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Lead Exposure as a Risk Factor for Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis · Epidemiology · Lead exposure · Environment

Abstract
Background: The etiology of amyotrophic lateral sclerosis (ALS) likely involves an environmental component. We qualitatively assessed literature on ALS and lead exposure. Problems of study design make case reports and studies of lead in blood or tissues difficult to interpret. Most previous case-control studies found an association of ALS with self-reported occupational exposure to lead, with increased risks of 2- to >4-fold. However, these results may have been affected by recall bias. ***Objective:*** To address inconsistencies among published reports, we used both lead biomarkers and interview data to assess lead exposure, and we evaluated the role of genetic susceptibility to lead. ***Methods:*** We conducted a case-control study in New England in 1993–1996 with 109 ALS cases and 256 population-based controls. We measured blood and bone lead levels, the latter using X-ray fluorescence, and interviewed participants regarding sources of lead exposure. ***Results:*** In our study, ALS was associated with self-reported occupational lead exposure, with a dose response for cumulative days of exposure. ALS was also associated with blood and bone lead levels, with a 1.9-fold increase in risk for each μg/dl increment in blood lead and a 2.3- to 3.6-fold increase for each doubling of bone lead. A polymorphism in the δ-aminolevulinic acid dehydratase gene was associated with a 1.9-fold increase in ALS risk. ***Conclusion:*** These results, together with previous studies, suggest that lead exposure plays a role in the etiology of ALS. An increase in mobilization of lead from bone into blood may play a role in the acute onset of disease.

Background

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting the motor neurons of the brain and spinal cord. The etiology of ALS is largely unknown, although both genetic and environmental factors may play a role. Recent studies of familial ALS, which constitute 5–10% of cases, have identified several genes associated with the disease [1]. However, variations in gene sequence or expression explain only a small proportion of ALS, suggesting that environmental exposures are also important.

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The descriptive epidemiology of ALS provides additional support for an environmental etiology [2, 3]. ALS is a rare disease: worldwide, the annual incidence is about 1–2 per 100,000 in the general population. Incidence generally increases with age, and risk of ALS in men is approximately 1.5-fold that in women. Some studies suggest that age-specific incidence of ALS is increasing over time, but the possibility that this apparent secular trend is due to better diagnosis or reporting cannot be excluded. In general there is little geographical variation in incidence: small differences in the rates reported for different countries are probably due to differences in the quality of the data. A notable exception is the very high incidence of ALS-Parkinson's dementia complex among the indigenous people of Guam. Most evidence suggests that this complex is due to an environmental exposure, possibly a component of cycad palm seeds [4]. The higher incidence in men and the secular trend also suggest an environmental etiology for ALS.

Outside of Guam, relatively few studies have investigated the relationship of ALS to environmental exposures. A number of occupations have been associated with ALS, including farming, welding or other work with metals, and electrical work [5]. Most studies have found that ALS is not associated with physical activity or trauma [6, 7], but a recent study showed a remarkably increased risk among Italian soccer players with presumed higher rates of head trauma [8]. Recent studies have suggested that US soldiers who were deployed during the first Gulf War are at increased risk of ALS, although the particular exposures involved are still unclear [9]. Some evidence supports associations of ALS with specific environmental exposures, including pesticides [10–13] and electromagnetic fields or related exposures, for example electric shocks [14–19]. A number of studies have evaluated the role of heavy metals, particularly lead and mercury [5].

The hypothesis that lead exposure is associated with ALS has a long history. Case reports have documented occurrence of an ALS-like syndrome following lead poisoning [20–24]. Many of these cases showed some improvement following chelation therapy, which lowers blood lead levels, suggesting that they did not have ALS but rather a neurotoxic response to continuing lead exposure. In contrast, a large case series reported that 24 of 31 cases had a history of metal exposure but showed no elevation in lead levels in blood or urine and no improvement in symptoms following chelation [25]. This result suggested that past exposure to lead might play a role in initiating a disease process that would later become clinically apparent in the presence of relatively normal lead levels. These case reports are difficult to interpret in the absence of controls.

Several early studies investigated the relationship of ALS to lead levels in various body compartments, including blood, cerebrospinal fluid, nerve or muscle, and bone [22, 26–35]. Results were inconsistent but generally showed little elevation in lead levels in ALS patients compared to controls. Most of the positive studies came from one group [27, 28, 31]. All these early studies were less than ideal from an epidemiologic perspective: they were very small (the largest had 25 cases); they sometimes used inappropriate controls; they were unable to address potential confounding, and some used potentially problematic techniques to measure lead.

