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Blood levels of trace metals and amyotrophic lateral sclerosis

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ABSTRACT

Some trace metals may increase risk of amyotrophic lateral sclerosis (ALS), whereas others may be beneficial. Our goal was to examine associations of ALS with blood levels of selenium (Se), zinc (Zn), copper (Cu), and manganese (Mn). We conducted a case-control study of 163 neurologist confirmed patients from the National Registry of Veterans with ALS and 229 frequency-matched veteran controls. We measured metal levels in blood using inductively coupled plasma mass spectrometry and estimated odds ratios (ORs) and 95% confidence intervals (CIs) for associations between ALS and a doubling of metal levels using unconditional logistic regression, adjusting for age, gender, and race/ethnicity. ALS was inversely associated with both Se (OR = 0.4, 95% CI: 0.2–0.8) and Zn (OR = 0.4, 95% CI: 0.2–0.8). Inverse associations with Se were stronger in patients with bulbar compared to spinal onset, worse function, longer diagnostic delay, and longer collection delay; inverse associations with Zn were stronger for those with worse function and longer collection delay. In contrast, ALS was positively associated with Cu (OR = 3.4, 95% CI: 1.5–7.9). For Mn, no linear trend was evident (OR = 0.9, 95% CI: 0.6–1.3, P_{trend} = 0.51). Associations of Se, Zn, Cu, and Mn with ALS were independent of one another. Adjustment for lead levels attenuated the positive association of ALS with Cu but did not change associations with Se, Zn, or Mn. In conclusion, Se and Zn were inversely associated with ALS, particularly among those with worse function, suggesting that supplementation with these metals may benefit such patients, while Cu was positively associated with ALS. Deficiencies of Se and Zn and excess Cu may have a role in ALS etiology.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting motor neurons in the brain and spinal cord (Wijesekera and Leigh, 2009). Most patients are in their fifties and sixties, and ALS is more common in men than women (Wijesekera and Leigh, 2009). ALS has a complex etiology: about 10% of patients have a family history of ALS, and their disease may have a genetic origin; the remaining 90% are likely due to a combination of genetic and environmental factors, with the latter playing an important role (Al-Chalabi and Hardiman, 2013).

Metal exposure is a potentially relevant environmental factor, although previous studies have produced inconsistent results, perhaps partly attributable to limited exposure assessment

Abbreviations: AIC, Akaike information criterion; ALS, amyotrophic lateral sclerosis; ALSFRS-R revised, ALS functional rating scale; CI, confidence interval; CSF, cerebral spinal fluid; Cu, copper; GENEVA, Genes and Environmental Exposures in Veterans with ALS; ICPMS, inductively coupled plasma mass spectrometry; Mn, manganese; MND, motor neuron disease; NHANES, National Health and Nutrition Examination Survey ns not statistically significant; OR, odds ratio; Pb, lead; RBCs, red blood cells; RR, relative risk; SD, standard deviation; Se, selenium; SOD1, superoxide dismutase 1; VA, Department of Veterans Affairs; VALE, veterans with ALS and lead exposure; Zn, zinc.

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(Sutedja et al., 2009). Few studies have evaluated metal exposure using levels measured in blood or other tissues. Lead (Pb), the metal most often studied using measured levels, is generally positively associated with ALS risk (Fang et al., 2010; Kamel et al., 2005). Studies of other metals, including selenium (Se), zinc (Zn), copper (Cu), and manganese (Mn), have primarily evaluated the presumed toxic effects of high exposures (Bergomi et al., 2002; Garzillo et al., 2014; Kapaki et al., 1997; Nagata et al., 1985; Pamphlett et al., 2001; Roos et al., 2013; see Supplemental Table 1). Metal deficiencies could also plausibly increase ALS risk because metals are essential trace elements and play a role in mitigating oxidative stress as well as other critical cell processes (Jellinger, 2013). Previous studies have yielded inconsistent results, possibly because of small sample sizes, choice of comparison groups, or the different types of samples used for measurement (Supplemental Table 1). No previous study has evaluated modification of the ALS-metal associations by clinical features, such as site of onset.

For the present analysis, we used data from the Veterans with ALS and Lead Exposure (VALE) study, a case-control study of US military veterans (Fang et al., 2010). We focused on four trace metals, Se, Zn, Cu, and Mn: Se because it has been reported to increase ALS risk (Vinceti et al., 2010); Zn and Cu because they are co-factors for superoxide dismutase (SOD1), an enzyme implicated in familial ALS (Trumbull and Beckman, 2009); and Mn because it has been implicated in neurodegenerative disease (Jellinger, 2013). In addition, Se, Zn, and Cu all affect oxidative stress (Jellinger, 2013; Navarro-Alarcon and Cabrera-Vique, 2008), a mechanism potentially involved in ALS pathogenesis (Wijesekera and Leigh, 2009). We report associations of ALS with these metals, variation of these associations by clinical features and effects of Pb on the relationships between ALS and each of the four metals.

