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A Meta-Analysis of Observational Studies of the Association Between Chronic Occupational Exposure to Lead and Amyotrophic Lateral Sclerosis

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Objective: The association between occupational exposure to lead and amyotrophic lateral sclerosis (ALS) was examined through systematic review and meta-analyses of relevant epidemiological studies and reported according to PRISMA guidelines. **Methods:** Relevant studies were searched in multiple bibliographic databases through September 2013; additional articles were tracked through PubMed until submission. All records were screened in DistillerSR, and the data extracted from included articles were synthesized with meta-analysis. **Results:** The risk of developing ALS among individuals with a history of exposure to lead was almost doubled (odds ratio, 1.81; 95% confidence interval, 1.39 to 2.36) on the basis of nine included case-control studies with specific lead exposure information, with no apparent heterogeneity across included studies ($I^2 = 14\%$). The attributable risk of ALS because of exposure to lead was estimated to be 5%. **Conclusions:** Previous exposure to lead may be a risk factor for ALS.

Learning Objectives

- Become familiar with previous research on the association between exposure to lead and heavy metals and the risk of amyotrophic lateral sclerosis (ALS).
- Summarize the findings of the new meta-analysis of studies of the association between occupational lead exposure and ALS risk.
- Discuss the authors' conclusions on the magnitude of the association, the findings of adjusted analyses, and the fraction of ALS cases attributable to lead.

Amyotrophic lateral sclerosis (ALS), a disease term often used interchangeably with motor neuron disease, is a rare multifactorial degenerative condition of motor neurons, characterized by rapid and irreversible progression. It presents as either a familial (fALS) or a sporadic (sALS) form, accounting for 5% to 10% and 90% to 95% of all ALS cases, respectively.[1] Amyotrophic lateral sclerosis begins with either a bulbar or a spinal onset in one third and two thirds of the ALS cases, respectively. In rare instances (about 1% of ALS cases), the initial noticeable clinical symptom is respiratory failure.[2] The disease usually occurs in adults, with an average age at onset of 60 years.[3] The average age at onset is higher among bulbar than among spinal cases.[3] Survival after diagnosis is about 2 to 3 years for bulbar onset cases and 3 to 5 years for spinal onset cases.[3]

The causes of ALS remain largely unknown, except for a small proportion (about 10%) of cases (including both sALS and fALS cases) that are related to monogenic mutations.[4] This type of monogenic mutation has been found in about two dozen genes.[5–8] In white patients with ALS, the two most common monogenic mutated genes are *C9orf72* and *SOD1*; mutations in these two genes are responsible for 35% and 25% of fALS cases and 6% and 2% of sALS cases, respectively.[3,4,9–11] The mutation in *C9orf72*, which is rare in nonwhite populations,[11] arose in Scandinavia several thousand years ago.[12,13] In nonwhite populations, *SOD1* is the most common mutated gene in patients with ALS.[14] Nevertheless, most (90%) sALS cases are believed to be caused by polygenic variants/polymorphisms, environmental risk factors, and perhaps stochastic factors that exert their influence only in genetically susceptible individuals.[15] Environmental and genetic factors are thought to play equally important roles in the development of ALS.[16,17] Although many polymorphic gene variants (such as *PON1* and *VEGF*) and environmental factors (such as pesticides, heavy metals, trauma, smoking, and electric shock) have been reported to be associated with ALS, none have been conclusively determined to cause ALS.[18–23]

Prior exposure to heavy metals, including lead, has long been suspected to be associated with an increased risk of ALS. Case reports have associated the onset of ALS with exposure of several heavy metals, including selenium, mercury, lead, aluminum, and manganese.[22] Epidemiological studies investigating the association between prior exposure to lead and ALS began about five decades ago, after a series of ALS cases with antecedent exposure to lead were reported as early as 100 years ago.[24–26] Since then, nearly two dozen observational epidemiological studies of ALS, in which assessment of exposure to lead was retrospective, have been conducted.[26–46] Most studies showed that reported occupational exposure to lead was associated with a higher risk of developing ALS,[34,42,47] although in some studies this was not statistically significant.[32,36,42] The major challenge in linking lead and ALS is the retrospective ascertainment of historical lead exposures. Although some studies measured lead levels in blood or other body fluids—these levels may not accurately reflect previous intensity or duration of exposure to lead—it is perhaps not surprising that some previous studies did not observe differences in lead levels between cases and controls.[34]

A recent study of the spatial distribution of ALS cases showed a gradient in the incidence of ALS in the vicinity of a lead smelting factory in a county of Missouri state.[48] In a case-control study in

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Boston, Kamel and colleagues found a dose–response relationship between ALS and self-reported estimates of exposure to lead[33,34,45]; this linear association was also observed in measured lead levels in blood and bone.[46] These findings have shed new light on the association between previous exposure to lead and ALS. The purpose of this study is to describe the association between prior exposure to lead and ALS by combining multiple relevant observational epidemiological studies using systematic review and meta-analyses.

