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Comparative assessment of blood Metal/metalloid levels, clinical heterogeneity, and disease severity in amyotrophic lateral sclerosis patients

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ABSTRACT

Amyotrophic lateral sclerosis is an unremitting neurodegenerative (ND) disease characterized by progressive and fatal loss of motor neuron function. While underlying mechanisms for ALS susceptibility are complex, current understanding suggests that interactions between age, genetic, and environmental factors may be the key. Environmental exposure to metal/metalloids has been implicated in various ND diseases including ALS, Alzheimer's Disease (AD), and Parkinson's Disease (PD). However, most of currently available population-based ALS studies in relation to metal exposure are based on individuals from European ancestry, while East Asian populations, especially cohorts from China, are less well-characterized. This study aims to examine the association between metal/metalloid levels and ALS onset by evaluating blood cadmium (Cd), lead (Pb), Cu, Zn, calcium (Ca), magnesium (Mg), and iron (Fe) levels in controls and sporadic ALS patients from North Western China. We report that Cu and Fe levels are found at higher levels in ALS patients compared to the controls. Spinal and bulbar onset patients show significant difference in Ca levels. Moreover, Cd, Pb, Cu, and Ca levels are positively correlated with high disease severity. Results from this study may provide new insights for understanding not only the role of metal/metalloids in ALS susceptibility, but also progression and forms of onset.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a complex and relentless neurodegenerative disease characterized by the progressive loss of upper and lower motor neurons in the motor cortex, brain stem, and spinal cord. Survival time is extremely short as patients typically die of respiratory paralysis between 3–5 years after initial diagnosis(Urdinguio et al., 2009; Wijesekera and Leigh, 2009). Degeneration of upper motor neurons in the motor cortex results in muscle spasticity and rigidness, while failure of lower motor neurons in the stem and spinal cord will lead to muscle atrophy due to loss of synaptic connectivity (Brown and Al-Chalabi, 2017). Based on clinical heterogeneity and location of onset, ALS can be further classified into spinal and bulbar forms. The majority of patients show spinal onset or muscle atrophy and weakness that begin in the limbs and trunk; while only about 30 % are classified as bulbar onset which first show diminished speech and swallow abilities (Shellikeri et al., 2017). Clinically, bulbar onset is considered the more severe form of ALS, characterized by worse prognosis and shorter survival (Mitsumoto and Del Bene, 2000; Goldstein et al., 2002; Shellikeri et al., 2017). Spinal and bulbar onset can be sporadic (sALS) or familial (fALS), although most (90–95 %) of ALS cases show no family history. Inherited ALS is usually a dominant trait passed on to male and female offspring at 1:1 ratio, while sALS affects men more than women. Global incidence rate is 1–2.6 in 100,000 people, and men are 20 % more likely to develop the disease than women (Wijesekera and Leigh, 2009; Portaro et al., 2020). Population-based studies have shown that ALS prevalence and incidence can vary across ethnicities and continents. ALS incidence in Europe (2.2 per 100,000) is higher than both East Asia (0.89 per 100,000) and South Asia (0.79 per 100,000) (Logroscino and Piccininni, 2019). In China, while the currently estimated incidence is 1.62 per 100,

Abbreviations: AD, Alzheimer's Diseaes; ALS, Amyotrophic lateral sclerosis; BBB, Blood brain barrier; Ca, Calcium; Cd, Cadmium; CNS, Central nervous system; CSF, Cerebral spinal fluid; Cu, Copper; DMT1, Divalent metal transporter; fALS, Familial ALS; Fe, Iron; Mg, Magnesium; ND, Neurodegenerative disease; Pb, Lead; PD, Parkinson's Disease; PDI, Protein disulphide isomerase; ROS, Reactive oxygen species; sALS, Sporadic ALS; TFR1, Transferrin receptor 1; Zn, Zinc.

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000 person-years (95 %, 1.58–1.67), by 2040, the number of cases is projected to increase by 46.3 % (Logroscino and Piccininni, 2019; Xu et al., 2020).

