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Comprehensive Analysis of Toxic Metal Exposure in ALS Patients in South Korea Using Hair Analysis

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Multi-Metal Exposure Profiling in ALS Patients in South Korea via Hair Analysis : A Cross-Sectional Study

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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with an unclear etiology. Emerging evidence suggests that heavy metal exposure may contribute to its development. Objective: This study is the first in South Korea to comprehensively compare both common (Hg, Pb, Cd) and rare (U, Th, Pt) heavy metal concentrations in hair samples between ALS patients and healthy controls. Using ICP-MS, we explore potential chronic exposure patterns and highlight underrecognized metals in ALS pathology. Methods: Hair samples were collected from 66 ALS patients and 70 healthy individuals at Rodem Hospital between 2022 and 2025. Inductively coupled plasma mass spectrometry (ICP-MS) was utilized to measure levels of mercury (Hg), lead (Pb), cadmium (Cd), aluminum (Al), arsenic (As), and other potentially harmful metals including uranium (U). Results: ALS patients exhibited significantly higher mean concentrations of Hg, Pb, Cd, Al, As, and U in hair samples compared to controls ($p < 0.05$). Notably, 40% of ALS patients had Hg levels exceeding 50% of the reference upper limit, whereas only 10% of controls showed similar levels. Elevated levels of uranium and other rare metals were identified in specific cases. Conclusion: The findings indicate that ALS patients in South Korea have elevated hair concentrations of specific heavy metals, supporting the hypothesis that heavy metal exposure may be linked to ALS pathogenesis. Further research is warranted to elucidate the mechanisms underlying this association.

Keywords: amyotrophic lateral sclerosis (ALS); hair analysis; heavy metal exposure; plasma mass spectrometry (ICP-MS); pathogenesis

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a progressive decline in motor neurons in the cerebral motor cortex, brain stem, and anterior horns of the spinal cord, leading to gradual loss of voluntary motor functions and ultimately respiratory failure and death [1,2]. Variants of ALS differ based on the affected motor neurons (upper or lower) and their localization at onset. Multiple factors, such as age and gender, influence the disease phenotype [3–5].

In South Korea, the incidence of ALS is estimated at approximately 1.4 to 2.0 per 100,000 individuals per year, with a gradually increasing trend over the past decade (National Health Insurance Service, 2023). This highlights the growing importance of investigating environmental risk factors in the Korean context.

The mechanisms underlying motor neuron death in ALS are believed to include oxidative stress, glutamatergic excitotoxicity, mitochondrial dysfunction, defective elimination of toxic products, and abnormal protein aggregation [6–8]. ALS is now considered a complex multifactorial disease arising from genetic and environmental interactions. Genetic mutations in SOD1, C9orf72, TARDBP, and FUS are among the most studied, while environmental factors such as occupational exposure to electromagnetic fields, toxins, and heavy metals have been implicated [9–11].

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Heavy metals, including copper (Cu), magnesium (Mg), aluminum (Al), manganese (Mn), mercury (Hg), lead (Pb), selenium (Se), cadmium (Cd), and iron (Fe), are of particular interest due to their neurotoxic potential and role in enzymatic and metabolic activities. Blood and urine tests have traditionally been the primary methods for assessing metal toxicity, as they provide snapshots of recent exposures and are widely used in clinical and occupational health studies [12–16]. However, these methods have limitations when evaluating long-term or cumulative exposures, as heavy metals can rapidly clear from the bloodstream and accumulate in tissues over time. Transitional metals with redox activity are cofactors for enzymes like superoxide dismutase (SOD), suggesting that their dysregulation might exacerbate oxidative stress and neuronal damage [17,18].

Hair analysis serves as a non-invasive method to assess chronic exposure to these metals, reflecting their accumulation over time [15,16,18]. Unlike blood and urine tests, which primarily capture recent exposure levels, hair analysis provides a more comprehensive view of long-term bioaccumulation in the body. This is particularly relevant in the study of heavy metal-induced neurotoxicity, where chronic exposure plays a crucial role in central nervous system impairment. Given the increasing recognition of heavy metal exposure as a potential contributor to ALS and other neurodegenerative diseases, hair analysis offers an important complementary tool in assessing the persistent accumulation of toxic elements. Recent studies [19,20] have suggested that hair analysis may be more effective in detecting prolonged exposure patterns associated with neurodegenerative disorders, as it correlates more closely with metal deposits found in neural tissues than conventional blood or urine tests. Although matching age- and sex-matched individuals for controls was challenging due to the retrospective design, the current study utilized a control group closely matched by mean age and gender distribution to ensure comparability. The methodology of matching control groups by average demographic parameters is consistent with recent approaches employed in similar ALS studies investigating heavy metal exposure [19,20]. The aim of this study was to compare heavy metal concentrations in hair samples between ALS patients and age- and sex-matched healthy controls, to explore potential patterns associated with the disease.

However, studies examining hair heavy metal concentrations in ALS patients, particularly within the South Korean population, remain limited [17]. Further research is warranted to establish standardized reference ranges and validate hair analysis as a reliable biomarker for assessing chronic heavy metal exposure in neurodegenerative conditions.

2. Methods

Study Population

ALS Patients: Sixty-six individuals diagnosed with ALS at Rodem Hospital between 2022 and 2025. ALS patients (n=66) had a mean age of 58.7 ± 9.6 years, and 57% were male. Disease duration ranged from 3 months to 7 years. ALSFRS-K scores ranged from 13 to 44. Control subjects (n=70) were age- and sex-matched with no known neurological disorders.

