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# Metals Dyshomeostasis in Alzheimer's Disease: A Systematic Review

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## Introduction

Dementia represents a major healthcare problem, involving 46.8 million people worldwide, with over 9.9 million new cases every year, one every 3.2 seconds.[1] Dementia is a leading cause of disability and dependency in the elderly, leading to high physical, psychological, social and economic impact on caregivers and families. It is also responsible for higher direct and indirect costs to society. The estimated worldwide cost of dementia is US $818 billion and is expected to reach one trillion dollars by 2018.[1]

Alzheimer's disease (AD) is the most frequent cause of dementia, involving 50 to 75 per cent of the global burden of disease,[2] with almost 46 billion people affected worldwide[3] and 1.54 million deaths globally.[4] Indeed, according to WHO,[4] Alzheimer's disease is the seventh cause of death among high-income countries. Therefore, preventing AD is a main issue: unfortunately, the detailed knowledge of its pathogenesis that would be necessary in order to implement rational preventive measures, is not yet available.

AD is a neurodegenerative disorder, characterized by progressive brain deposition of the amyloid β peptide (Aβ), which is generated by proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretases. The abnormal aggregation and accumulation of neurotoxic Aβ has been proposed as the primary driving force for AD in the amyloid hypothesis.[5]

Amyloid-β is also a major constituent of senile plaques. According to recent studies, the cerebral accumulation of metal ions might contribute to Aβ aggregation, through the production of a reactive oxygen species (ROS).[6] Although there is a plethora of studies concerning pathogenesis of AD, it has not been completely clarified yet because of the complex interaction between endogenous (age, family history, genetic factors, gender) and exogenous (environment) factors that could contribute to the development of this illness.[7] Genetic predisposition seems to be the most important risk factor of Alzheimer's disease, contributing 70% of the overall risk of AD, with the remaining 30% likely to be caused by lifestyle and chronic exposure to environmental factors.[8] The "brain trace metal dyshomeostasis" is a fascinating, although controversial, hypothesis to explain the residual part of ethiopathogenesis of Alzheimer's disease. Although the role of metals as hazardous substances is largely accepted, with even the Agency for Toxic Substances and Disease Registry (ATSDR) including arsenic, lead, mercury, and cadmium in the first ten places on the annual list of hazardous substances,[9] much has yet to be clarified as to their impact on neurodegenerative disorders. Some evidence of an association with AD seems to be available only for aluminium. According to a recent metanalysis undertaken on 34 studies involving 1,208 participants, aluminium levels were significantly higher in serum, brain, and the CSF of patients with Alzheimer's disease.[10]

The aim of this systematic review is to evaluate the currently available studies concerning metal concentration, except for aluminium, in biological human matrices (blood, serum, hair, brain tissue, nails, cerebrospinal fluid) of AD and non-AD subjects.

## Methods

This systematic review was performed according to a-priori protocol, designed following the PRISMA guidelines.[11]

Pubmed/Medline, Scopus and Cochrane databases were examined on 21st November 2016, through a combination of keywords and relevant medical subject heading (MeSH) terms, as extensively shown in Table 1. Search inclusion criteria were restricted to studies on humans, published in the last 10 years, written in English and with an abstract available.

The flow diagram of the complete process selection is shown in Figure 1.

Only original studies measuring metal concentrations – except for Aluminium – in biological matrices (blood, urine, hair, nails, cerebrospinal fluid, brain tissue) in AD subjects were considered suitable for inclusion. Cases followed Alzheimer's disease international diagnostic criteria: NINCDS—ADRDA, DSM-IV, or BRAAK criteria for *post-mortem* studies.

Four authors (CA, RdD, MM, FP) independently extracted the articles that met the eligibility criteria. Disagreements on the potential relevance of the selected studies were resolved by consensus or by discussion with a fifth author (FL). Furthermore, overlapping articles were excluded.

Additionally, five further works coming from the examination of references of the previews articles were included.[16,47,48,52,53]

The mean difference in metal concentrations with p value $<0.05$ was considered as statistically significant result.

A quality assessment of the included studies was performed using the NIH specific tool[12] for observational studies evaluation.