Mechanisms of ALS occurrence are complex and inconclusive, and the presence of various forms of the disease reflect potential coaction between genetic and environmental factors. Some of the currently proposed mechanisms include oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, neuroinflammation, abnormal RNA processing, axonal transport dysfunction, and misfolded protein aggregation (Mancuso and Navarro, 2015; Oggiano et al., 2021). However, less is known about the triggering risk factors underlying these molecular processes. Current research suggests that onset of sporadic ALS may be associated with environmental factors such as exposure to metals, metalloids, pesticides, cyanobacterial toxins, and viruses (Vinceti et al., 2012; Oggiano et al., 2021). In particular, metal/metalloids can accumulate and compromise normal brain development and function by disrupting brain energy metabolism, mitochondrial function, DNA damage repair, and neuronal calcium-ion dyshomeostasis (Ijomone et al., 2020). Various epidemiology studies have demonstrated potential correlations (positive and negative) between metal exposure and ALS onset. A recent study suggested that high environmental contaminations of copper (Cu), zinc (Zn), chromium (Cr), and nickel (Ni) may be correlated with high ALS incidence in patients from Northern Italy (Tesauro et al., 2021). Another population-based case-control study on Italian ALS patients found a positive correlation between disease incidence and metal (Pb, Hg, Se) exposure (Filippini et al., 2020). A study based in the United States showed positive association between blood Cu levels and ALS (Peters et al., 2016). On the other hand, a study based in Denmark did not find occupational metals (Cr, Fe, Ni) to be associated with ALS incidence (Dickerson et al., 2020). Seemingly inconsistent results from currently available studies underscore the need for further investigation, especially from different geographical cohorts. Notably, most of population-based epidemiological studies on ALS involve participants with European ancestry. Currently, there is a lack in metal-associated population-based studies centered around Chinese ALS cohorts. This study aims to evaluate potential correlations between blood metal/metalloid levels and ALS onset, with a particular focus on patients from North Western China. Atomic absorption spectrometer was employed to assess levels of cadmium (Cd), lead (Pb), Cu, Zn, calcium (Ca), magnesium (Mg), and iron (Fe) in sALS patients and corresponding controls. More important, our analyses add important insights for potential associations between blood metal/metalloid levels, different forms of onset, as well as degree of severity in ALS patients, which may contribute to strategies for ALS diagnosis, prevention, and treatment.

2. Subjects and methods

2.1. Study population

Forty-six population-based sporadic cases identified with definite, probable, or possible ALS according to the revised El Escorial criteria were matched with 30 age- and sex-matched controls for this study. All participants are of the same ethnicity, and were recruited from the First Affiliated Hospital of Xi'an Jiaotong University in the northwestern region of China. Baseline demographic information were provided during each participant's first visitation. All clinical data (diagnosis, spinal/bulbar onset, ALSFRS-r score) as well as personal data (age, sex, exposure history, etc.) were conducted and collected by medical professionals. This study was approved by the Institutional Ethical Committee of Xi'an Jiaotong University. All participants gave written informed consent.

2.2. Biological samples and metal/metalloid analysis

Whole blood samples were collected from ALS cases and controls in trace-metal free ethylenediaminetetraacetic acid (EDTA) vacutainer BD tubes. Blood samples were immediately placed in ice containers and transferred to the laboratory for analysis at First Affiliated Hospital of Xi'an Jiaotong University. We employed atomic absorption spectrometry for detection of blood Cd, Pb, Cu, Zn, Ca, Mg, and Fe. Graphite furnace atomic absorption spectrometry (BH2200S, BoHui, China) was used to measure Cdand lead concentrations. Elements Cu, Zn, Ca, Mg, and Fe were measured using BoHui 5300 analyzer. Commercial standard reference materials, related reagents, and calibrators were all purchased from Beijing Bohui Innovation Biotechnology Co., Ltd. (Beijing, China). Limits of detection for the metals are as follows: Pb (<100ug/L), Cd (<5ug/L), Cu (<9.3 mg/L), Zn (<12.4 mg/L), Ca (<93 mg/L), Mg (<62 mg/L), and Fe (<620 mg/L).

2.3. Statistical analysis

All statistical analyses and graphs were carried out using Graphpad Prism 8.0 software and R (ggplot2 package). Demographic information was analyzed and presented in frequencies and percentages. Each metal/metalloid was analyzed independently using nonparametric pairwise Mann-Whitney tests. The results are presented as medians and interquartile ranges (25th and 75th). Linear regression was used to assess the correlation between Cu and Fe, as well as pesticide exposure and ALSFS-r scores. A two-tailed p-value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic information

As presented in Table 1, 76 participants were enrolled for this study, 30 were controls and 46 were ALS patients. There was an equal number of female and male participants in the control group aged between 47–85 (median = 60.7). The ALS group had a slightly higher percentage of males (65.11 %) compared to females (34.89 %) with a median age of 61 (41–81). The median disease duration was 10 months (2−24 months). We further subdivided the ALS patients into spinal (n = 38) and bulbar (n = 8) onset groups. Interestingly, male patients are significantly higher in the spinal group (71.05 %) compared to the bulbar group (20 %). No significant differences were found for age and sex between control, ALS, spinal onset, and bulbar onset groups.