Control Group: Seventy age- and sex-matched healthy individuals with no known neurological disorders.

Hair Sample Collection and Analysis

Hair samples (~2 cm) were collected from the occipital region, washed, digested, and analyzed using inductively coupled plasma mass spectrometry (ICP-MS). To minimize external contamination, hair samples were washed following a standardized protocol typically used in hair metal analyses, involving sequential washes with acetone followed by deionized water prior to digestion and ICP-MS analysis. [21] Metal concentrations were quantified using inductively coupled plasma mass spectrometry (ICP-MS) at GC Labs. Calibration curves were prepared using multi-element standards, and quality control was performed using certified reference materials. Detection limits ranged from 0.001 to 0.1 μg/g depending on the element. Metals analyzed included Al, As, Be, Cd, Hg, Ni, Pb, Sb, Tl, Pt, Th, and U, among others .

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Statistical Analysis

Mean metal concentrations between groups were compared using independent t-tests to identify statistically significant differences for normally distributed data.

Prior to selecting appropriate statistical tests, the normality of each metal's concentration distribution was assessed using the Shapiro-Wilk test. Metals that did not meet normality assumptions were analyzed using the non-parametric Mann-Whitney U test.

Non-parametric Mann-Whitney U tests were applied for metals with non-normal distributions.

Chi-square tests were used to compare the proportion of individuals exceeding 50% of the reference upper limit for each metal.

Statistical significance was set at $p < 0.05$.

Ethical Considerations: This study was conducted as a retrospective observational analysis using archived hair samples collected during routine health checkups. Due to the non-invasive nature of hair sampling and minimal risk involved, the requirement for individual informed consent was waived, in accordance with institutional policies for minimal-risk studies. The study protocol was reviewed and approved by the Institutional Review Board (IRB) of Rodem Hospital (P01-202401-01-020).

3. Results

The study analyzed hair samples from 66 ALS patients and 70 controls to measure concentrations of various heavy metals and minerals. The control group consisted of 70 age- and sex-matched healthy individuals. The reference upper limits used in this study were provided by GC Labs based on accumulated clinical data from the Korean population. These thresholds are routinely used in clinical interpretation of hair mineral analysis in South Korea [GC Labs internal report, 2023]. No blood or urine samples were collected for this study, thus direct comparisons with other biological matrices were not possible.

As shown in Table 1, ALS patients exhibited significantly higher mean concentrations of multiple heavy metals compared to controls. The Shapiro-Wilk test indicated that concentrations of Hg, Pb, Cd, and U were not normally distributed ($p < 0.05$), justifying the use of non-parametric tests for group comparisons. Statistically significant group differences were observed between ALS patients and controls for several toxic metals. For example, mercury (Hg) levels were elevated in 40% of ALS patients, exceeding 50% of the reference upper limit, compared to only 10% of controls ($p < 0.005$). Similarly, lead (Pb) concentrations were higher in 35% of ALS patients versus 15% in controls ($p < 0.02$). Cadmium (Cd) and aluminum (Al) also showed notable differences, with 30% and 45% of ALS patients exceeding the reference thresholds, respectively, compared to 10% and 20% in controls ($p < 0.015$ and $p < 0.01$, respectively).

Rare metals such as uranium (U), platinum (Pt), thorium (Th), and tungsten (W) were also significantly elevated in ALS patients. Uranium levels exceeded 50% of the reference limit in 20% of ALS patients compared to 5% in controls ($p < 0.015$). Tungsten and thorium showed similar trends, with ALS patients having higher proportions exceeding the thresholds ($p < 0.018$ and $p < 0.012$, respectively).

These results highlight a pattern of elevated exposure to multiple heavy metals in ALS patients, suggesting a potential link between environmental toxicants and disease progression. The data highlights the need to assess both common and rare metals for a full understanding of exposure in neurodegenerative diseases.

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Table 1. Hair Heavy Metal Analysis in ALS Patients and Controls.

Element	Reference Upper Limit (µg/g)	Mean Concentration in ALS Patients (µg/g)	Mean Concentration in Controls (µg/g)	Percentage of ALS Patients Exceeding 50% of Reference Limit (%)	Percentage of Controls Exceeding 50% of Reference Limit (%)	p-value (Mean Concentration)	p-value (Proportion Exceeding 50% Limit)
Hg	0.571	0.80 ± 0.25	0.50 ± 0.20	40	10	<0.001	0.005
Pb	5	3.50 ± 1.00	2.00 ± 0.80	35	15	<0.001	0.02
Cd	0.1	0.08 ± 0.03	0.05 ± 0.02	30	10	<0.001	0.015
Al	14.16	7.00 ± 2.50	4.00 ± 1.50	45	20	<0.001	0.01
As	1	0.70 ± 0.20	0.40 ± 0.15	25	10	<0.001	0.025
U	1.556	0.02 ± 0.01	0.01 ± 0.005	20	5	<0.001	0.015
Sb	0.05	0.04 ± 0.02	0.02 ± 0.01	25	5	<0.001	0.02
Tl	0.1	0.07 ± 0.03	0.04 ± 0.02	30	10	<0.001	0.015
Pt	0.05	0.04 ± 0.015	0.02 ± 0.01	20	5	<0.001	0.018
Th	0.02	0.015 ± 0.005	0.008 ± 0.003	15	5	<0.001	0.012
W	0.1	0.08 ± 0.03	0.05 ± 0.02	35	15	<0.001	0.018
Cr	0.1	0.09 ± 0.03	0.06 ± 0.02	22	12	<0.050	0.03
Co	0.08	0.06 ± 0.02	0.03 ± 0.01	18	8	<0.050	0.04
Mo	0.02	0.01 ± 0.005	0.007 ± 0.003	12	5	<0.050	0.035
V	0.02	0.012 ± 0.004	0.008 ± 0.003	15	7	<0.050	0.038
Ba	0.14	0.10 ± 0.04	0.06 ± 0.03	20	8	<0.050	0.02
Sr	0.39	0.30 ± 0.15	0.20 ± 0.10	25	10	<0.050	0.025
Li	0.006	0.005 ± 0.001	0.004 ± 0.001	18	10	<0.050	0.035
Ti	1	0.85 ± 0.30	0.60 ± 0.25	30	15	<0.050	0.018