METHODS

Search Strategy

The search strategy was initially developed in Medline using search terms selected to identify relevant scientific publications, including systematic reviews, meta-analyses, and observational (case-control, cohort, and cross-sectional) studies (see Supplemental Digital Content 1, http://links.lww.com/JOM/A175). Once the search strategy was sufficiently well developed, it was used to search other bibliographic databases, with minor modifications to adapt to the requirements of those databases. The search included disease-relevant terms (amyotrophic lateral sclerosis, ALS, motor neuron disease, or Lou Gehrig's disease), and terms of environmental risk factors to identify all environmental risk-related studies. The articles related to exposure to lead were further identified with lead exposure-related terms (see below).

Databases Searched

The following databases were searched through September 2013: Medline, PubMed, EMBASE, Toxiline/toxnet, Ageline, Proquest (including dissertations), PsycInfo, and Google Scholar. Relevant articles were also hand-searched from reference lists and other databases, or through contacting to article authors. Similar articles were tracked with PubMed until the submission of this article.

Screen and Selection of Retrieved Articles

Duplicate articles were identified using Reference Manager by comparing authors and titles in adjacent references, after sorting references by author name; duplicates were then removed. The retained articles were screened in DistillerSR using predesigned screening forms. Level 1 screening was conducted by reading the titles in relation to the inclusion/exclusion criteria (English language, human study, relevant disease terms, environmental risk factor terms, and observational epidemiological study [cohort, case-control, cross-sectional, but not an intervention study or a review or commentary]). Level 2 screening was conducted by examining abstracts with respect to the inclusion/exclusion criteria mentioned above. Level 3 screening was conducted by examining the complete article and applying additional inclusion/exclusion criteria (relating to population characteristics, case ascertainment, environmental risk factors [studies of military personnel were excluded because of the highly selected nature of such populations, and studies with ALS confined to Guam were excluded because the disease on that island and surrounding areas might be associated with specific local risk factors], data analysis methods, and key results and conclusions] using two reviewers. During this step, if we found that an article had only fALS cases, or an article was with only an ecological study design, then the article was excluded. After this step, the retained articles were examined to carefully identify terms related to exposure to lead (lead, heavy metal, solder, soldering, Pb, weld, or welding) by using "find" function in the PDF file or reading through the Methods and Results sections of the article.

Quality Assessment, Data Extraction, and Meta-Analysis

Because ALS is a rare neurological condition, it is important to determine whether information on the same sample of patients with ALS has been reported in more than one article among the set of retained articles. If so, then the earliest article was included in the meta-analysis in the interests of minimizing the potential information fidelity decay with the time when reconstructing historical lead exposure profiles in later studies. The quality of articles selected for meta-analysis was evaluated using a system with a total 20 points, developed by our research team (see Supplemental Digital Content 2, http://links.lww.com/JOM/A176)[49,50] as a modification of the quality assessment tool of Downs and Black.[51] Information on study participants, study design, data collection, and synthesis was used to evaluate the comparability between cases and controls, risk factor estimation, and the control of the potential biases and confounding. More detail about the items evaluated for each article is presented in Supplemental Digital Content 2, http://links.lww.com/JOM/A176.