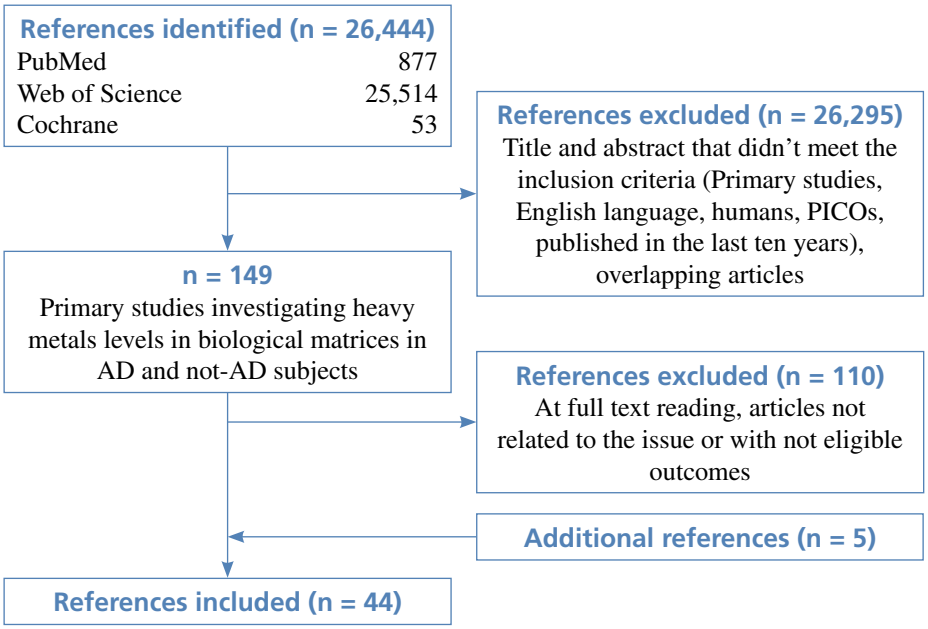


**Figure 1.** Flow diagram of study selection

## Results

Forty-four articles meeting the eligibility criteria were identified: 39 from search engines and 5 additional studies checked from reference lists of the included works. (Table 1; Figure 1).

All the studies were of low to moderate quality, according to the NIH tool score:[12] the median score of the selected studies being 5.5 out of 12.

The main characteristics of the included studies are summarized in Table 2-16.

### Arsenic (As)

As shown in table 2, an overall number of four studies evaluating arsenic concentrations in biological matrices were considered eligible. Three of the included studies[14,15,16] were achieved on serum samples. Park et al. and Paglia et al. found no statistically significant difference (p=0.309 and p=0.312, respectively) in metalloid concentration between AD subjects and healthy controls in two large populations of 207 (89 cases and 118 controls) and 118 subjects (34 AD, 20 MCI, 24 SMC, and 40 non-AD) respectively. A similar result occurred in Baum research: among the 85 enrolled subjects, 44 with a diagnosis of Alzheimer's disease and 41 healthy controls, there were no relevant differences in arsenic blood concentration between the two groups: a mean level of 35.7 nmol/L in AD subjects compared with 38.7 nmol/L in healthy controls. A post mortem case control study[13] carried out by Szabo et al. on 31 brain samples showed significantly lower concentration both in frontal cortex and in ventricular fluid brain regions (p=0.033 and p=0.044, respectively) of AD patients when compared with non-AD subjects (frontal cortex: 145 ng/g vs 159 ng/g; ventricular fluid: 18 ng/ml vs 13 ng/ml, respectively).

**Table 1.** Search strategy.

| Search engine | Keywords | Limits |
|---|---|---|
| Pubmed/Medline | Alzheimer in title/abstract AND copper in title/abstract OR zinc in title/abstract | Published date from January 2007 to November 2016, English language, humans, primary studies (case report, clinical study, clinical trial, comparative study, controlled clinical trial, multicenter study, observational study, randomized controlled trial, twin study), available abstract |
| Pubmed/Medline | Alzheimer disease as MeSH term AND copper as MeSH term OR zinc as MeSH term | Published date from January 2007 to November 2016, English language, humans, primary studies (case report, clinical study, clinical trial, comparative study, controlled clinical trial, multicenter study, observational study, randomized controlled trial, twin study), available abstract |
| Web of science | Alzheimer in title AND Zinc in title OR copper in title | Publishied date from January 2007 to November 2016, available abstract |
| Cochrane | Alzheimer in title/abstract/keywords AND copper in title/abstract/keywords OR zinc in title/abstract/keywords | Publishied date from January 2007 to November 2016, available abstract |
| Cochrane | Alzheimer disease as MeSH term AND copper as MeSH term OR zinc as MeSH term | Publishied date from January 2007 to November 2016, available abstract |