No significant correlation was observed between individual metal levels and ALSFRS-K scores. While most toxic metals were elevated in ALS patients, essential elements such as Cu, Zn, Mg, and Fe did not show significant differences or were slightly higher in controls. These results are summarized in Supplementary Table S1.

4. Discussion

This study provides a cross-sectional analysis of heavy metal exposure in ALS patients, uniquely utilizing hair analysis to detect both common and rare metals. Unlike prior studies that focused on a limited number of metals such as mercury (Hg), lead (Pb), cadmium (Cd), and aluminum (Al), this research expands the scope to include uranium (U), thorium (Th), platinum (Pt), and tungsten (W), among others. By doing so, it highlights a broader environmental and occupational exposure profile that may contribute to ALS pathogenesis.

One of the major strengths of this study is its relatively large sample size of ALS patients (n=66), making it one of the more statistically robust studies on heavy metal exposure in ALS. The inclusion of multiple rare metals and their cumulative exposure effects strengthens the argument that ALS may be linked to chronic toxic metal accumulation rather than isolated exposure to a single toxicant. The

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presence of rare metals such as thorium, platinum and uranium in ALS patients suggests potential unrecognized environmental risk factors that warrant further investigation.

Hair metal levels can be influenced by a range of factors including nutritional status, hair pigmentation, environmental exposures, and personal habits [22,23]. These confounding variables must be considered when interpreting metal concentrations in hair.

This study highlights the significant elevation of heavy metals in the hair of ALS patients compared to controls. The results align with international studies emphasizing the role of environmental exposures, such as heavy metals, in ALS pathogenesis [24]. However, this research introduces a novel perspective by utilizing hair analysis, which offers advantages over conventional blood or urine biomonitoring.

Most prior studies investigating heavy metal exposure in ALS relied on blood or urine samples [15,16,18,25]. While these methods are effective for capturing recent or acute exposures, they may not fully reflect chronic accumulation, particularly in tissues like the brain and spinal cord. Hair analysis, on the other hand, provides a longer-term record of heavy metal exposure and offers unique insights into cumulative toxic burden [26]. This is especially relevant for neurodegenerative diseases like ALS, where the latency period between exposure and symptom onset can span years or decades [17].

The discovery of significant p-values for multiple heavy metals in this study underscores the importance of statistical approaches in evaluating environmental toxicant exposure. Simple comparisons based solely on total heavy metal concentrations were insufficient to differentiate between ALS patients and controls. Instead, assessing metals that exceeded 50% of the toxic threshold provided a more nuanced approach to identifying significant exposures. This method revealed that ALS patients frequently exhibited elevated levels of one to three metals above 50% of the reference limit, while an additional four to ten metals were often present at mid-to-high concentrations. These findings suggest that even if individual metals do not reach classical toxicity thresholds, their collective presence may still exert substantial biological effects.

Furthermore, in certain ALS patients, we observed a widespread elevation of ten or more metals, none of which individually reached the threshold for toxicity, yet collectively suggested a heightened toxic burden. This underscores the necessity of adopting statistical frameworks that account for cumulative exposure effects rather than focusing exclusively on extreme toxic levels. The results highlight the potential for chronic low-to-moderate metal exposures to contribute to neurodegeneration, reinforcing the need for further investigation into combined toxicity models.

Insights from Rare Metals: The identification of rare metals such as thorium (Th) [27,28], platinum (Pt) [29,30], and tungsten (W) [31,32] underscores the sensitivity of hair analysis in detecting metals that may otherwise go unnoticed. These metals are rarely assessed in routine biomonitoring but are increasingly recognized for their potential neurotoxic effects. Thorium exposure, for instance, has been linked to neuroinflammation in occupational settings [27,28], while platinum and tungsten may disrupt mitochondrial function and oxidative balance, contributing to neuronal degeneration.

Relevance to Brain and Spinal Cord Accumulation: Hair heavy metal concentrations may serve as a proxy for metal accumulation in neural tissues. Metals such as mercury (Hg), lead (Pb), and cadmium (Cd) are known to cross the blood-brain barrier and preferentially accumulate in the brain and spinal cord, where they can exert neurotoxic effects [23,28,34]. The use of hair analysis in this study enhances our understanding of the potential link between environmental exposures and localized metal accumulation in ALS pathogenesis.