3.2. Metal/metalloid concentration analysis

Blood levels of Cd, Pb, Cu, Zn, Mg, Ca, and Fe were measured by atomic absorption spectrometer. As shown in Table 2, Cu (12.32 μg/L, p = 0.02) and Fe (8.52 μg/L, p = 0.04) levels are significantly higher in the ALS group compared to the control group (Cu = 9.27 μg/L, Fe = 7.88 μg/L). A distribution graph of all the metals are also presented in Fig. 1. Since both Cu and Fe levels were found significantly different between control and ALS groups, we tested the potential correlational

Table 1
Baseline demographic characteristics for ALS cases and Controls.

	Control Non-ALS (N = 30)	Total (N = 46)	ALS Spinal onset (N = 38)	Bulbar onset (N = 8)
Gender Females	15(50 %)	15(34.89 %)	11(28.95 %)	5(62.5 %)
Males	15(50 %)	28(65.11 %)	27(71.05 %)	3(37.5 %)
Median age (range in years)	60.7 (47−85)	59.4 (41−81)	60.5 (35−81)	60.6 (43−74)
Median disease duration (range in months)	–	11.5 (2−24)	9.5 (2−24)	12(6−16)

Table 2
Blood metal/metalloid levels (median, 25th, and 75th percentile) in for ALS cases and Controls.

Metal/metalloid (μg/L)	Control N = 30 Median (IQR)	ALS N = 46 Median (IQR)	p-value
Cd	0.78(0.12–1.81)	0.89(0.31–1.95)	0.65
Pb	37.52(25.66–60.79)	37.58(27.13–64.22)	0.57
Cu*	9.27(6.59–11.59)	12.32(9.15–15.06)	0.02
Zn	97.90(88.89–109.6)	96.95(88.18–109.5)	0.96
Ca	1.54(1.44–1.62)	1.53(1.34–1.67)	0.95
Mg	1.57(1.37–1.71)	1.58(1.43–1.68)	0.48
Fe*	7.88(7.14–8.57)	8.52(7.53–9.27)	0.04

relationship between these two metals. Interestingly, Cu and Fe levels are positively correlated ($p = 0.003$), which may suggest potential synergistic or mixture effects of the two (Fig. 2). In addition to Cu and Fe, we analyzed the correlation between all metals in ALS patients, and found significant and positive correlation between Mg and Fe ($p < 0.0001$), Mg and Zn (<0.0001), Cu and Ca ($p = 0.0002$), Mg and Cu ($p = 0.0005$), Zn and Fe ($p < 0.0001$), as well as Cd and Ca ($p = 0.04$). We present this data in Fig. 3.

We further divided the ALS group into spinal and bulbar onset subgroups for pair-wise comparison analysis to examine whether metal levels differed by disease onset. Results in Table 3 indicate Cu levels in the spinal (11.71 μg/L, $p = 0.04$) and bulbar (14.64 μg/L, $p = 0.02$) groups are significantly higher than that of the control group (9.27 μg/L). On the other hand, Fe levels were only found significantly different between the spinal (8.7 μg/L) and control (7.88, $p = 0.01$) groups. These results suggest that high Fe levels found in ALS patients may be specific to spinal onset while Cu levels are found high in both bulbar and spinal patients. Interestingly, Ca levels in the bulbar (1.68 μg/L) group were higher than that of the spinal (1.5 μg/L, $p = 0.04$) group. No significant difference between spinal and bulbar groups or between bulbar and control groups were found for the rest of the metal/metalloids. We also present the concentration distribution of all metals in Fig. 4.

In addition to origin of onset, we divided the ALS patients into two groups based on high (ALSFRS-r < 32, n = 5) and low (ALSFRS-r > 32, n = 41) disease severity to investigate potential correlations between disease severity and blood metal/metalloid levels. Notably, high severity patients showed significantly higher blood levels of Cd (2.14 μg/L, $p = 0.03$), Pb (83.19 μg/L, $p = 0.005$), Ca (1.63 μg/L, $p = 0.03$), and Cu (15.61 μg/L, $p = 0.045$) compared to the low severity group (Cd = 0.83 μg/L, Pb = 34.22 μg/L, Ca = 1.48 μg/L, and Cu = 11.71 μg/L) (Table 4). However, there was no significant difference in Fe levels for the two groups. Moreover, using linear regression, we found that pesticide exposure is positively correlated with ALS disease severity ($p = 0.04$) (Fig. 5).

