p=0.869
Cr	0.04±0.02	0.04 [0.0]	0.03±0.02	0.03 [0.0]	0.037±0.02	0.035 [0.0]	χ^2=8.175 **p=0.017 [1-2]***
Ni	0.28±0.11	0.27 [0.2]	0.30±0.12	0.30 [0.2]	0.25±0.12	0.25 [0.2]	F=2.663 p=0.072
Sb	0.16±0.08	0.12 [0.1]	0.11±0.07	0.10 [0.1]	0.11±0.07	0.10 [0.1]	χ^2=9.075 **p=0.011 [1-2,3]***
Sn	0.26±0.15	0.24 [0.2]	0.22±0.11	0.22 [0.1]	0.23±0.10	0.23 [0.1]	χ^2=1.364 p=0.506
Cu	129.74±26.76	128.7 [36.8]	124.65±25.70	124.4 [39.0]	127.66±22.1	128.1 [28.1]	F=0.733 p=0.481
Mn	1.24±0.63	1.10 [1.0]	1.27±0.66	0.98 [1.4]	1.25±0.48	1.15 [0.7]	χ^2=0.665 p=0.717
Zn	94.69±27.32	88.9 [44.0]	91.88±27.39	83.0 [33.4]	104.71±25.25	100.8 [40.7]	χ^2=13.002 **p=0.002 [1,2-3]***

[1] For data with a normal distribution, the ANOVA test (F-table value) statistics were employed to compare measurement values of three or more independent groups. In cases where the data did not follow a normal distribution, the Kruskal-Wallis H test (χ2-table value) statistics were used to compare measurement values of three or more independent groups.

* [1-2] – statistically significant difference between 1 and 2 groups, [1-2,3] – statistically significant difference between 1 and 2, 1 and 3 groups, 1 – patient group, 2 – control 1 group, 3 – control 2 group

tient and control groups is presented in Table 4. The relationship between Al, Sb, Cr, Cd and Zn blood levels and the patient and control groups is shown in Figure 1.

There is no statistically significant relationship between patients' heavy

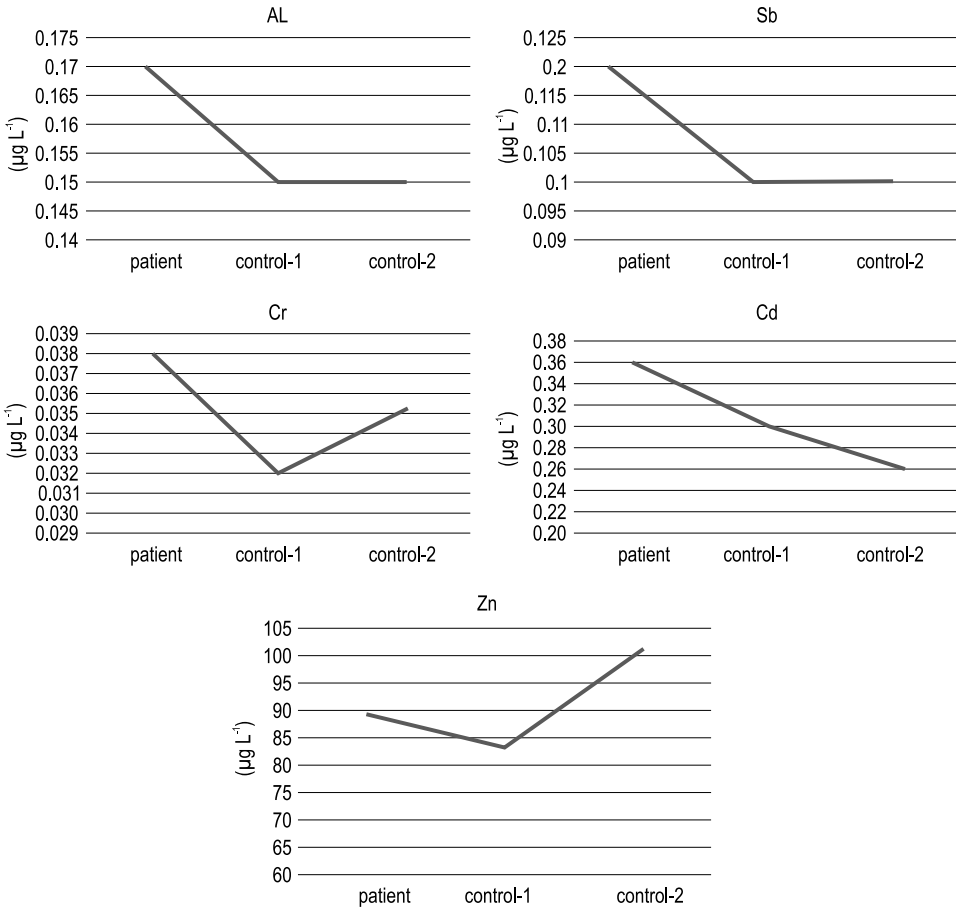

Fig. 1. Relationships between Al, Sb, Cr, Cd and Zn blood levels (µg L^{-1}) in patient and control groups

metal blood levels and TETRAS, FTM Scores (p>0.05). Results of the examination of relationships between heavy metal levels in patients' blood and clinical parameters are presented in Table 5.