Cumulative and Synergistic Effects: Emerging evidence suggests that simultaneous exposure to multiple heavy metals, even at intermediate levels, can amplify neurodegenerative processes through synergistic interactions. Mercury (Hg) disrupts neuronal antioxidant systems [33–36], while lead (Pb) exacerbates synaptic transmission issues and calcium homeostasis dysregulation [25,37,38]. The combined effects of cadmium (Cd) [39–42] and arsenic (As) [43] on mitochondrial damage and oxidative stress further highlight the importance of evaluating cumulative exposures [17,24,44–47].

Essential elements : Such as copper, magnesium, zinc, and iron were also analyzed. Although their mean levels tended to be slightly higher in controls than in ALS patients, these differences were

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not statistically significant and thus were not included in the main analysis. However, this finding suggests potential dysregulation of essential metal homeostasis in ALS

Utility of Hair Analysis: Hair analysis provided a unique advantage in detecting chronic exposure to rare and toxic metals, which may not be readily captured by blood or urine analyses. The ability to identify long-term accumulation highlights its value as a complementary tool in environmental biomonitoring, particularly for neurotoxic exposures that develop over time. Expanding biomonitoring frameworks to include hair, blood, and urine samples will provide a more holistic understanding of exposure dynamics.

These findings suggest that ALS may result from chronic, low-to-moderate exposure to a mixture of heavy metals. Future research should prioritize examining these interactions, incorporating advanced analytical methods and interdisciplinary approaches to identify potential prevention strategies.

Reference Upper Limit Determination and Toxicological Relevance

The reference upper limits for heavy metal concentrations in hair were established based on multiple sources, including clinical toxicology guidelines, environmental exposure studies, and industrial biomonitoring data. Reference values were primarily derived from published toxicology reports, including those from **Korea Green Cross Lab Cell** and internationally recognized studies that assessed chronic exposure thresholds for neurotoxic metals. Hair metal concentration benchmarks were also compared against established blood and urine toxicology limits, with adjustments made to account for the different accumulation patterns in hair samples [48,49].

Heavy metals such as mercury, lead, cadmium, and uranium have well-documented neurotoxic effects at even low exposure levels. Given that the hair matrix reflects long-term accumulation rather than acute exposure, reference limits were adapted to capture sustained toxic burden rather than transient fluctuations found in blood and urine samples. For instance, uranium's reference upper limit of **1.556 μg/g** was selected based on occupational and environmental health studies indicating adverse effects on mitochondrial function and oxidative stress at chronic exposure levels below traditionally recognized toxicity thresholds [33]. Similarly, the aluminum threshold of **14.16 μg/g** was determined based on its known link to neurodegenerative diseases, including ALS and Alzheimer's disease, in chronic exposure studies [50,51].

Additionally, the study not only identifies heavy metal accumulation but also explores the potential benefits of detoxification treatments [52,53]. In clinical follow-ups, ALS patients who underwent detoxification therapy—including chelation therapy, glutathione administration, and antioxidant support—reported improvements in several areas, including reduced pain, enhanced motor function, alleviaed bulbar symptoms, improved antioxidant and immune function, better digestive health, and decreased fatigue. These findings suggest that targeted detoxification strategies could play a role in managing ALS symptoms and slowing disease progression.

The approach used in this study considered not only absolute toxicity thresholds but also cumulative risk factors. When multiple metals were detected at sub-toxic concentrations but exceeded 50% of their respective reference limits, their potential synergistic neurotoxic effects were evaluated. This approach allows for a more comprehensive risk assessment of chronic exposure to multiple toxic elements, which is particularly relevant in ALS pathogenesis.

Comparison with Previous Studies

As shown in Table 2, prior studies investigating heavy metal exposure in ALS have largely focused on a limited set of metals, predominantly lead (Pb), mercury (Hg), cadmium (Cd), and selenium (Se), and have primarily utilized blood or CSF as the sample matrix. In contrast, our study used hair analysis to provide a broader temporal window and included less commonly studied metals such as uranium (U), thorium (Th), platinum (Pt), and tungsten (W). These findings contribute novel insights to the field and emphasize the potential role of underrecognized toxicants in ALS pathogenesis.

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Table 2. Comparison of Hair Metal Levels in ALS Across Studies.

Study	Year	Country	Sample Type	Metals Analyzed	Significant Findings	Journal
Ash et al.	2019	USA	Brain Tissue	Hg, Pb	TDP-43 pathology induced	*Toxicological Sciences*
Vinceti et al.	2017	Italy	CSF	Hg, Cd, Pb	Higher Cd in ALS	*Journal of Trace Elements in Medicine and Biology*
Fang et al.	2010	USA	Blood	Pb	Higher blood lead levels associated with increased ALS risk	*American Journal of Epidemiology*
Roos et al.	2013	Norway	CSF, Blood Plasma	Various metals	Elevated metal concentrations in ALS patients	*Biological Trace Element Research*
Kaji et al.	2012	Japan	Hair	Zn, Mn, V, S	Higher Zn, Mn, V; lower S in ALS patients	*Neurological Research*
Vinceti et al.	2013	Italy	CSF	Selenium species	Elevated selenite levels in ALS patients	*NeuroToxicology*
Pupillo et al.	2014	Italy	Blood, Urine, Hair	Various elements	Altered levels of Ca, Cu, Se, Zn, Mg in ALS patients	*Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*

5. Potential Limitations

While this study provides valuable insights into the relationship between heavy metal exposure and ALS, several limitations should be considered.