4. Discussion

In this study, we evaluated concentrations of various metals/metalloids in ALS cases and controls. Results suggest that ALS patients retain higher levels of Cu and Fe compared to the control group. Notedly, there was no correlation between age and any of the metals in our data analysis. Moreover, our data show that spinal onset patients have higher Cu and Fe levels compared to the control group, while bulbar onset patients only showed higher Cu levels than the control group. These

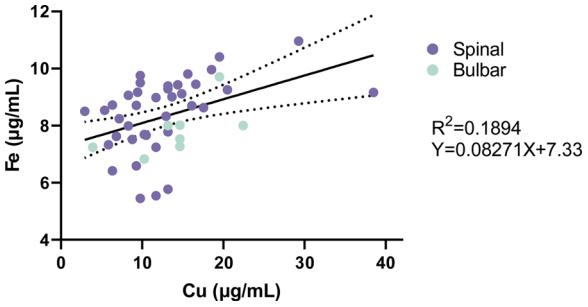

Fig. 2. The scatter plot illustrates the positive correlation between Cu and Fe levels in both spinal and bulbar onset patients. Linear regression of the slope is significant, $p = 0.003$.

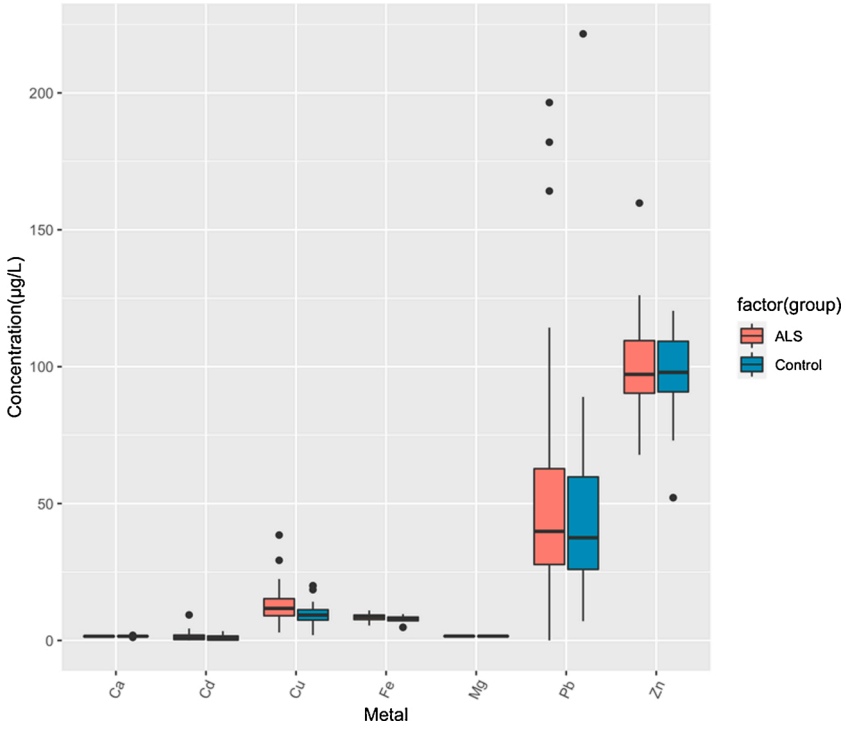

Fig. 1. The illustration provides metal/metalloid concentrations on one graph to demonstrate their distributions among ALS patients and controls.