There is a statistically significant, weak/moderate positive correlation between disease duration and Al (µg L^{-1}) blood levels (p<0.05). A statistically significant, very weak negative correlation was found between disease duration and Sb (µg L^{-1}) blood levels (p<0.05). There was no statistically significant correlation between disease duration and blood levels of the other heavy metals. The relationship between patients' heavy metal blood levels and disease duration is presented in Table 6.

Table 5

Examination of relationships between heavy metal levels in patients' blood (μg L^{-1}) and clinical parameters

Correlation* TETRAS		Patient group (n=110)	
		FTM	
Al	r	0.101	0.154
	p	0.293	0.109
Cd	r	0.159	0.072
	p	0.097	0.545
Pb	r	0.027	0.009
	p	0.777	0.930
Cr	r	-0.060	-0.028
	p	0.531	0.774
Ni	r	0.079	0.072
	p	0.413	0.453
Sb	r	-0.100	-0.079
	p	0.297	0.411
Sn	r	0.061	0.153
	p	0.524	0.103
Cu	r	0.022	-0.098
	p	0.822	0.309
Mn	r	0.007	0.027
	p	0.943	0.777
Zn	r	-0.090	-0.045
	p	0.350	0.640

Abbreviations: FTM – Fahn-Tolosa-Marin Clinical Rating Scale for Tremor, TETRAS – the Essential Tremor Rating Assessment Scale

* The correlation between two quantitative variables with a normal distribution was investigated using the Pearson correlation coefficient. In cases where at least one variable did not exhibit a normal distribution, the Spearman correlation coefficient was employed.

DISCUSSION

In our study, the levels of Al, Cd, Cr, and Sb in the patient group were found to be statistically significantly higher compared to both control groups. However, as far as we know, there is no clinical or laboratory study in the literature evaluating the relationship between these metals and ET. Considering these results, we believe that there may be an etiological effect of between Al, Cd, Cr, and Sb levels and ET.

In studies investigating the relationship between ET and heavy metals, lead (Pb) has been predominantly examined, and there is a noticeable lack of scientific research on the relationship between other heavy metals and

Table 6

Relationships between heavy metal levels in patients' blood (μg L^{-1}) and disease duration

Correlation*		Patients group (n=110)
		disease duration (year)
Al	r	0.227
	p	**0.017**
Cd	r	0.002
	p	0.982
Pb	r	-0.001
	p	0.990
Cr	r	0.127
	p	0.185
Ni	r	0.055
	p	0.570
Sb	r	-0.221
	p	**0.020**
Sn	r	0.030
	p	0.758
Cu	r	0.019
	p	0.841
Mn	r	-0.184
	p	0.054
Zn	r	-0.018
	p	0.852

* In examining the relationships between two quantitative variables that did not follow a normal distribution, the Spearman correlation coefficient was employed.

ET (Louis et al. 2003, Dogu et al. 2007). ET is most commonly diagnosed between the ages of 20 and 60, showing a bimodal distribution with a higher incidence in young adults and those over 60 (Louis et al. 2010). In our study's patient group the mean age of 52 years and disease duration of 13 years were consistent with the literature (Bain et al. 1994, Benito-León et al. 2005, Louis et al. 2010).

The most frequently observed comorbidity in the patients' medical history was hypertension, seen in 21.5%, followed by anxiety disorder at a rate of 13.4%. Tremor history in first-degree relatives was present in 41.8% of the patient group, and in 6.3%, it was observed in second-degree relatives. These findings are consistent with the expected results for a disease with a genetic predisposition, aligning with the literature (Gulcher et al. 1997, Higgins et al. 1997).

Regarding clinical findings, 72% of the patients exhibited tremor in the upper extremities, 20% in both upper extremities and the head, 1.8% in both upper and lower extremities, 1.8% in the upper extremities, head, and jaw, and 0.9% in both upper and lower extremities and the head. These findings

are consistent with the known distribution of ET in the body and align with previous studies (Louis et al. 2001, Bhatia et al. 2018). The treatment regimens used by the patients are in line with the recommended treatment for ET and existing literature (Deuschl et al. 2011).

Having a family history is known to be a risk factor for ET (Louis et al. 2001, Lorenz et al. 2004). Although there was no statistically significant difference in terms of family history between the patient and control groups in our study, it was observed that approximately half of the patient group had a positive family history. Family history was more prevalent among first-degree relatives compared to second-degree relatives.