Nine independent case-control studies have examined the association of ALS with lead exposure [10, 12, 14, 26, 36–40]. In all but one [10], risk of ALS was elevated at least 2-fold in individuals with lead exposure; in two studies [39, 40] risk was elevated more than 4-fold. A potential limitation of these studies is their reliance on self-reports to assess lead exposure. Information on exposure was collected using interviews or questionnaires that generally focused on occupational exposure, although other sources of exposure were sometimes also considered. Self-reports of exposure may present a problem because cases may remember their exposures more clearly than controls, creating the appearance of an association where none in fact exists. This problem is illustrated by results from the study by McGuire et al. [12]. They collected both self-reports of lead exposure and complete occupational histories that were reviewed by a panel of industrial hygienists. The two methods of exposure assessment gave inconsistent results: risk of ALS was increased 1.9-fold for self-reported exposure but only 1.1-fold for the panel's assessment of lead exposure.

Together these studies suggested that lead poisoning is not a common factor in ALS and that ALS cases do not have large elevations in lead levels after diagnosis. However, these results do not preclude the possibility that a past exposure to high levels of lead, or chronic exposure to low lead levels, might play some role in ALS etiology. The case-control studies suggested that ALS might be associated with occupational lead exposure. Inconsistencies among all these studies, potentially due to limitations in exposure assessment, prompted us to conduct a further case-control study of ALS with a different approach. Our study used both biomarkers of lead and interview data to evaluate lead exposure. We measured bone lead, which is considered to reflect cumulative lifetime exposure, and

blood lead, to assess recent exposure, as well as conducting interviews on sources of lead exposure.

Methods

We conducted a case-control study in New England, USA, in 1993–1996, enrolling 109 ALS cases from two Boston clinics [41]. Diagnosis of ALS by board-certified neurologists specializing in motor neuron disease was based on the World Federation of Neurology criteria, i.e., on the presence of progressive disease with both upper and lower motor neuron signs. More than 80% of cases were first diagnosed within 1 year of enrollment and the remainder within 2 years. Cases were required to live in New England, to be mentally competent, and to speak English. We compared the cases to 256 population controls who were identified by random telephone screening and frequency matched to the cases on the basis of age, sex, and region within New England. Eligibility criteria were the same as for cases; in addition we excluded individuals with any neurodegenerative disease from the control group.

Data collection involved both a structured interview and measurements of blood and bone lead. The interview collected information on demographics, medical history, lifestyle, hobbies, residential history, and occupational history; its main focus was exposure to lead and other neurotoxicants. We measured bone lead in both tibia and patella [42] using K X-ray fluorescence (XRF) and blood lead using atomic absorption spectrometry. We collected whole blood as a source of DNA for studies of genetic susceptibility to lead. DNA was isolated and genetic polymorphisms identified using PCR-RFLP [43]. Of eligible cases, 71% participated in the study; virtually all completed both the interview and the laboratory portions of the study. Of eligible controls, 74% completed the interview but only 41% completed the laboratory portions of the study.

Data were analyzed using logistic regression. All models included the matching variables age, sex, and region within New England. Models also included education ($\leq$ high school vs. $>$ high school). Models for bone and blood lead included a continuous variable measuring current physical activity (hours per day spent sitting, lying down or sleeping). Blood and bone lead levels were modeled as continuous variables after transformation using $\log_2([Pb] + 32)$, where [Pb] is lead concentration [41]. Results are expressed as adjusted odds ratios (ORs) with 95% confidence intervals (CIs).

Results and Discussion

Table 1 shows the association of ALS with self-reported occupational exposure to lead [41]. Cases were 1.9 times more likely than controls to report ever having had a job involving lead exposure, consistent with other reports. There was also a dose response for lifetime days of on-the-job exposure; exposure for 2,000 or more days (approximately 8 years of 5-day weeks) was associated with a 2.3-fold increase in risk of ALS. We collected a complete occupational history from study participants and identified jobs likely involving lead exposure, for example battery manufacture or reclamation, soldering, working with firearms or on a firing range, painting, or paint removal. This approach produced virtually identical results to the self-reported data: ever having had a job involving lead exposure was associated with a 2-fold increase in risk of ALS. The dose response for lifetime days of exposure and the findings for occupational history suggest that the association with self-reported occupational exposure was not due to recall bias.