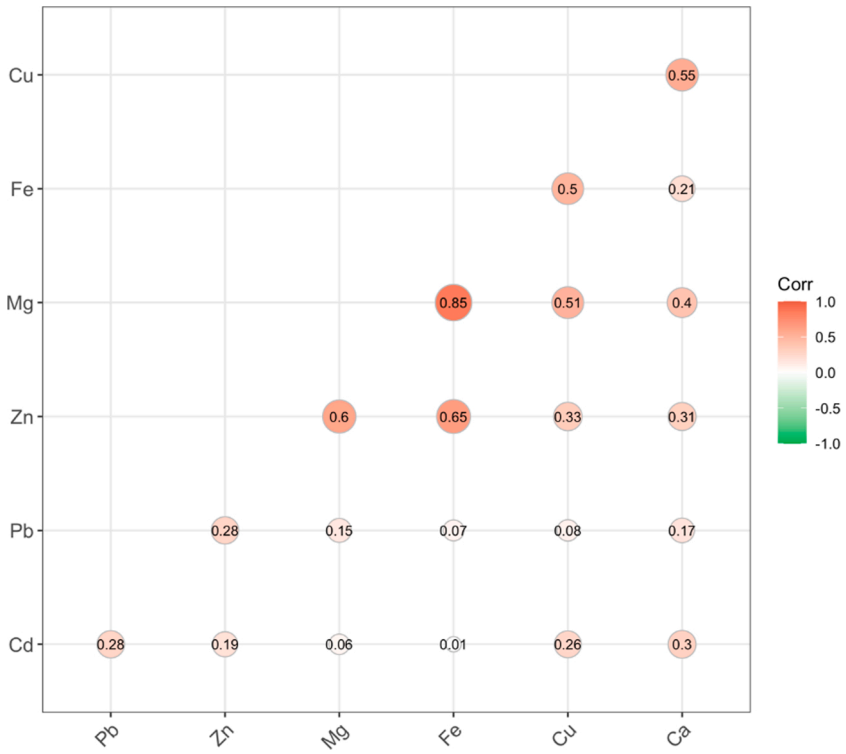

Fig. 3. The graph shows correlation between metals in ALS patients. All values above 0.3 indicate $p < 0.05$ significance.

Table 3
Distribution of blood meta/metalloid levels(median, 25th, and 75th percentile) by spinal and bulbar onset.

Metal/metalloid (μg/L)	Control N = 30 Median (IQR)	Spinal onset N = 38 Median (IQR)	Bulbar onset N = 8 Median (IQR)
Cd	0.78(0.12–1.81)	0.89(0.31–1.98)	0.91(0.03–1.98)
Pb	37.52 (25.66–60.79)	41.32 (27.29–62.44)	34.33 (26.06–80.75)
Cu	9.27 (1.95–11.59)	11.71 (8.66–15.06)	14.64 (10.98–18.30)
Zn	97.90 (88.89–109.6)	97.19 (87.59–111.65)	96.72 (89.48–101)
Ca	1.54(1.44–1.62)	1.50(1.32–1.62)	1.68(1.5–1.73)
Mg	1.57(1.37–1.71)	1.57(1.42–1.68)	1.61(1.47–1.78)
Fe	7.88(7.14–8.57)	8.70(7.65–9.32)	7.77(7.25–8.0)

* Mann-Whitney test show significant differences in Control vs Spinal onset for Cu($p = 0.04$) and Fe ($p = 0.01$), Control vs Bulbar onset for Cu ($p = 0.02$), and Spinal vs Bulbar onset for Ca($p = 0.04$).

results suggest that difference in Fe levels between ALS and control participants may be due to high levels found in spinal patients rather than bulbar patients. Moreover, bulbar patients showed significantly higher Ca levels than spinal patients, which suggests that Ca homeostasis may be an important factor to consider for differential ALS onset. Notably, bulbar patients have a significantly higher percentage of female patients compared to male patients. It would be interesting for future studies may consider looking into the role of gender and Ca homeostasis in ALS onset. Copper imbalance has been implicated in various neurodegenerative diseases including Alzheimer's Disease, Parkinson's Disease, Menkes Disease, and Wilson's Disease; however, its role in ALS pathogenesis is unclear (Telianidis et al., 2013; Chang and Hahn, 2017; Bisaglia and Bubacco, 2020). As a heavy and an essential metal, depending on its levels in the human body, copper can be either necessary or toxic. Copper acts as a cofactor for various enzymes due to its oxidoreductase activity, and is involved in various physiological processes including neurotransmission, aerobic metabolism, iron homeostasis, erythrocyte formation, peptide amidation, and antioxidant defense (Giampietro et al., 2018; Gromadzka et al., 2020). In the central nervous system (CNS), copper is evidenced to modulate synaptic activity, neurite outgrowth, and neurotransmission (Gaier et al., 2013; Opazo et al., 2014; Scheiber et al., 2014; Giampietro et al., 2018). While copper is present in all parts of the brain, its levels are particularly high in the hippocampus, cerebellum, basal ganglia, synaptic membranes, pyramidal neurons, and cerebellar granular neurons (Madsen and Gitlin, 2007; Giampietro et al., 2018). While Cu is important for CNS development and function, excess levels can be harmful. Our results also showed a positive correlation between Cu and Fe levels. Cu and Fe are known co-factors for enzymes necessary for myelin formation and neurotransmitter synthesis (Gaggelli et al., 2006; Lutsenko et al., 2010; Skjørringe et al., 2012). Considering similarity in their physiochemical properties, the molecular functions and metabolism between Cu and Fe may be closely intertwined. Notably, evidence suggests that Cu levels are elevated under iron deficiency conditions, and that Cu accumulation can influence iron transport, ferroxidase biosynthesis, as well as iron-regulatory hormone hepcidin expression and activity. The positive correlation between Cu and Fe levels found in ALS patient blood samples may indicate potential interaction between the two. Fe is also important for regulating various biological processes including oxygen transportation, oxidative stress generation, and oxygen transportation (Ward et al., 2014; Bu et al., 2019). In the CNS, blood Fe travels through the blood brain barrier (BBB) via transferrin receptor 1 (TFR1)- and divalent metal transporter 1 (DMT1)-mediated endocytosis (Ke and Qian, 2007; Moos et al., 2007; Leitner and Connor, 2012; Bu et al., 2019). Fe overload in the brain can be stimulated via elevated DMT1 levels due to neuroinflammation signals as well as increased BBB permeability as a result of aging (Nagata et al., 1985; Lee et al., 2004; Nemeth et al., 2004; Bu et al., 2019). As a redox metal, Fe is capable of generating reactive oxygen species (ROS) such as hydroxyl radial and TNF-a secretion to induce oxidative stress- and neuroinflammation-mediated neuronal cell damage and death (Lee et al., 2015; Popović-Bijelić et al., 2016; Bu et al., 2019). In addition, ROS can participate in a positive feedback fashion to maintain iron load by inducing the release of iron from mitochondrial