In the present meta-analysis, comparisons were based on subjects ever-exposed versus never-exposed to lead through the occupational environment on the basis of the results from included articles. Relevant result information was abstracted from the selected articles, including lead author, country, study type, study period, recruitment method, case ascertainment, control selection, response rates, data analysis methods, risk factor information, study results, and conclusions. A 5% of random sample of the studies from which data had been abstracted was verified by a second reviewer. The odds ratio (OR) for ALS in relation to lead exposure was used as the summary measure of risk in these meta-analyses. The primary meta-analysis was based on random-effects modeling, with a fixed-effects model run as a secondary analysis using Review Manager 5.1.[52] Heterogeneity across included studies was estimated by Tau^2, χ^2, and I^2. Forest plots and relevant supporting statistics were examined. Funnel plots were used to evaluate possible publication bias. Meta-analyses for subgroups (on the basis of publication year, exposure to lead vs to heavy metals, adjusted risk estimate) were considered if at least three articles were available for each category. To investigate whether the quality of studies influences the risk estimates from meta-analysis, the articles were divided into two groups, representing articles with higher and lower quality, respectively. If two articles had the identical median quality score, then the more recent article (which would have benefited from the experience of previous investigations and better controlled analyses) was allocated to the higher quality score group to achieve two groups of equal size.

Attributable Risk of ALS Because of Previous Exposure to Lead

The combined prevalence data for meta-analysis were used to estimate the attributable risk (AR) because of previous exposure to lead among ALS cases.[53] The assumptions underlying this calculation are the following[54,55]: (1) the disease prevalence in the general population is low (usually <5%); (2) previous exposure to lead is causally associated with ALS; (3) ALS cases and controls included in the selected observational studies are representative of the total population of patients with ALS and general population; and (4) all excess exposure events among ALS cases as compared with controls are responsible for the development of ALS among those who reported being exposed to lead. Under these assumptions, this attributable risk is given by

$$AR(\%) = 100 \times P \times (r-1)/[P \times r + (1-P)],$$

where P denotes the prevalence of exposure to lead among control subjects and r denotes the relative risk (RR) for ALS because of exposure to lead. The RR could be replaced by the OR, which is a close approximation to the RR when the OR is not large.

RESULTS

Summary of Literature Search

The results of this systematic review and meta-analysis were reported according to the PRISMA guidelines.[56] Details of the literature search, article screening, article evaluation, data extraction, and data analyses described in the Methods section are summarized in Fig. 1. All of the selected articles are case-control studies, except for two large cohort studies (see Supplemental Digital Content 3, Supplemental Table 1, http://links.lww.com/JOM/A177).[43,44] Of the 21 selected articles, four articles used the same ALS subjects[33,34,45,46]; the earliest report was selected for inclusion in the meta-analysis,[33] leaving 18 articles for further evaluation. Of the 18 retained articles, nine articles used the risk term "lead," four articles used the terms relating to exposure to "heavy metals," and five articles did not provide primary prevalence data, but only summary risk estimates. The meta-analysis was therefore conducted with the nine articles, specifically addressing the risk associated with occupational exposure to lead. Sensitivity analyses were conducted to evaluate the effect of various categories of publications, including the four articles focusing on occupational exposure to heavy metals of any type, including lead, and the effect of the study quality.

Meta-Analysis of Association Between Previous Exposure to Lead and ALS

The results of meta-analyses on the basis of nine included articles related to occupational exposure to lead revealed that the odds of developing ALS were significantly higher among subjects with a history of occupational exposure to lead than among unexposed subjects (OR, 1.81; 95% confidence interval, 1.39 to 2.36, on the basis of a random-effects model [Fig. 2]; the corresponding estimate on the basis of a fixed-effects model was identical [OR, 1.81; 95% confidence interval, 1.42 to 2.29], with no significant heterogeneity across included studies [$P = 0.32$, $I^2 = 14\%$]), and with no apparent publication bias indicated by the funnel plots (data not shown). A similar increase in the risk of ALS was also found on the basis of the meta-analysis of four articles using heavy metals as the risk factor (OR, 2.13; 95% confidence interval, 1.33 to 2.42) (Fig. 2). Thus, combining the nine articles focusing on lead exposure with the four articles focusing on exposure to all heavy metals yields an OR of 1.87 (1.57 to 2.33). Similar results were obtained using the fixed-effects model (data not shown).