Table 1. Association of ALS with self-reported occupational exposure to lead in a case-control study conducted in New England, USA, 1993–1996[1]

	Cases		Controls		Adjusted OR	95% CI
	n	%	n	%		
Ever had a job with lead exposure						
No	67	66	193	78	1.0	referent
Yes	35	34	54	22	1.9	1.1–3.3
Lifetime days of lead exposure						
0	67	66	193	78	1.0	referent
1–399	8	8	17	7	1.6	0.6–3.9
400–1,999	11	11	17	7	1.9	0.8–4.3
2,000+	16	16	20	8	2.3	1.1–4.9
Trend						p = 0.02

[1] Adjusted ORs and 95% CIs were calculated by logistic regression controlling for age, sex, region within New England, and education. This table is reproduced with permission from Kamel et al. [41].

In contrast to results for occupational exposure, ALS was not associated with potential residential or recreational sources of lead exposure [41]. Using a complete residential history, we examined characteristics that might entail exposure, for example the age of the residence and its location in a large city, in a high traffic area, or near a highway or battery plant. None of these characteristics was associated with increased risk of ALS. Similarly, we found no association of ALS with recreational activities that might involve lead exposure, including hunting, target shooting either in general or on an indoor range, casting bullets or reloading ammunition, fishing with lead weights, glazing pottery or other ceramics, or soldering. Our finding that ALS was related to occupational but not residential or recreational lead exposure is not surprising. Occupational exposure is likely to involve higher levels of lead and more constant and prolonged exposure. Occupational exposure is also easier to quan-

Table 2. Association of ALS with blood and bone lead levels in a case-control study conducted in New England, USA, 1993–1996[1]

	Cases mean ± SE	Controls mean ± SE	Adjusted OR	95% CI
Blood lead, μg/dl	5.2 ± 0.4	3.4 ± 0.4	1.9	1.4–2.6
Patella lead, μg/g	20.5 ± 2.1	16.7 ± 2.0	3.6	0.6–20.6
Tibia lead, μg/g	14.9 ± 1.6	11.1 ± 1.6	2.3	0.4–14.5

[1] Means were adjusted for age, sex and region within New England; values for 60-year-old men from Boston are presented. Adjusted ORs and 95% CIs were calculated by logistic regression controlling for age, sex, region within New England, education, and physical activity. ORs were calculated for each unit increase in blood lead or in log-transformed bone lead; the latter is approximately equivalent to a doubling of bone lead.

tify with interview techniques than residential or recreational exposure. For example, residential measures like age of housing are relatively crude markers for lead exposure, so there is probably more misclassification, making it more difficult to observe an effect.

Table 2 shows the association of ALS with blood and bone lead levels [41]. The range of blood lead levels was <1 to 14 μg/dl, of patella lead levels –4 to 107 μg/g, and of tibia lead levels –7 to 61 μg/g (very low bone lead levels can be estimated as negative values [42]). Mean lead levels were higher in ALS cases than controls for both blood and bone. The relationship of ALS to blood lead was strong and statistically significant, with a 1.9-fold increase in risk for each μg/dl increase in blood lead. The associations of ALS with bone lead were numerically greater, with a 3.6-fold increase in risk of ALS for each doubling of patella lead, and a 2.3-fold increase in risk for each doubling of tibia lead. However, these relationships were actually weaker than the association with blood lead, since they were calculated for a greater increment in bone lead. Variability associated with bone lead measurements may have contributed to the imprecision of the associations of ALS with bone lead.

We were concerned that the association with blood lead might represent an effect rather than a cause of ALS. A decline in physical activity as cases became weaker might lead to bone demineralization with release of lead from bone into blood and consequent elevation in blood lead levels. However, we found that associations of ALS with bone and blood lead levels were actually stronger after adjusting for current levels of physical activity. We also found that among cases there was no correlation of lead levels with time since diagnosis and that the associations we found were essentially unchanged after excluding cases who had been diagnosed more than 1 year before enrollment in the study. So reverse causality appears to be an unlikely explanation for the strong association of ALS with blood lead levels.

We found these results surprising. We had hypothesized that ALS would be associated with cumulative lead exposure, represented by bone lead, but we found a stronger and more reliable association with blood lead. A possible explanation is that the elevation in blood lead in ALS cases is simply a reflection of higher bone lead. Blood lead is considered to indicate recent exposure, but in older individuals with no obvious sources of external exposure, like most of the participants in our study, bone lead is actually the largest source of lead in blood [44]. Future research on this issue could use markers of bone resorption to evaluate whether the combination of bone resorption and bone lead predicts ALS better than bone lead alone. Another interesting possibility is that pre-existing differences in lead metabolism between cases and controls make lead more likely to be mobilized from bone in people who then go on to develop ALS. These pre-existing differences could be genetic.