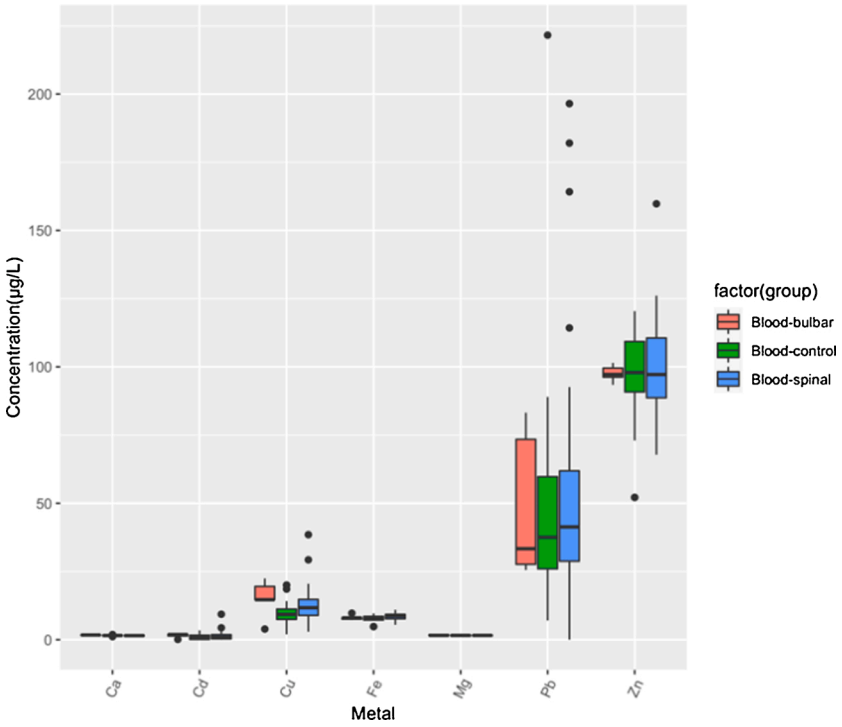

Fig. 4. The illustration provides metal/metalloid concentrations on one graph to demonstrate their distributions among bulbar onset, spinal onset patients, and controls.

Table 4
Distribution of blood metal/metalloid levels (median, 25th, and 75th percentile) by ALS disease severity.

Metal/metalloid (μg/L)	High Severity N = 5 Median (IQR)	Low Severity N = 41 Median (IQR)	p-value
Cd*	2.14(1.49–2.24)	0.83(.29–1.69)	0.03
Pb*	83.19(69.08–92.41)	34.22(25.58–55.36)	0.005
Cu*	15.61(12.45–24.4)	11.71(8.54–14.64)	0.045
Zn	118.5(92.21–140.6)	96.71(87–106.9)	0.11
Ca*	1.63(1.61–1.72)	1.48(1.32–1.65)	0.03
Mg	1.94(1.51–2)	1.57(1.42–1.67)	0.08
Fe	9.81(7.86–10.69)	8.50(7.43–9.17)	0.07