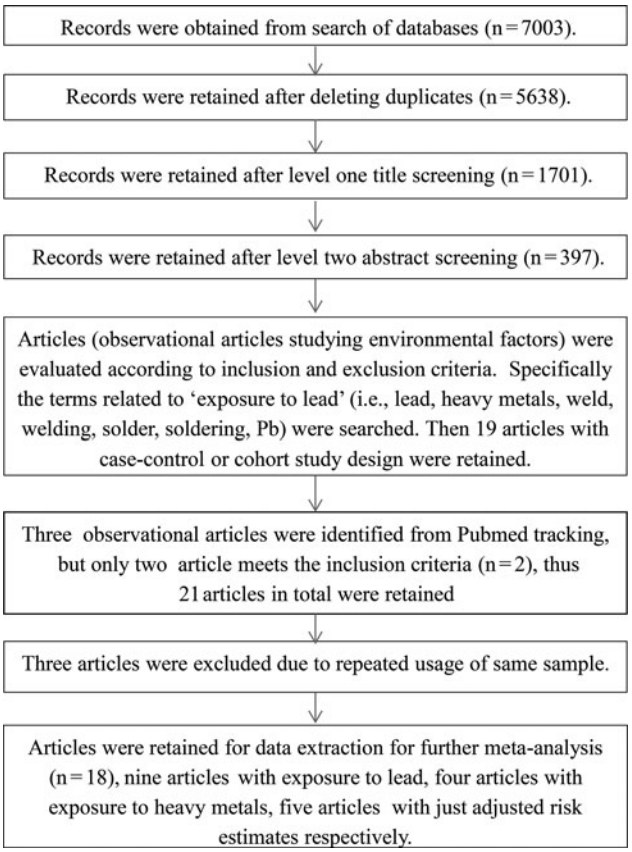


FIGURE 1. Literature search, screen, evaluation, data extraction, and data analysis flow chart for the meta-analysis of observational studies of the association between previous exposure and ALS.[56]

Quality of Articles Seems Not to Significantly Affect the Conclusion

As indicated in Fig. 2, the risk of ALS because of exposure to lead is not materially different from the risk because of exposure to heavy metals. We therefore used all 12 studies to evaluate whether study quality affects the risk estimate of developing ALS. The median quality score among these 12 articles was 7 of a possible total of 20 points, and two articles were assigned this score. We arbitrarily allocated the newer article to the high-quality group to balance the group sizes. A new article became available during the course of the peer review of this article, which is assigned to the high-quality group because its quality score was 14, thus the meta-analysis was updated and included this article.[57] This yielded a total of 7 and 6 articles in the higher and lower quality groups, respectively (Fig. 3). The meta-analysis revealed that the publication quality did not significantly change the estimate of risk for ALS, although the risk derived from articles with higher quality (OR, 1.71 [1.32 to 2.23]) is slightly lower than the risk derived from articles with lower quality (OR, 2.23 [1.54 to 3.21]) (Fig. 3).

Adjusted Risk Estimates Are Somewhat Attenuated

Of the 18 included studies for this article, five articles provided adjusted risk estimates (adjusted for sex and age, for example) only (either RRs or ORs) either for the risk factor "lead" or for the risk factor "heavy metals."[38,39,42–44] Two other articles also provided adjusted risk estimates, along with the prevalence of exposure to lead among cases and controls. One of these two studies has been included in Fig. 2;[37] the other[46] used the same sample of patients as the article authored with Kamel,[33] which was therefore excluded from the analysis in Fig. 2. We conducted an additional meta-analysis with the estimates from these six studies to assess the degree of agreement with the results for the 13 studies in Fig. 2. The results of this analysis indicate that the risk is increased by about 40% (OR, 1.41 [1.21 to 1.65]) (Fig. 4), slightly lower (but not significantly different) than the estimate from Fig. 2 and with no heterogeneity ($I^2 = 0$) among the included studies or evidence of publication bias (Funnel plot, figure not shown).

Attributable Risk Because of Previous Exposure to Lead

A total of 191 ALS cases from a population of 1228 individuals with ALS had a history of previous exposure to lead, whereas a total 160 controls from 1544 normal subjects had previous exposure to lead, on the basis of the nine studies using lead rather than heavy metals as the exposure metric in Fig. 2. The AR because of previous exposure to lead is calculated on the basis of these four values using the formula given in the Methods section. The prevalence of exposure is given by $P = 160/1544 = 0.1036$, and the relative risk is $RR = [(191/1228)/(160/1544)] = 1.5009$. The AR is then estimated to be $4.9\% = [0.1036 \times 0.5009/(0.1036 \times 1.5009 + 0.8964)] \times 100\%$ of total ALS cases. This AR estimate implies that 61 ALS cases of