2. Materials and methods

2.1. Study population

VALE patients came from the US Department of Veterans Affairs (VA) National Registry of Veterans with ALS (VA Registry) (Allen et al., 2008). Veterans or their caretakers who passed a telephone screening questionnaire were asked to provide medical records, and approximately half donated blood samples for a DNA bank (DiMartino et al., 2007). Neurologists specializing in motor neuron disease (MND) used information from the medical records to assign a diagnosis using an algorithm based on the revised El Escorial Criteria (Brooks et al., 2000). The VALE study included a subset of VA Registry patients who donated blood samples between January and September 2007. Our main analyses focused on 163 patients with clinically definite, probable, or possible ALS. We also considered a broader group of MND patients consisting of these ALS patients plus 30 persons diagnosed with progressive muscular atrophy; patients with primary lateral sclerosis were excluded from all analyses.

VALE controls came from the Genes and Environmental Exposures in Veterans with ALS (GENEVA) study (Schmidt et al., 2008). GENEVA identified controls from an age-stratified random sample of 10,000 US veterans obtained from the Veterans Benefit Administration in June 2005. Veterans free of ALS and other neurological disorders were frequency matched to GENEVA patients on age within five years (age at diagnosis for patients and age at interview for controls), and use of the VA health care system before diagnosis/interview (as a proxy for socioeconomic status). For VALE, controls already enrolled in GENEVA were contacted between May 2007 and May 2008 and invited to donate a blood sample during a home visit. Of 359 controls contacted, 252 consented to participate, and 229 donated a blood sample.

Institutional Review Boards of the National Institute of Environmental Health Science, the Durham VA Medical Center, Duke University, and Copernicus Group approved VALE. All study participants provided written informed consent.

2.2. Blood collection and metal measurements

For VALE patients, blood collection procedures were the same as those for other VA Registry patients (Allen et al., 2008) except for the addition of a whole blood sample in a 6-mL BD Vacutainer blue-top Trace Element metal-free tube (Becton, Dickinson and Company, Franklin Lakes, New Jersey) for metal measurement; this tube was collected first. Like patients, VALE controls provided two samples collected during home visits: a 6-mL whole blood sample in a metal-free tube, collected first, and a 9-mL plasma sample. Samples for cases and controls were processed similarly. For both, blood samples were chilled and shipped cold. Upon arrival at the lab, plasma samples were separated and whole blood and plasma samples were frozen within 48 h of blood draw and stored at −80 °C until assay.

The present analysis focused on Se, Zn, Cu, and Mn; Pb was considered as a covariate because it has commonly been associated with ALS. We determined metal concentrations in 1.0 ml whole blood by inductively coupled plasma mass spectrometry (ICPMS) as previously described (Fang et al., 2010). All batches included both cases and controls. Sample contamination was minimized by using a class 100 plastic hood and trace metal-free reagents (oxidants, 18-MΩ-quality deionized water, and ultrex-grade acids). Detection limits were 0.113 μg/dl for Se, 6.24 μg/dl for Zn, 0.234 μg/dl for Cu, and 0.124 μg/dl for Mn. Assay precision was good, with percent relative standard deviation ~2% for all four metals. To additionally monitor precision, 5% of cases and controls underwent preparation and analysis twice; agreement was ≥95% for all duplicates except for Zn, where agreement was ≥80%. Because the assay was optimized for Pb measurement, no external standards for other metals were evaluated. However,values for reagent blanks, included in all batches, were below the detection limit for all four metals. Accuracy was evaluated as percent relative error using spiked blood samples; values were 68% for Se, 5% for Zn, −17% for Cu, and −8% for Zn. The high recovery for Se, contributing to the increased% relative error, is due to spectral interference that can impart a consistent positive bias to biological samples unless a tuning gas is used. This gas can interfere with sensitivity for detection of Pb and hence was not used. Historical data for Se from the laboratory indicated% relative error of −18% for spiked reagent blanks and 10% for spiked blood samples.

2.3. Covariates

Information on covariates including age, gender, race (White, other), ethnicity (Hispanic, non-Hispanic), and smoking (ever, never) was collected by interview for both patients and controls. We combined race and ethnicity to create a single race/ethnicity variable (non-Hispanic White, other).

For patients, we obtained clinical information such as site of onset (spinal, bulbar) and dates of symptom onset and first diagnosis from medical records. We used the latter two variables to create the variable diagnostic delay, defined as the time from symptom onset to diagnosis and categorized as ≤1 vs. >1 year. We defined collection delay as the time from diagnosis to blood collection and dichotomized it at the median (≤13 vs. >13 months).

Patients enrolled in the VA Registry completed follow-up interviews at approximately six month intervals. During each interview, participants' functional status was monitored using the revised ALS Functional Rating Scale (ALSFRS-R) (Gordon et al., 2004). The ALSFRS-R score has a possible range 0–48 with a lower