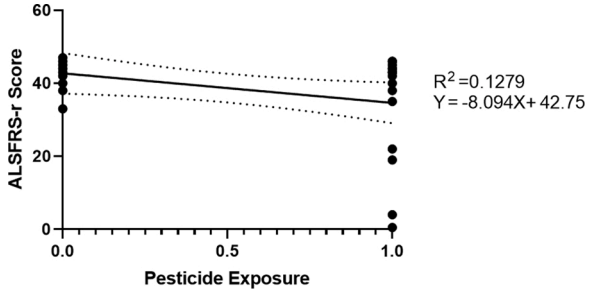

Fig. 5. The graph demonstrates simple linear regression of pesticide exposure on ALSFRS-r score. On the X-axis, 0 indicates no exposure history, 1 indicates previous pesticide exposure history. Linear regression of the slope is significant, p = 0.04.

iron-sulfur cluster proteins (Horowitz and Greenamyre, 2010; Bu et al., 2019). Heightened Fe levels in ALS patients have been reported in serum, plasma, and cerebral spinal fluid (CSF) (Kokić et al., 2005; Goodall et al., 2008; Qureshi et al., 2008; Mitchell et al., 2010; Hozumi et al., 2011; Bu et al., 2019). Moreover, autopsy and MRI results have also demonstrated increased iron deposition in the frontal cortex, spinal cord, and motor cortex (Yasui et al., 1993; Ince et al., 1994; Kasarskis et al., 1995; Ignjatović et al., 2013; Bu et al., 2019). On the contrary, low Fe levels in blood, hair, and urine samples of ALS patients have also been reported (Oggiano et al., 2018). Our results indicate the ALS patients show higher blood Fe levels than the control group. And similar to Patti et al., we did not find a significant difference in Fe levels between spinal and bulbar patients (Patti et al., 2020). However, we did find that spinal onset patients demonstrated significantly higher levels than the control group. This may suggest that spinal onset patients may be the main contributor for high Fe levels found in the total ALS group. Role of Fe and its interacting function with copper in different forms of ALS may be an interesting point of investigation for future studies. Moreover, we also found positive correlations between Mg and Fe, Mg and Zn, Cu and Ca, Mg and Cu, Zn and Fe, and Cd and Ca. Interestingly, many of these metals, such as Zn and Mg, were not found at significantly high levels in the ALS group compared to the control group. However, the positive correlations between these metals may present mixture effects since interactions among different metals can change both toxicokinetic and toxicodynamic. Future studies may consider examining the group effect of metal/metalloid mixtures on ALS disease pathogenesis.

In addition to examining the correlation between metal/metalloids and different forms of ALS onset, we also analyzed blood metal/metalloid levels based on ALS severity. As our results indicate, high severity ALS patients showed higher Cu, Cd, Pb, and Ca levels compared to those in the low severity group. Unlike Cu and Fe, Cd is a heavy metal with no biological role but can be highly toxic for neuronal cells even at low concentrations. Notably, Cd is classified as a group I human carcinogen by the International Agency for Research on Cancer (2012; Oggiano et al., 2021). Human exposure to Cd can occur via inhalation, ingestion, or skin absorption as a result of smoking or involuntary occupational exposure such as working with Cd-containing pesticides or in Ni-Cd battery factories (Bar-Sela et al., 2001; Wang et al., 2015; Oggiano et al., 2021). Once inside the body, Cd can be absorbed through the intestine and accumulate in the liver, kidney, and brain due to absence of natural elimination mechanisms and its ability to cross the BBB