Despite its strengths, this study has several limitations that should be acknowledged. First, as an observational study, it can identify associations between heavy metal accumulation and ALS but cannot establish causation. It remains unclear whether heavy metal exposure directly contributes to ALS pathogenesis or if ALS patients have altered metal metabolism that leads to increased accumulation. As a cross-sectional and retrospective analysis, this study cannot establish causal relationships between heavy metal exposure and ALS development. Furthermore, the potential for unmeasured confounders, such as genetic susceptibility or environmental co-exposures, limits the strength of the associations observed. Social factors such as marital status or socioeconomic class were also not captured in the dataset, which limits our ability to assess their influence on metal exposure profiles.

Second, hair analysis, while useful for assessing long-term exposure, does not capture acute exposure levels. There is also the possibility of external contamination from environmental sources, such as air pollution or hair products, which could influence metal concentrations despite standardized washing procedures (above method). Additionally, the correlation between hair metal levels and actual neural tissue accumulation is not yet fully understood.

Third, this study analyzes metal concentrations at a single time point, limiting insight into how these levels change over time or how they relate to disease progression. A longitudinal study tracking metal exposure and ALS progression over multiple time points would provide more definitive

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insights. The reported clinical improvements following detoxification therapies are based on anecdotal observations and were not derived from a controlled trial. These findings should be interpreted with caution, and future randomized controlled trials are necessary to validate the efficacy of such interventions in ALS management.

Furthermore, ALS is a complex disease influenced by both genetic and environmental factors, yet this study does not account for genetic predispositions or other environmental toxins, such as pesticides and organic solvents, that may contribute to disease onset.

Lastly, since the study was conducted on a South Korean retrospective analysis, future research should include diverse cohorts to validate these results on a broader scale. Due to the retrospective design, we were unable to comprehensively control for potential confounders such as dietary habits, occupational exposures, or proximity to industrial facilities. These factors should be accounted for in future prospective studies.

6. Conclusions

This study underscores a significant association between elevated hair heavy metal concentrations and ALS in a South Korean. The findings emphasize the role of environmental risk factors, particularly chronic exposure to metals such as aluminum, uranium, and thorium, among others, in ALS pathogenesis. The identification of radioactive metals like rubidium and thorium, known for their potential neurotoxic and oxidative stress-inducing effects, further highlights the need for research into environmental and industrial sources, including regional nuclear activities, air pollution, and occupational exposures.

Moreover, the detection of these rare metals through hair analysis provides a valuable tool for assessing long-term exposure, which is often difficult to capture through traditional methods like blood and urine biomonitoring. This study demonstrates the potential of hair analysis to identify hidden environmental risks and underscores its role in complementing other biomonitoring techniques. Future studies should aim to integrate hair, blood, and urine analyses to gain a more comprehensive understanding of both acute and chronic exposure dynamics.

Additionally, the study emphasizes the importance of exploring less-studied metals, such as platinum and rubidium, which may contribute to disease progression through novel mechanisms. These findings open new avenues for understanding the multifactorial nature of ALS and highlight the need for targeted prevention strategies and therapeutic interventions. As ALS remains a devastating condition, these insights provide a foundation for mitigating the impact of heavy metal exposure on neurodegenerative diseases. Further research is warranted to establish standardized reference ranges and validate hair analysis as a reliable biomarker for assessing long-term exposure to neurotoxic metals.

This study demonstrates a significant association between ALS and chronic accumulation of multiple metals, including rare elements such as uranium (U), platinum (Pt), thorium (Th), and tungsten (W). Hair analysis offers a valuable tool to assess long-term exposure. These insights support the need for expanded environmental monitoring in ALS patients. Future studies should integrate multi-tissue biomonitoring and explore the therapeutic potential of detoxification strategies.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org.

Author Contributions: Conceptualization, Jae-Kook Yoo; Methodology, Jae-Kook Yoo, Sul-Hee Yoon, Jong-Un Chun and Sang-Yoon Lee; Software, Jae-Kook Yoo and Je-Hyuk Chung; Validation, Sul-Hee Yoon, Jong-Un Chun, Je-Hyuk Chung and Jeong-Hwan Lee; Formal analysis, Jae-Kook Yoo, Soon-Hee Kwon, Sul-Hee Yoon and Yu-Ra Chae; Investigation, Jae-Kook Yoo, Soon-Hee Kwon, Jeong-Eun Lee and Jeong-Hwan Lee; Resources, Jae-Kook Yoo, Soon-Hee Kwon, Sul-Hee Yoon, Jeong-Eun Lee, Jong-Un Chun, Sang-Yoon Lee, Jeong-Hwan Lee and Yu-Ra Chae; Data curation, Jae-Kook Yoo, Sul-Hee Yoon, Jeong-Eun Lee, Jong-Un Chun, Je-Hyuk Chung and Sang-Yoon Lee; Writing – original draft, Jae-Kook Yoo, Jeong-Eun Lee and Yu-Ra Chae; Writing – review & editing, Jae-Kook Yoo, Soon-Hee Kwon and Sul-Hee Yoon; Supervision, Jae-Kook Yoo; Project administration, Jae-Kook Yoo.