**Table 2.** Summary results on Arsenic (As).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [13] | Case-control post-mortem | N=31 (16 AD, 15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [As] 145 ng/g FC (AD); 159 ng/g FC (non AD) [As] 18 ng/mL VF (AD);13 ng/mL VF (non AD) | Significantly lower [As] in frontal cortex (N.R.; p=0.033) and in ventricular fluid (N.R.; p=0.044) of AD subjects | N.A. | 4 out of 12 |
| [14] | Case-control | N=207 (89 AD, 118 non AD) | NINCDS-ADRDA | Serum | [As] 28.08µg/L (AD); 28.66µg/L (non AD) | No significant difference in [As] among AD and non AD subjects (N.R.; p=0.309) | Age | 6 out of 12 |
| [15] | Case-control | N=85 (44 AD,41 non AD) | NINCDS-ADRDA | Serum | [As]35.7 nmol/L(AD); 38.7 nmol/L(non AD) | No statistically significant variation. | Age Gender | 5 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [As] 3.55 µg/L (AD); 3.13 µg/L (non AD) | No statistically significant variation (F=1.204; p=0.312) | Age Sex Education | 6 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease;NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; N.R.= Not Reported

**Cadmium (Cd)**

Among the five studies assessing cadmium concentration in blood samples of patients with Alzheimer's disease compared with healthy controls, no significant differences emerged between the two groups ($p>0.05$). Only one paper[21] reported different results, showing a statistically significant ($p=0.001$) increase in cadmium levels among AD patients. Of note, this study included only 39 subjects, having the smallest size among the considered studies. Only one study[19] investigated variations in cadmium cerebrospinal fluid level, showing no statistically significant difference between AD and non AD subjects.

Concerning nails and hair, Koseoglu et al. carried out a case control study on 122 subjects with and without dementia, showing a statistically significant decrease in metal concentration in patients with Alzheimer's disease compared with healthy controls. Two post mortem studies analysing cadmium levels in brain tissue gave no univocal results. Szabo et al. found a statistically significant ($p=0.031$) decrease in Cd frontal cortex levels in AD patients, whereas. Akatsu et al. reported a significant ($p<0.05$) increase in amygdala region of AD compared with non-AD subjects.

**Chrome (Cr)**

As shown in Table 4, five articles evaluating a difference in chrome concentration between cases and controls met the eligibility criteria. In 2014 Gonzalez-Dominguez et al. carried out a case-control study to assess serum levels of the metal in 65 subjects: 25 with diagnosis of Alzheimer's disease, 15 with mild cognitive impairment and 25 healthy patients without dementia. No statistically significant difference in chrome blood concentration was found among the three groups. Similar results were reported by Baum et al., 2010 and by Paglia et al., 2016. Finally, post mortem case control studies by Szabo et al. and Akatsu et al. on brain tissue, found no substantial difference in chrome content, in particular in frontal cortex, ventricular fluid, hippocampus and amygdala.

**Cobalt (Co)**

As shown in Table 5, three studies[16,19,22] focused on blood samples (plasma and serum). Alimonti et al. found a significant decrease in serum cobalt among AD subjects in a population of 177 (53 AD and 124 controls), while the studies carried out by Gerhardsson et al. in 2008 and by Paglia et al. in 2016 showed no substantial changes in metal concentration between the two groups. A recent case control study (2017) by Koseoglu et al. focused on Co levels in other biological matrices, hair and nails. The research was undertaken on 122 subjects (62 AD and 60 non-AD) and showed a statistically significant decrease in cobalt concentration ($p < 0.001$) among AD patients in hair. On the contrary, no real variations emerged by comparison of nail samples between the two investigated groups.