To explore this possibility, we examined a polymorphism in the δ-aminolevulinic acid dehydratase gene *(ALAD)* that has been implicated in lead susceptibility [45]. Lead is bound to ALAD protein in red blood cells, and the variant ALAD 2, found in 10% of the Caucasian population, binds lead more tightly than the wild-type ALAD 1. This differential binding affects the toxicokinetics of lead, so that more lead remains in blood and less is taken up in bone in *ALAD 2* carriers. The effect of this change on the toxicity of lead is unclear. Tighter binding of lead to the variant allele might make lead less available to target tissues and hence less toxic, so that the variant allele would have a protective effect. On the other hand, tighter binding of lead to the variant allele means that more lead remains in blood, available to target tissues, so that risk might be increased in *ALAD 2* carriers. Only a few studies have actually investigated the effect of the polymorphism on the toxicity of lead, with respect to several health outcomes, and most have found little effect [46–51].

We found no difference in blood lead levels associated with genotype [43]. However, bone lead levels were reduced from 14 to 7.3 μg/g for patella lead and from 14 to 8.6 μg/g for tibia lead in *ALAD 2* carriers compared to *ALAD 1-1* homozygotes. These results are consistent with previous studies suggesting that the polymorphism con-

Table 3. Association of ALS with a polymorphism in the δ-aminolevulinic acid dehydratase *(ALAD)* gene in a case-control study conducted in New England, USA, 1993–1996[1]

	Cases		Controls		Adjusted OR	95% CI
	n	%	n	%		
ALAD 1-1	80	78	31	82	1.0	referent
ALAD 1-2 and *ALAD 2-2*	23	22	7	19	1.9	0.60–6.3

[1] Adjusted ORs and 95% CIs were calculated by logistic regression controlling for age, sex, region within New England, education, and physical activity.

sistently reduces bone lead levels but affects blood lead levels only at higher concentrations [52, 53]. Table 3 shows that ALAD 2 carriers had a 1.9-fold increase in risk of ALS. When both lead levels and genotype were included in models, results for lead levels were unchanged and the association of ALS with genotype was stronger, but lead level did not interact with genotype. The results do not provide direct support for modification by *ALAD 2* of the ALS-lead relationship, although our power to investigate effect modification was limited. On the other hand, the association of ALS with the *ALAD* polymorphism itself may be related to a subtle change in lead toxicokinetics over a lifetime that is difficult to observe in a cross-sectional study.

Mechanisms involved in lead neurotoxicity are similar to those involved in ALS pathogenesis, supporting a role for lead exposure in ALS [54]. Lead promotes mitochondrial dysfunction, oxidative stress, and excitotoxicity. Lead affects glia as well as neurons, particularly astrocytes, which sequester lead in young animals. Initially sequestration may serve to reduce lead neurotoxicity, but later in life, or under conditions when astrocytes become activated, stored lead may be released and be a source of exposure to neurons. Many of these mechanisms – mitochondrial dysfunction, excitotoxicity, oxidative stress – may involve disruption of intracellular calcium homeostasis. Lead can bind to many calcium-sensitive sites and substitute for calcium in many intracellular reactions; these sites include voltage-sensitive calcium channels, synaptotagmin, the calcium sensor for exocytosis, protein kinase C, phospholipase C, and calmodulin [55]. These effects take place at concentrations comparable to those seen in exposed people and are thus physiologically relevant.

In summary, we found that ALS was associated with occupational but not residential or recreational lead exposure and with increased blood and bone lead levels. Genetic susceptibility to lead may contribute to these associations. These results are consistent with previous reports and a role for lead exposure in ALS pathophysiology is biologically plausible. The role of environmental neurotoxicants in neurologic disease is often considered to involve cumulative exposure over long periods of time. Aspects of the disease process in ALS are rapid, however. The number of motor units declines rapidly and abruptly during the first year of clinically apparent disease [56]; the loss of motor units in the SOD mouse is also rapid [57]. Although this loss may reflect the accumulation of damage during a preclinical period, the hypothesis that elevated blood lead levels may contribute to or accelerate this process on an acute basis deserves further consideration.

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