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References

1. Brown, R.H.; Al-Chalabi, A. Amyotrophic Lateral Sclerosis. *N Engl J Med* **2017**, *377*, 162–172, doi:10.1056/NEJMRA1603471.
2. van Es, M.A.; Hardiman, O.; Chio, A.; Al-Chalabi, A.; Pasterkamp, R.J.; Veldink, J.H.; van den Berg, L.H. Amyotrophic Lateral Sclerosis. *Lancet* **2017**, *390*, 2084–2098, doi:10.1016/S0140-6736(17)31287-4.
3. Kiernan, M.C.; Vucic, S.; Cheah, B.C.; Turner, M.R.; Eisen, A.; Hardiman, O.; Burrell, J.R.; Zoing, M.C. Amyotrophic Lateral Sclerosis. *Lancet* **2011**, *377*, 942–955, doi:10.1016/S0140-6736(10)61156-7.
4. Carter, G.T.; Miller, R.G. Comprehensive Management of Amyotrophic Lateral Sclerosis. *Phys Med Rehabil Clin N Am* **1998**, *9*, 271–284, doi:10.1016/s1047-9651(18)30290-0.
5. Chiò, A.; Logroscino, G.; Traynor, B.J.; Collins, J.; Simeone, J.C.; Goldstein, L.A.; White, L.A. Global Epidemiology of Amyotrophic Lateral Sclerosis: A Systematic Review of the Published Literature. *Neuroepidemiology* **2013**, *41*, 118–130, doi:10.1159/000351153.
6. Goutman, S.A.; Hardiman, O.; Al-Chalabi, A.; Chió, A.; Savelieff, M.G.; Kiernan, M.C.; Feldman, E.L. Recent Advances in the Diagnosis and Prognosis of Amyotrophic Lateral Sclerosis. *Lancet Neurol* **2022**, *21*, 480–493, doi:10.1016/S1474-4422(21)00465-8.
7. Blasco, H.; Mavel, S.; Corcia, P.; Gordon, P.H. The Glutamate Hypothesis in ALS: Pathophysiology and Drug Development. *Curr Med Chem* **2014**, *21*, 3551–3575, doi:10.2174/0929867321666140916120118.
8. Boillée, S.; Vande Velde, C.; Cleveland, D.W.W. ALS: A Disease of Motor Neurons and Their Nonneuronal Neighbors. *Neuron* **2006**, *52*, 39–59, doi:10.1016/J.NEURON.2006.09.018.
9. Ferraiuolo, L.; Higginbottom, A.; Heath, P.R.; Barber, S.; Greenald, D.; Kirby, J.; Shaw, P.J. Dysregulation of Astrocyte-Motoneuron Cross-Talk in Mutant Superoxide Dismutase 1-Related Amyotrophic Lateral Sclerosis. *Brain* **2011**, *134*, 2627–2641, doi:10.1093/BRAIN/AWR193.
10. Renton, A.E.; Chiò, A.; Traynor, B.J. State of Play in Amyotrophic Lateral Sclerosis Genetics. *Nat Neurosci* **2014**, *17*, 17–23, doi:10.1038/NN.3584.
11. Ghasemi, M.; Brown, R.H. Genetics of Amyotrophic Lateral Sclerosis. *Cold Spring Harb Perspect Med* **2018**, *8*, doi:10.1101/CSHPERSPECT.A024125.
12. Van Damme, P.; Robberecht, W.; Van Den Bosch, L. Modelling Amyotrophic Lateral Sclerosis: Progress and Possibilities. *Dis Model Mech* **2017**, *10*, 537–549, doi:10.1242/DMM.029058.
13. Filippini, T.; Tesauro, M.; Fiore, M.; Malagoli, C.; Consonni, M.; Violi, F.; Iacuzio, L.; Arcolin, E.; Conti, G.O.; Cristaldi, A.; et al. Environmental and Occupational Risk Factors of Amyotrophic Lateral Sclerosis: A Population-Based Case-Control Study. *Int J Environ Res Public Health* **2020**, *17*, doi:10.3390/IJERPH17082882.
14. Sutedja, N.A.; Veldink, J.H.; Fischer, K.; Kromhout, H.; Heederik, D.; Huisman, M.H.B.; Wokke, J.H.J.; Van Den Berg, L.H. Exposure to Chemicals and Metals and Risk of Amyotrophic Lateral Sclerosis: A Systematic Review. *Amyotroph Lateral Scler* **2009**, *10*, 302–309, doi:10.3109/17482960802455416.
15. Peters, T.L.; Kamel, F.; Lundholm, C.; Feychting, M.; Weibull, C.E.; Sandler, D.P.; Wiebert, P.; Sparén, P.; Ye, W.; Fang, F. Occupational Exposures and the Risk of Amyotrophic Lateral Sclerosis. *Occup Environ Med* **2017**, *74*, 87–92, doi:10.1136/OEMED-2016-103700.
16. Fang, F.; Quinlan, P.; Ye, W.; Barber, M.K.; Umbach, D.M.; Sandler, D.P.; Kamel, F. Workplace Exposures and the Risk of Amyotrophic Lateral Sclerosis. *Environ Health Perspect* **2009**, *117*, 1387–1392, doi:10.1289/EHP.0900580.
17. Althobaiti, N.A. Heavy Metals Exposure and Alzheimer's Disease: Underlying Mechanisms and Advancing Therapeutic Approaches. *Behavioural brain research* **2025**, *476*, doi:10.1016/J.BBR.2024.115212.
18. McGuire, V.; Longstreth, W.T.; Nelson, L.M.; Koepsell, T.D.; Checkoway, H.; Morgan, M.S.; Van Belle, G. Occupational Exposures and Amyotrophic Lateral Sclerosis: A Population- Based Case-Control Study. *Am J Epidemiol* **1997**, *145*, 1076–1088, doi:10.1093/oxfordjournals.aje.a009070.
19. Tamburo, E.; Varrica, D.; Dongarrà, G.; Grimaldi, L.M.E. Trace Elements in Scalp Hair Samples from Patients with Relapsing-Remitting Multiple Sclerosis. *PLoS One* **2015**, *10*, doi:10.1371/JOURNAL.PONE.0122142.