Finally, two studies[19,23] assessing metal levels in cerebrospinal fluid on large population samples [N=314 (cases+controls) and N=318 (cases+controls), respectively] did not detect statistically significant differences between cases and controls.

**Copper (Cu)**

Copper is the most widely investigated metal, with 36 studies assessing its concentrations in different biological matrices (blood, cerebrospinal fluid, hair, nails and brain tissue). As shown in Table 6, most studies assessed copper levels in blood, serum or plasma. The results were extremely variable with 42% of the studies (N=11) showing a significant increase in total and free copper in AD subjects compared with non-AD, 42% (N=11) showing no statistically significant difference between the two groups, and 16% (N=6) showing a significant decrease in AD patients. Also the most robust study in terms of sample population[19,33,40,43] gave no univocal results. It must be pointed out that the number of subjects included in studies showing decreased concentrations was generally much lower than in studies showing an increase or no difference. Concerning cerebrospinal fluid, three case control studies[19,23,35] found no substantial difference in CSF copper concentration between AD subjects and healthy controls whereas only one study[31] showed significantly higher copper levels amongst cases compared with the control group.

Few studies have been made on hair or nail matrices. Koseoglu et al.[20] evaluated copper levels both in hair and nails on a population of 122 people, reporting a significant decrease in patients with Alzheimer's disease. On the contrary, Koc et

**Table 3.** Summary results on Cadmium (Cd).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [13] | Case-control post-mortem | N=31 (16 AD, 15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [Cd] 20 ng/g in FC (AD); 30 ng/g in FC (non AD); [Cd] <LOQ in VF (AD); <LOQ in VF (non AD) | Significantly lower [Cd] is in AD than in non ADsubjects, in frontal cortex (N.R.; p=0.031) | N.A. | 4 out of 12 |
| [14] | Case-control | N=207 (89AD, 118 non AD) | NINCDS-ADRDA | Serum | [Cd] 0.048µg/L (AD); 0.040µg/L (non AD) | No significant difference in [Cd] among AD and non AD subjects (N.R.; p=0.084) | Age | 6 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Cd] 0.03 µg/L (AD); 0.02 (non AD) | No statistically significant difference (N.R.; p=0.069) | Age Sex Education | 6 out of 12 |
| [17] | Case-Control | N=65 (25 AD, 15 MCI, 25 non AD) | NINCDS-ADRDA | Serum | [Cd]= 0.0799µg/L (AD); 0.0640µg/L (non AD) | No statistically significant difference in [Cd] among AD and non ADsubjects. | N.A. | 5 out of 12 |
| [18] | Case-Control post-mortem | N= 45 (18 AD, 11 DLB, 16 non AD) | BRAAK | Brain tissue (hippocampus, amygdala) | Hippocampus [Cd] 1.0 µM of wet tissue (AD); 0.7 µM of wet tissue (non AD) Amygdala [Cd] 1.0 µM of wet tissue (AD); 0.7 µM of wet tissue (non AD) | Statistically significant increase in amygdala [Cd] among AD and non-AD subjects (N.R.; p<0.05) | N.A. | 5 out of 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc.,) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Cd] 0,05 µg/L (AD);0.5 µg/L (AD+vasc); 0.05 µg/L (non AD) CSF [Cd] <0.04 µg/L (AD subjects);<0.4 µg/L (AD+vasc) <0.04 µg/L (non AD) | No significant difference in [Cd] among AD and non ADsubjects | Age | 5 out of 12 |
| [20] | Case-control | N= 122 (62 AD, 60 non AD) | NINCDS-ADRDA | Nail Hair | Nail [Cd] 0.39 µg/g (AD); 0.73 µg/g (non AD) Hair [Cd] 0.21 µg/g (AD); 0.31 µg/g (non AD) | Statistically significant difference in [Cd] (N.R.; p < 0.001) in AD subjects nails Statistically significant lower [Cd]( N.R.; p < 0.01) in AD subjects hair | N.A. | 7 out of 12 |
| [21] | Case-control | N= 39 (24 AD, 15 non AD) | NINCDS-ADRDA | Serum | [Cd] 0.0032 µg/dL(AD); 0.0011 µg/dL (non AD) | [Cd] in AD subjects were significantly higher (N.R.; p=0.001) | Age | 5 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available;LOQ = Limit of Quantification; CSF = Cerebrospinal Fluid; N.R.= Not Reported