(Al-Saleh and Shinwari, 2001; Ebert-McNeill et al., 2012; Wang and Du, 2013). In the nervous system, Cd accumulation has been correlated with both cognitive and motor decline such as diminished learning, slowed vasomotor and olfactory functions (Wang and Du, 2013). Previous studies have reported high Cd levels in ALS patients, while others found no association between Cd and ALS etiology (Bar-Sela et al., 2001; Bergomi et al., 2002; Gellein et al., 2003; Roos et al., 2013; Oggiano et al., 2021). Our finding that Cd level is high in more severe ALS patients is in accordance with a previously published study (Oggiano et al., 2018). At the molecular level, Cd is known to elicit a wide array of toxic effects including cell necrosis and apoptosis(López et al., 2003), ROS generation(Chen et al., 2008), mitochondrial membrane impairment (Al-Nasser, 2000), lipid peroxidation(Shukla et al., 1996), as well as Ca, Zn, and Cu imbalance (Cannino et al., 2009; Zhang et al., 2012; Yuan et al., 2013). Oxidative stress has been implicated as a potential risk factor for ALS, and Cd is known to induce ROS 36 times more than that of sodium (Na) and Mg(Pogue et al., 2012; Oggiano et al., 2021). Excessive Cd accumulation has also been demonstrated to induce neuron apoptosis through positive regulation of mitochondrial voltage dependent anion channel 1 (VDAC1) protein, endonucleases, caspase 9, cytochrome c, Bax, Bcl-2, and inverse regulation of protein disulphide isomerase (PDI) (Huang et al., 2006; Rahman et al., 2017; Oggiano et al., 2021). Interestingly, Cd overload has also been implicated to induce overexpression of Ca-binding protein S100A2 as well as Ca release from intracellular storage (Yuan et al., 2013; Forcella et al., 2020; Oggiano et al., 2021). This is in line with our results showing that Cd and Ca are both found at high levels in severe ALS patients. While Ca is an essential nutrient for the human body, heightened intracellular Ca levels can induce ROS production, alter mitochondrial membrane potential, and dysregulate cytochrome oxidase function, leading to cell apoptosis(Xu et al., 2011; Yuan et al., 2013; Oggiano et al., 2021). High calcium levels have been reported in various neurodegenerative diseases including PD (Calvo-Rodriguez et al., 2020), HD(Bano et al., 2011), AD (Berridge, 2010; Popugaeva et al., 2015), and multiple sclerosis (Kurnellas et al., 2007; Parihar et al., 2008; Patai et al., 2017). Most important, Ca dyshomeostasis has been evidenced to facilitate abnormal protein misfolding and toxic aggregation in ALS patients(Leal et al., 2013; Tedeschi et al., 2019). Interestingly, other than Cd-mediated calcium increase, various proposed pathological mechanisms of ALS such as, oxidative stress, mitochondrial dysfunction, excitotoxicity, and neuroinflammation can promote calcium overload (Patai et al., 2017).

In addition to Cd, Cu, and Ca, Pb is also found at high levels in high severity ALS patients. Similar to Cd, Pb is highly carcinogenic and does not have any function in the human body, although it can readily cross the BBB and accumulate in neuronal and glial cells. People can be exposed to Pbs in either occupational or environmental settings, and anthropogenic sources of which include fossil fuel, arms, and metal alloy industries. As a cumulative toxicant, Pb can distribute and reside in various tissues and organs including kidney, liver, and the brain. Various quantitative epidemiological studies have investigated the potential role of Pb in ALS pathogenesis; however, current results seem inconclusive as some report high (Fang et al., 2010; Garzillo et al., 2014; Bocca et al., 2015; Eum et al., 2015; Peters et al., 2016; Fang et al., 2017; Oggiano et al., 2018) and others have reported low (De Benedetti et al., 2017; Farace et al., 2020) levels of Pb in various human matrices (Crinnion, 2011; Grashow et al., 2015; Ji et al., 2015; Vinceti et al., 2017; Farace et al., 2020). Our results suggest that while Pb levels are not significantly different between ALS and control groups, it may play a role in disease progression as Pb levels are found at higher levels in more severe patients. In addition, we found a positive correlation between pesticide exposure and ALS disease severity, which may be a potential explanation for higher levels of Cd, Pb, Ca, and Cu levels found in high severity patients. However, our dataset was limited in that there was no pesticide exposure information for participants in the control group, thus we were unable to correlate pesticide exposure to ALS onset. In addition, due limited information on the degree and length of pesticide exposure, we were only able to divide the ALS patients based on “never” or “previous/current” exposure.

Overall, results from our study suggest that high blood Cu and Fe levels may be potential risk factors for ALS, and that Cd, Pb, Ca, and Cu may play important roles in ALS progression based on their correlation with high disease severity. Future studies should consider integrating sequencing data to examine the interacting effects of genetic and environmental risk factors on ALS susceptibility.

5. Authors contribution

Qiao Yi Chen, and Jingxia Dang conceived the study. Material preparation, data collection, and analysis were performed by Xing Qin, Peng Wu, Ting Wen, Rui Jia, Ronghua Zhang, Jiaoting Jin, Fangfang Hu. The manuscript was written by Qiao Yi Chen, Xing Qin, Peng Wu, and Jingxia Dang, and all authors provided comments on draft versions. All authors read and approved the final manuscript.

6. Ethics approval

This study was approved by the Institutional Ethical Committee of Xi’an Jiaotong University. All participants gave written informed consent.

7. Consent to participate and publish

Informed consent was obtained from all individual participants included in this study.

8. Authors agreement

Qiao Yi Chen, and Jingxia Dang conceived the study. Material preparation, data collection, and analysis were performed by Xing Qin, Peng Wu, Ting Wen, Rui Jia, Ronghua Zhang, Jiaoting Jin, Fangfang Hu. The manuscript was written by Qiao Yi Chen, Xing Qin, Peng Wu, and Jingxia Dang, and all authors provided comments on draft versions. All authors read and approved the final manuscript.

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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