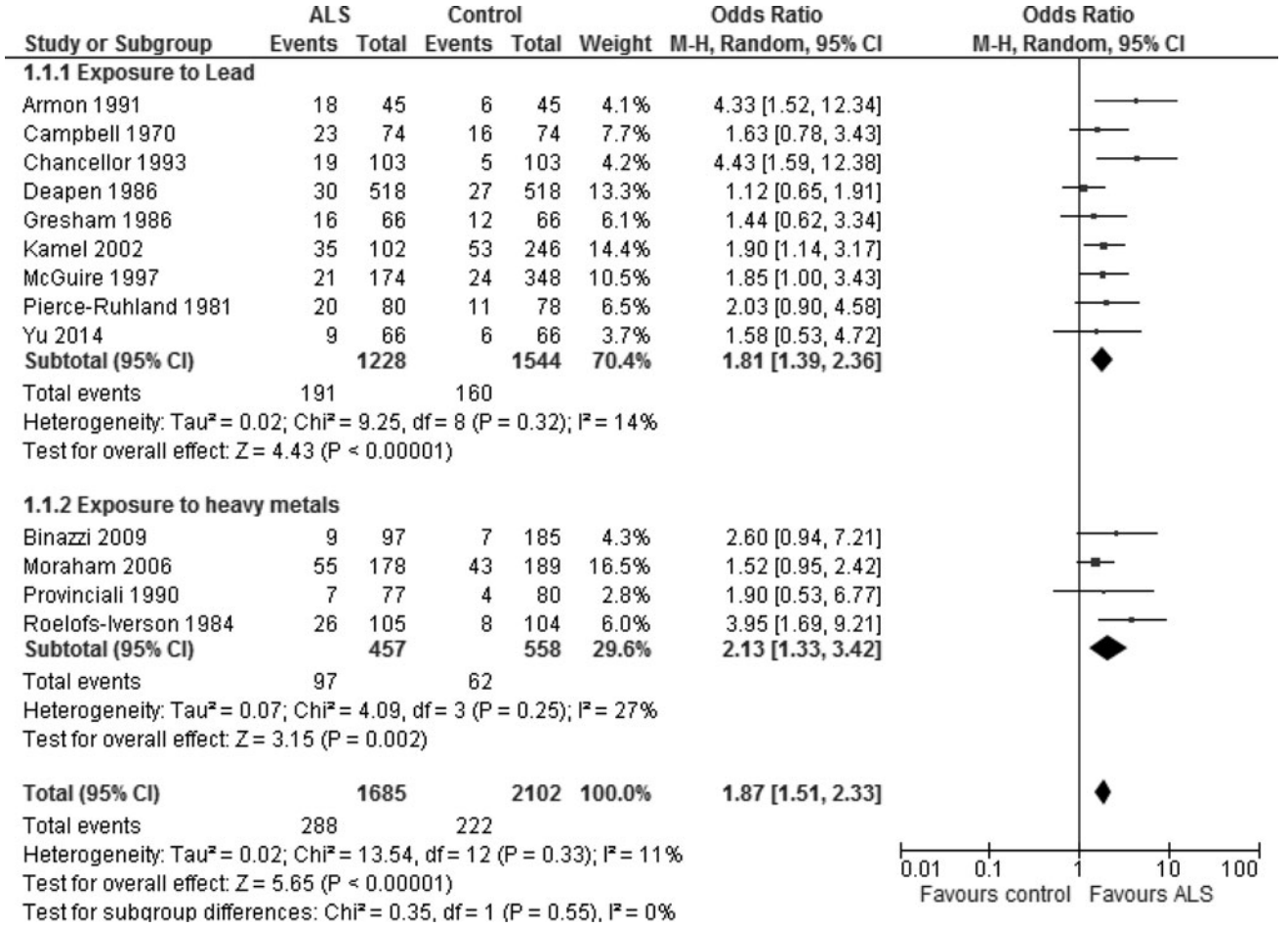


FIGURE 2. Previous exposure to lead/heavy metals is associated with increased risk of developing ALS. Exposure data were extracted from 13 case-control studies in which exposure to lead (9 studies) or heavy metals (4 studies) and the risk of ALS was assessed. No evidence of heterogeneity across the included studies was observed in a meta-analysis using a random-effects model. Nor was their evidence of significant publication bias (data not shown).

the total 1228 ALS cases from all included studies might have been caused by previous exposure to lead.