al.,[38] assessing metal concentration in hair on a smaller sample (N=78) found no significant difference between AD and non-AD subjects. Finally, a number of post mortem case control studies (N=7), were carried out on brain tissue. More than 70% of eligible articles (5 out of 7)[6,18,36,39,41] found a statistically significant decrease in copper brain concentration among AD subjects when compared with healthy controls. Only two studies[13,29] showed no relevant variations in metal levels between cases and controls.

**Iron (Fe)**

As reported in Table 7, an overall number of 25 articles on iron were considered eligible. Most of the included studies (18 out of 25) were carried out on blood as a biological matrix. One study,[50] published in 2014 by Faux et al., was carried out on a large population (N=1,112: 211 with diagnosis of Alzheimer's disease, 133 with mild cognitive impairment, and 768 healthy controls). In this study there was no evidence of any significant difference in blood iron concentration between AD and non-AD subjects. Non univocal results were reported in the remaining studies. Indeed, ten studies[17,19,24,27,37,43,45,50,52,53] didn't show substantial differences between cases and controls. Six studies[15,16,22,25,32,51] showed a significant decrease in blood iron levels of AD patients when compared with healthy controls. Conversely, only one study[21] showed a significant increase of iron in AD subjects compared with control group (p=0.001).

Concerning hair and nails, a recent study (2017) by Koseoglu et al. gave evidence of a significant decrease of iron levels (p <

**Table 4.** Summary results on Chrome (Cr).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [13] | Case-control post-mortem | N=31 (16 AD, 15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | FC[Cr] 154 ng/g [AD]; 91 ng/g (non AD) VF[Cr] 17.5 ng/ml [AD];14.4 ng/ml (non AD) | No statistically significant difference in among AD and non ADsubjects. | N.A. | 4 out of 12 |
| [15] | Case-control | N=85 (44 AD,41 non AD) | NINCDS-ADRDA | Serum | [Cr] 22.7 nmol/L (AD); 17.2 nmol/L(non AD) | No statistically significant difference in among AD and non AD subjects. | Age Gender | 5 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC,40 non AD) | NINCDS-ADRDA | Blood | [Cr]= 0.10 µg/L (AD); 0.21(non AD) | No statistically significant difference. | Age Sex Education | 6 out 12 |
| [17] | Case-Control | N=65 (25 AD, 15 MCI, 25 non AD) | NINCDS-ADRDA | Serum | [Cr] 0.207µg/L (AD); 0.212µg/L (non AD) | No statistically significant difference in among AD and non ADsubjects. | N.A. | 5 out of 12 |
| [18] | Case-Control post-mortem | N= 45 (18 AD, 11 DLB, 16 non AD) | BRAAK | Brain tissue (hippocampus, amygdala) | Hippocampus [Cr] 0.2 µM of wet tissue (AD); 0.3 µM of wet tissue (non AD) Amygdala [Cr] 0.7µM of wet tissue (AD); 0.4 µM of wet tissue (non AD) | No statistically significant difference in among AD and non AD subjects. | N.A. | 5 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DLB= Dementia with Lewy bodies.; N.R.= Not Reported