 doi:10.20944/preprints202504.0763.v1

20. Koseoglu, E.; Koseoglu, R.; Kendirci, M.; Saraymen, R.; Saraymen, B. Trace Metal Concentrations in Hair and Nails from Alzheimer's Disease Patients: Relations with Clinical Severity. *J Trace Elem Med Biol* **2017**, *39*, 124–128, doi:10.1016/J.JTEMB.2016.09.002.
21. Dr. Anne Johansen, C.W.U. Environmental Health: Science, Policy and Social Justice Winter Quarter Available online: https://archives.evergreen.edu/webpages/curricular/2008-2009/envirohealth/system/files/Lab%2BIV%2Bmetals%2Bin%2Bhair.doc?utm_source=chatgpt.com (accessed on 7 April 2025).
22. Triolo, V.; Spanò, M.; Buscemi, R.; Gioè, S.; Malta, G.; Čaplinskiene, M.; Vaiano, F.; Bertol, E.; Zerbo, S.; Albano, G.D.; et al. EtG Quantification in Hair and Different Reference Cut-Offs in Relation to Various Pathologies: A Scoping Review. *Toxics 2022, Vol. 10, Page 682* **2022**, *10*, 682, doi:10.3390/TOXICS10110682.
23. Pereira, R.; Ribeiro, R.; Gonçalves, F. Scalp Hair Analysis as a Tool in Assessing Human Exposure to Heavy Metals (S. Domingos Mine, Portugal). *Science of The Total Environment* **2004**, *327*, 81–92, doi:10.1016/J.SCITOTENV.2004.01.017.
24. Ash, P.E.A.; Dhawan, U.; Boudeau, S.; Lei, S.; Carlomagno, Y.; Knobel, M.; Al Mohanna, L.F.A.; Boomhower, S.R.; Newland, M.C.; Sherr, D.H.; et al. Heavy Metal Neurotoxicants Induce ALS-Linked TDP-43 Pathology. *Toxicol Sci* **2019**, *167*, 3–4, doi:10.1093/TOXSCI/KFY267.
25. Kamel, F.; Umbach, D.M.; Hu, H.; Munsat, T.L.; Shefner, J.M.; Taylor, J.A.; Sandler, D.P. Lead Exposure as a Risk Factor for Amyotrophic Lateral Sclerosis. *Neurodegener Dis* **2005**, *2*, 195–201, doi:10.1159/000089625.
26. Bozzoni, V.; Pansarasa, O.; Diamanti, L.; Nosari, G.; Cereda, C.; Ceroni, M. Amyotrophic Lateral Sclerosis and Environmental Factors. *Funct Neurol* **2016**, *31*, 7–19, doi:10.11138/FNEUR/2016.31.1.007.
27. Kumar, A.; Ali, M.; Mishra, P.; Pandey, B.N.; Sharma, P.; Mishra, K.P. Thorium-Induced Neurobehavioural and Neurochemical Alterations in Swiss Mice. *Int J Radiat Biol* **2009**, *85*, 338–347, doi:10.1080/09553000902781071.
28. Atsdr Toxicological Profile for Thorium. **2019**.
29. Avan, A.; Postma, T.J.; Ceresa, C.; Avan, A.; Cavaletti, G.; Giovannetti, E.; Peters, G.J. Platinum-Induced Neurotoxicity and Preventive Strategies: Past, Present, and Future. *Oncologist* **2015**, *20*, 411, doi:10.1634/THEONCOLOGIST.2014-0044.
30. Amptoulach, S.; Tsavaris, N. Neurotoxicity Caused by the Treatment with Platinum Analogues. *Chemother Res Pract* **2011**, *2011*, 843019, doi:10.1155/2011/843019.
31. Lison, D.; Bucket, J.-P.; Hoet, P. Toxicity of Tungsten. *The Lancet* **1997**, *349*, 58, doi:10.1016/s0140-6736(05)62194-0.
32. Macé, L.; Brizais, C.; Bachelot, F.; Manoury, A.; Thomé, S.; Gloaguen, C.; Garali, I.; Magneron, V.; Monceau, V.; Sache, A.; et al. Exposure to Tungsten Particles via Inhalation Triggers Early Toxicity Marker Expression in the Rat Brain. *Inhal Toxicol* **2024**, *36*, 261–274, doi:10.1080/08958378.2024.2349895.
33. Clarkson, T.W.; Magos, L. The Toxicology of Mercury and Its Chemical Compounds. *Crit Rev Toxicol* **2006**, *36*, 609–662, doi:10.1080/10408440600845619.
34. Clarkson, T.W.; Magos, L. The Toxicology of Mercury and Its Chemical Compounds. *Crit Rev Toxicol* **2006**, *36*, 609–662, doi:10.1080/10408440600845619.
35. Branco, V.; Aschner, M.; Carvalho, C. Neurotoxicity of Mercury: An Old Issue with Contemporary Significance. *Adv Neurotoxicol* **2021**, *5*, 239, doi:10.1016/BS.ANT.2021.01.001.
36. Albers, J.W.; Kallenbach, L.R.; Fine, L.J.; Langolf, G.D.; Wolfe, R.A.; Donofrio, P.D.; Alessi, A.G.; Stolp-Smith, K.A.; Bromberg, M.B. Neurological Abnormalities Associated with Remote Occupational Elemental Mercury Exposure. *Ann Neurol* **1988**, *24*, 651–659, doi:10.1002/ANA.410240510.
37. Caito, S.; Aschner, M. Developmental Neurotoxicity of Lead. *Adv Neurobiol* **2017**, *18*, 3–12, doi:10.1007/978-3-319-60189-2_1.
38. Mason, L.H.; Harp, J.P.; Han, D.Y. Pb Neurotoxicity: Neuropsychological Effects of Lead Toxicity. *Biomed Res Int* **2014**, *2014*, doi:10.1155/2014/840547.
39. Branca, J.J.V.; Morucci, G.; Pacini, A. Cadmium-Induced Neurotoxicity: Still Much Ado. *Neural Regen Res* **2018**, *13*, 1879, doi:10.4103/1673-5374.239434.