DISCUSSION

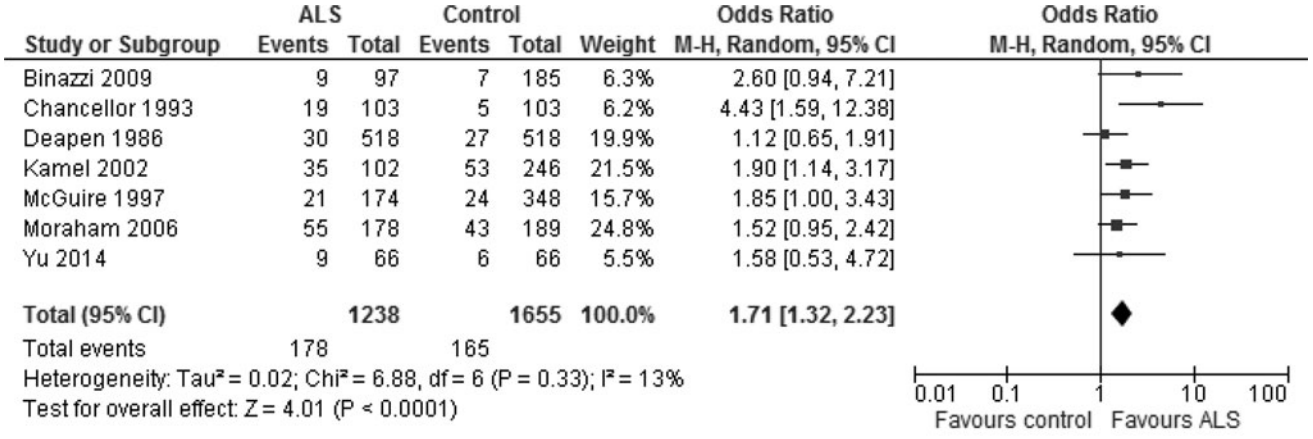
The present meta-analysis of nine case-control studies with specific lead exposure information indicates that the risk of developing ALS is almost doubled after occupational exposure to lead, compared with unexposed controls. The estimated risk for ALS because of exposure to lead is not materially different from that calculated on the basis of exposure to all heavy metals. In addition, we found that the estimated risk on the basis of the articles of lower quality is not significantly different from the estimated risk on the basis of the articles of higher quality, although the former estimate is slightly higher than the latter (Fig. 3). The available data suggest that about 5% of all sporadic ALS cases may be attributable to occupational exposure to lead, although the actual attributable fraction could be somewhat lower because of the assumptions in this calculation. Nonetheless, our results suggest that previous exposure to lead in the occupational environment is a significant risk factor for ALS. The following observations from the scientific evidence in literature support this association.

Lead Is Toxic to Motor Neurons in Humans

Motor neuron toxicity in humans after exposure to lead was recognized more than a century ago.[26] The classic form of lead neuropathy is characterized by weakness that initially involves primarily the wrist and finger extensors, but later spreads to other muscles.[58] Sensory involvement is minimal.[58] Motor neuropathy is more likely to develop after relatively short-term exposure to high lead concentrations, and evolves in a subacute fashion.[58] Many ALS-like or ALS cases with antecedent occupational or cosmetic exposure to lead have been documented.[24,59–62] For example, the study by Felmus et al[62] documented six ALS cases with antecedent occupational exposure to lead for periods of 8 to 34 years. A number of epidemiological studies on long-term exposure to lead have also shown an association with ALS.[34,42,47] These observations suggest that prior exposure to lead may be causally associated with the development of ALS.

Lead Level in Tissues Is Associated With an Increase in the Risk of ALS

Demonstrating a dose–dependent relationship between exposure to lead and ALS is important in detecting a causal relationship. An increasing positive relationship between lead exposure (on the basis of occupational history) and the risk of ALS has been shown in some epidemiological studies.[26,35] Nonetheless, the reliability and validity of the exposure ascertainment would be enhanced if supported by biomarkers of lead exposure measured in body tissues or fluids. Higher lead levels have been reported in muscle tissue,[63,64] cerebrospinal fluid (CSF), blood, and plasma/serum[64–68] in patients with ALS, as compared with controls.[26,69–74] Nevertheless, fewer