Aluminum (Al) is one of the heavy metals that we are frequently exposed to due to its presence in factory wastes, electrical transmission lines, kitchen utensils such as foil, cosmetic materials, explosives, drugs such as antacids, vaccines as adjuvants and food additives. It is known to primarily induce low-amplitude, high-frequency postural tremor in the central nervous system and can lead to neurological conditions such as dementia, amyotrophic lateral sclerosis, and parkinsonism (Shaw et al. 2013, Briffa et al. 2020). In our study, a statistically significant difference was found between the patient and control groups in terms of Al levels. Al blood level in the patient group is significantly higher than in both of the two control groups. These results indicate that Al is one of the etiological factors in essential tremor.

Cadmium (Cd) is another heavy metal frequently encountered through environmental factors. It is found in pesticides, batteries, industrial waste and coatings, particles that cause air pollution, cigarette smoke, shellfish (mussels, shrimp, etc.), mushrooms. It can lead to peripheral and central nervous system diseases such as olfactory dysfunction, neurodegenerative diseases (Alzheimer's Disease, Parkinson Disease), and peripheral polyneuropathy (Viaene et al. 2000, Leal et al. 2012, Briffa et al. 2020). In a study by Söderholm et al., an association was found between Cd exposure and subarachnoid hemorrhage. However, this relationship was linked to an increase in Cd levels in the blood due to smoking rather than smoking itself causing vascular pathologies (Söderholm et al. 2020). In our study, Cd blood level in the patient group was found to be significantly higher than in control-2 group. These results suggest that Cd is one of the etiological factors in essential tremor. Numerically, Cd blood level in control-1 group was higher than In Control-2 group, although this difference was not statistically significant. These findings highlight the common environmental exposure of ET patients and control-1 group, suggesting that control-1 group may carry a risk of increased Cd levels and the emergence of tremors. Recent studies on Cd levels have shown that they are higher in smokers (Viaene et al. 2000). In our study, although the smoking rate was the highest in control-2 group, blood Cd levels were higher in the patient group than in any control group. This situation contradicts the literature information.

Chromium (Cr) toxicity has been reported to cause sensorineural hearing

loss in the central nervous system, cognitive impairment, brain tumors, and peripheral neuropathy in patients with metal hip prostheses (Wise et al. 2022). In our study, Cr blood level in the patient group was found to be significantly higher than in control-1 group. Cr blood level in the patient group was higher than in control-2 group, but there was no statistically significant difference. These results indicate that Cr is one of the etiological factors in essential tremor.

Antimony (Sb) is a heavy metal that humans are exposed to less often than to the other metals mentioned. It causes sleep disorders and obstructive sleep apnea syndrome in the central nervous system (Scinicariello et al. 2017). In a study by Xu et al., exposure to Sb in an animal model resulted in the accumulation of amyloid beta and hyperphosphorylated tau proteins in the brain, leading to dementia pathology. However, there is currently no study conducted in humans (Xu et al. 2021). In our study, the Sb blood level in the patient group was found to be significantly higher than in either of the two control groups. These results suggest that Sb is one of the etiological factors in essential tremor. Although studies have shown that individuals using tobacco products (cigarettes, hookah, etc.) are exposed to Sb (Talio et al. 2019), in our study a high Sb level was not detected in smokers. But passive smoking was not evaluated in our study.

Zinc (Zn) is a heavy metal that we frequently encounter. It is a well-known factor in the pathophysiology of dementia, neurodegenerative disease of the central nervous system by increasing the formation of amyloid beta and hyperphosphorylated tau (Mezzaroba et al. 2019, Briffa et al. 2020, Narayanan et al. 2020).While there are a few publications suggesting that extracellular accumulation of Zn in traumatic brain injury and cerebrovascular diseases increases neurotoxicity, on the contrary, a neuroprotective role that reduces neuronal damage in the same pathologies is mentioned (Levenson et al. 2005, Galasso et al. 2007). Zinc deficiency is thought to cause hyperintense lesions in T2 sequences in brain MRI, loss of function in helper T lymphocytes (Th-1, Th-2), and may lead to Multiple Sclerosis with a decrease in Th-17 cells (Bredholt, Frederiksen 2016). In our study, Zn blood levels in control-2 group were significantly higher than in the patient and control-1 groups. The higher Zn blood levels in control-2 group compared to the patient group can be explained by the neuroprotective effect of Zn.