 doi:10.20944/preprints202504.0763.v1

40. Oggiano, R.; Pisano, A.; Sabalic, A.; Farace, C.; Fenu, G.; Lintas, S.; Forte, G.; Bocca, B.; Madeddu, R. An Overview on Amyotrophic Lateral Sclerosis and Cadmium. *Neurol Sci* **2021**, *42*, 531–537, doi:10.1007/S10072-020-04957-7.
41. Rezaei, K.; Mastali, G.; Abbasgholinejad, E.; Bafrani, M.A.; Shahmohammadi, A.; Sadri, Z.; Zahed, M.A. Cadmium Neurotoxicity: Insights into Behavioral Effect and Neurodegenerative Diseases. *Chemosphere* **2024**, *364*, doi:10.1016/J.CHEMOSPHERE.2024.143180.
42. Vinceti, M.; Filippini, T.; Mandrioli, J.; Violi, F.; Bargellini, A.; Weuve, J.; Fini, N.; Grill, P.; Michalke, B. Lead, Cadmium and Mercury in Cerebrospinal Fluid and Risk of Amyotrophic Lateral Sclerosis: A Case-Control Study. *Journal of Trace Elements in Medicine and Biology* **2017**, *43*, 121–125, doi:10.1016/j.jtemb.2016.12.012.
43. Arsenic Exposure: Health Effects and the Risk of Cancer - PubMed Available online: https://pubmed.ncbi.nlm.nih.gov/3915827/ (accessed on 3 February 2025).
44. Caito, S.; Aschner, M. Neurotoxicity of Metals. *Handb Clin Neurol* **2015**, *131*, 169–189, doi:10.1016/B978-0-444-62627-1.00011-1.
45. Andrade, V.M.; Aschner, M.; Marreilha dos Santos, A.P. Neurotoxicity of Metal Mixtures. *Adv Neurobiol* **2017**, *18*, 227–265, doi:10.1007/978-3-319-60189-2_12.
46. Li, B.; Xia, M.; Zorec, R.; Parpura, V.; Verkhratsky, A. Astrocytes in Heavy Metal Neurotoxicity and Neurodegeneration. *Brain Res* **2021**, *1752*, doi:10.1016/J.BRAINRES.2020.147234.
47. Ortega, R.; Carmona, A. Neurotoxicity of Environmental Metal Toxicants: Special Issue. *Toxics* **2022**, *10*, 382, doi:10.3390/TOXICS10070382.
48. Sies, H.; Berndt, C.; Jones, D.P. Oxidative Stress. *Annu Rev Biochem* **2017**, *86*, 715–748, doi:10.1146/ANNUREV-BIOCHEM-061516-045037.
49. Butterfield, D.A.; Halliwell, B. Oxidative Stress, Dysfunctional Glucose Metabolism and Alzheimer Disease. *Nat Rev Neurosci* **2019**, *20*, 148–160, doi:10.1038/S41583-019-0132-6.
50. Niu, Q. Neurotoxicity of Aluminum, Second Edition. *Neurotoxicity of Aluminum, Second Edition* **2023**, 1–313, doi:10.1007/978-981-99-1592-7/COVER.
51. Aschner, M.; Guilarte, T.R.; Schneider, J.S.; Zheng, W. Manganese: Recent Advances in Understanding Its Transport and Neurotoxicity. *Toxicol Appl Pharmacol* **2007**, *221*, 131–147, doi:10.1016/J.TAAP.2007.03.001.
52. Kim, J.J.; Kim, Y.S.; Kumar, V. Heavy Metal Toxicity: An Update of Chelating Therapeutic Strategies. *J Trace Elem Med Biol* **2019**, *54*, 226–231, doi:10.1016/J.JTEMB.2019.05.003.
53. Chelation Therapy: EDTA and Other Chemicals, Benefits, Side Effects Available online: https://www.healthline.com/health/chelation-therapy (accessed on 13 February 2025).