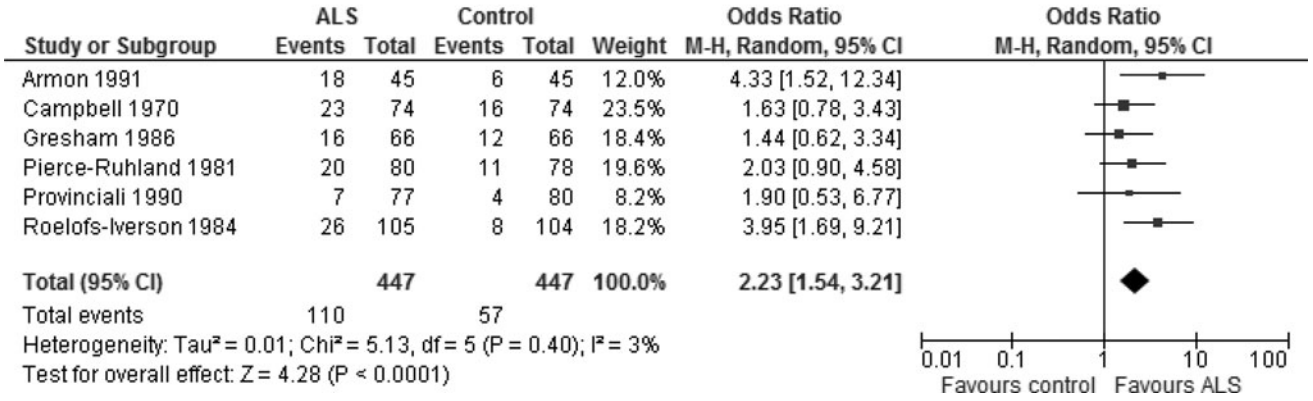


FIGURE 3. Article quality among included studies does not affect the risk estimate. Included studies were assessed and divided into two groups (one comprising seven articles and the other six) on the basis of quality scores. If two articles with an identical quality score need to allocate into two groups, then we arbitrarily allocated the newer article to the high-quality group, to balance the group sizes. The meta-analysis of articles (random-effects model) with higher quality scores is shown in the upper panel, and the meta-analysis of articles with lower scores is presented in the lower panel.

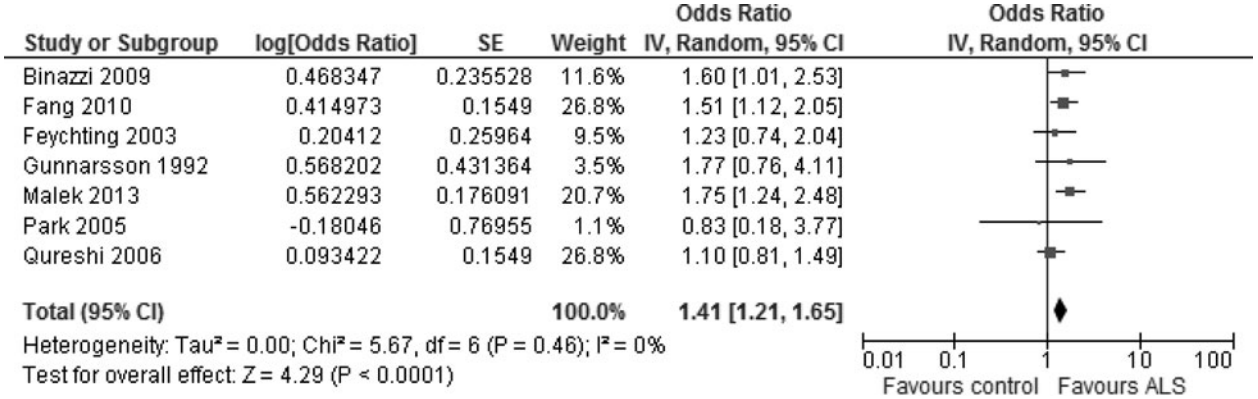


FIGURE 4. Meta-analysis using adjusted relative risk estimates provided by included articles. Seven articles were included in this analysis, including 5 case-control studies and 2 cohort studies. Two case-control studies presented risk estimates specifically for exposure to lead,[42,45] with the remaining case-control studies focusing on exposure to heavy metals, including lead. The two cohort studies gave estimates for exposure to heavy metals, including lead.[43,44] The relative risk (OR or RR) from 7 included articles were first transformed to the log value, then estimated the confidence error on the basis of confidence interval log (95% upper CL − 95% lower CL). The meta-analysis was conducted with RevMan 5.1.