Lead (Pb) is one of the heavy metals with the most widespread exposure. It is found in ceramics, paints, batteries, industrial alloys, electronic devices, batteries, pesticides, sports equipment, ammunition and explosives, red meat, wine, shellfish and grains. It causes attention deficit, fatigue, sleep disorder, polyneuropathy, headache, delirium, seizure, changes in consciousness (from full alertness to coma), encephalopathy and tremor as a result of basal ganglia accumulation (Wani et al. 2015). It has been shown that lead exposure can cause acute and chronic progressive disorders accompanied by action tremor in laboratory animals and humans (Goldings et al.

1982). Previous studies by Louis et al. and Dogu et al. showed that ET patients had higher blood lead levels (Louis et al. 2003, Dogu et al. 2007). Interestingly, in our study, there was no statistically significant difference in lead levels between the patient and control groups. However, the lead blood level in the patient group was higher than in control-1 and control-2 groups, although it did not show statistical significance. We think that such tests may show statistical significance in a study with more patients and control groups.

Occupational exposure is a significant risk factor for heavy metal toxicity, particularly in industrial areas, metal processing, refining, casting-soldering works, mining, and farming (European Commission, 2009). In the literature, a study by Louis and colleagues evaluated blood lead levels in ET patients, and no statistically significant difference was found between ET and control group in terms of occupational exposure (Louis et al. 2003). In a study by Dogu et al., where they evaluated Pb blood levels in ET, retailers made up 6% and industrial workers constituted 2% of the contact occupational group. At the same time, by dividing the patients into two groups as active workers and retirees in terms of their occupational exposure, a statistically significant difference was detected between the ET and control groups in terms of active workers (Dogu et al. 2007). Nevertheless, in our study, no significant difference was found in the levels of other heavy metals in the patient group based on occupational exposure, except for Cr. The Cr blood levels of patients in the non-contact group are significantly higher than in the contact group. This situation may be attributed to the small number of participants in the contact group in patients.

Regarding the clinical severity of the disease, Louis and colleagues did not find a significant relationship between blood lead levels and TETRAS and FTM, while Dogu and colleagues found a positive correlation in their study (Louis et al. 2003, Dogu et al. 2007). In our study, no statistically significant relationship was found between the blood levels of all heavy metals and TETRAS, FTM scores in patients. However, in Dogu and colleagues' study, no significant relationship was found between the duration of the disease and blood lead levels (Dogu et al. 2007). We found a positive correlation between disease duration and Al blood levels, but to the best of our knowledge, there is no publication on this subject in the literature. Conversely, we found a negative correlation between disease duration and Sb blood levels.

Although heavy metals are known to be one of the characteristic etiological factors of ET, clinical studies have been limited to Pb blood levels. Various effects of all these heavy metals on the central and peripheral nervous systems have been previously reported. However, as far as it is known, there is no study investigating the relationship between these heavy metals and ET. A strong aspect of our study is that the relationship of ET not only with Pb but also with other heavy metals (Al, Cd, Cr, Ni, Sb, Sn, Cu, Mn, Zn) has been evaluated with a current and valid methodology. We hope that

our study will be pioneering in this regard. A major limiting factor of our study can be considered the inability to evaluate the effect of levels of heavy metals on other tissues such as bones and hair, due to the cumulative accumulation property of heavy metals.

CONCLUSIONS

Considering the results of our study, we think that more valuable results will be obtained with prospective, longitudinal clinical follow-up studies with a larger cohort and therefore more comprehensive evaluation. With a better understanding of the underlying mechanisms of the disease, investigating the etiological role of particular heavy metals will enable the establishment of novel therapeutic approaches to prevent or cure ET.

In many areas of metal pollution, chronic low-dose exposure to multiple elements is a major public health concern. Elucidating the mechanisms of heavy metal interactions is essential for health risk assessment and management of chemical mixtures. Hence, research is needed to further elucidate the molecular mechanisms and public health impact associated with human exposure to mixtures of toxic metals. It is particularly important to encourage studies in the areas of public health and human exposure.

Supplementary materials

Supporting Information Appendix 1, Tetras Scale – Elble, R. *et al.* (2012) 'Reliability of a new scale for essential tremor', *Movement Disorders*, 27(12), available: http://dx.doi.org/10.1002/mds.25162

Supporting Information Appendix 2, FTM Scale – Fahn, S., Tolosa, M., Marin, C. (1988) 'Clinical Rating Scale for Tremor', *Parkinson's Disease and Movement Disorders*. Baltimore-Munich:Urban&Schwarzenberg, p. 225-34.

Author contributions

I.K. – conception, data analysis, writing the manuscript, critical review of manuscript, A.A.G. – conception, critical review of manuscript, N.B. – data analysis, critical review of manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The author declares that there is no conflict of interest in the materials or methods used in this study or the findings in this paper.

Ethical compliance statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by Ethics Committee of Tekirdag Namik Kemal University Faculty of Medicine (Approval numbers: 2019.211.11.08)

All patients provided written informed consent prior to their inclusion. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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