than 30 subjects were included in most of these studies,[65–69,74] limiting their power to establishing dose–dependent increased risk of ALS. A report published in 2013 showed that CSF lead concentrations in patients with ALS were higher (even higher than in blood), further supporting the association between ALS and previous lead exposure. The higher CSF lead concentrations in patients with ALS suggest a net influx of lead from blood into the CSF, reflecting a possible mechanism for bioaccumulation of lead in patients with ALS,[68] with limited potential for elimination from the body. Such an association has also been observed in a case-control study of 108 ALS cases in the Boston area conducted by Kamel et al,[33,46] on the basis of measured blood lead concentrations. Taken together, these studies are compatible with the findings of the present meta-analysis.

Mechanisms Whereby Chronic Exposure to Lead Might Cause ALS

Chronic exposure to lead could cause damage to the renal, nervous, reproductive, endocrinal, and immunological systems,[75] possibly obfuscating a diagnosis of ALS in light of similar symptoms associated with other health conditions. Pure motor neuron effects after chronic exposure to lead have been observed in chickens,[76] which demonstrated motor neuron disease with characteristics similar to human ALS.[76] Lead deposition was identified in both the spinal cord and muscles in chickens exposed to lead. Spinal motor neuron degeneration (in the anterior horn cells), motor axonal loss, and atrophy of muscle tissue were also observed in the chickens exposed to lead. As noted previously, many ALS cases have been diagnosed after chronic exposure to lead.[24,59–62] Collectively, these results suggest that chronic exposure to lead could result in ALS in humans, in the absence of symptoms associated with exposure to lead being manifested in nonneuronal or neuronal tissues.

Nearly 200 mutations in metal-binding superoxide dismutase 1 (SOD1) have been linked to ALS.[7,77] Animal experiments found that treatment with lead could increase SOD1 expression of mRNA in mice,[78] and decrease SOD1 activity in rats,[79] suggesting that lead treatment might influence the normal folding process of SOD1 protein, and potentially cause the accumulation of unfolded or misfolded SOD1 protein, a primary mechanism resulting in the apoptosis of motor neurons.[80] As a potential link between lead exposure and ALS, we suggest that lead may trigger misfolding of metal-binding proteins,[81] such as SOD1, and induce a productive template for propagation of the misfolded protein.[82,83]

Limitations of This Study

This study is subject to several limitations. First, the observation of an increased risk of ALS in relation to prior exposure to lead on the basis of meta-analysis demonstrates only association, not causation. Nevertheless, the weight of evidence in support of a causal relationship is strengthened when the totality of evidence from clinical case reports, epidemiological studies, and toxicological studies is considered. Second, the accuracy of the risk estimate may be influenced by the quality of included studies, or the quality of the data in meta-analysis, although there is no significant difference among the three estimates (on the basis of articles with low or high quality, and the overall adjusted estimate, compared with the estimated risk on the basis of prevalence data only) in this study. The risk estimate from articles of high quality is attenuated with lower variation. The estimate from adjusted estimates by sex and age is the lowest estimate with lowest variation. To confirm this trend, more observational studies are required. Third, because the present meta-analyses included just two cohort studies, the recall bias in each of the included case-control studies in meta-analysis may influence the risk estimate. Fourth, there remains considerable uncertainty about the fraction of the ALS burden that may be attributed to lead in the general population. The AR estimate in this study represents the first AR estimate for previous occupational lead exposure to ALS; the extent to which this estimate is generalizable to encompass (generally lower) environmental lead exposures as well is unclear. Finally, although it is well known that ALS affects men more frequently than women, sex-specific estimates of the risk for ALS in individuals with previous lead exposure were not derived. Just two studies provided risk information for males and females separately,[35,40] and neither study reached a clear conclusion.

CONCLUSIONS

The results of the present meta-analysis of nine case-control studies suggest that previous exposure to lead in the occupational environment is a risk factor for ALS. Lead might not account for a large number of ALS cases at the present time, because lead pollution has been significantly reduced over the last three decades, and lead-containing products have been more stringently regulated. Confirmation of the present findings in future studies would serve both to elucidate the causes of ALS, and to support risk mitigation actions to further reduce the risk of ALS because of exposure to lead from occupational and other sources.

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