**Table 5.** Summary results on Cobalt (Co).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Co]= 0.39 µg/L (AD); 0.39 (non AD) | No statistically significant variation (F=0.614; p=0.607) | Age Sex Education | 6 out of 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Co] 0.87 µg/L(AD); 0.80 µg/L(AD+vasc); 1.1 µg/L(non AD) CSF [Co] 0.08 µg/L(AD); 0.07 µg/L(AD+vasc); 0.08 µg/L(non AD) | No statistically significant variation | N.A. | 5 out of 12 |
| [20] | Case-control | N= 122 (62 AD, 60 non AD) | NINCDS-ADRDA | Nail Hair | Nail [Co] 0.50 µg/g (AD); 0.62 µg/g (non AD) Hair [Co] 0.28 µg/g (AD); 0.41 µg/g (non AD) | AD subjects showed statistically significant lower [Co] (N.R.; $p < 0.001$) in hair | N.A. | 7 out of 12 |
| [22] | Case-control | N=177 (53 AD, 124 non AD) | NINCDS-ADRDA | Serum | [Co] 0.11 µg/L (AD); 0.16 µg/L (non AD) | Statistically significant decrease in [Co] in AD subjects | Age Gender Drug intake | 5 out of 12 |
| [23] | Case-control | N=318 (264 AD, 54 non AD) | NINCDS-ADRDA | CSF | [Co] 0.08 µg/L (AD); 0.07 µg/L (ADMV); [Co] 0.08 µg/L (non AD) | No statistically significant variation. | N.A. | 5 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision); N.R.= Not Reported

**Table 6.** Summary results on Copper (Cu).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [6] | Case-control post mortem | N=68 (42 AD: 14 Moderate and 28 Severe; 26 non AD) | BRAAK | Brain tissue (neocortex, Brodmann area 7) | [Cu] 20.7 mg/Kg (Moderate AD); 17.9 mg/Kg (Severe AD); 22.5 mg/Kg (non AD) | [Cu] is significantly decreased in severe AD subjects (N.R.; p<0.001). | N.A. | 5 out of 12 |
| [13] | Case-control post-mortem | N=31 (16AD, 15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [Cu]2112 ng/g FC (AD);2398 ng/g FC (Non AD) [Cu]117.5 ng/mL VF (AD);104.2 ng/mL VF (Non AD) | No statistically significant variation. | N.A. | 4 out of 12 |
| [15] | Case-control | N=85 (44 AD,41 non AD) | NINCDS-ADRDA | Serum | [Cu] 16.2 nmol/L (AD); 15.3 nmol/L (non AD) | No statisticall significant variations. | Age Sex | 5 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Cu] 815.75 µg/L (AD); 703.88 (non AD) | [Cu] is increased in AD subjects (F=3.013; p=0.033) | Age Sex Education | 6 out 12 |
| [17] | Case-Control | N=65 (25 AD, 15 MCI, 25 non AD) | NINCDS-ADRDA | Serum | [Cu] 1.126.8µg/L (AD); 1.058.5µg/L (non AD) | No statistically significant variation in [Cu] between AD and non AD subjects. | N.A. | 5 out of 12 |
| [18] | Case-Control post-mortem | N= 45 (18 AD, 11 DLB, 16 non AD) | BRAAK | Brain tissue (hippocampus, amygdala) | AD [Cu] Hippocampus= 29.5 µM of wet tissue; [Cu] Amygdala= 27.8 µM of wet tissue Non AD [Cu] Hippocampus=57.7 µM of wet tissue; [Cu] Amygdala= 53.2 µM of wet tissue | Statistically significant [Cu] reduction in AD subjects (N.R.; p<0.01 both for Hippocampus and Amygdala). | N.A. | 5 out of 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Cu] 1.2 mg/L (AD), 1.2 mg/L (AD + vasc) 1.2 mg/L (non AD) CSF [Cu] 18 mg/L (AD), 18 mg/L (AD + vasc) 18 mg/L (non AD) | No statistically significant variation. | Age | 5 out of 12 |
| [20] | Case-control | N= 122 (62 AD, 60 non AD) | NINCDS-ADRDA | Nail Hair | Nail [Cu]14.63 µg/g (AD) 40.04 µg/g (non AD) Hair [Cu]17.62 µg/g (AD), 22.83 µg/g (non AD) | Statistically significant difference in [Cu] (N.R.; p < 0.001) in nail. in AD subjects. Statistically significant lower hair [Cu] (N.R.; p < 0.01) in AD subjects | N.A. | 7 out of 12 |
| [21] | Case-control | N= 39 (24 AD, 15 non AD) | NINCDS-ADRDA | Serum | [Cu] 1.097 µg/dL (AD), 0.901 µg/dL (non AD) | No significant difference was found in [Cu] among AD and non AD subjects | N.A. | 5 out of 12 |
| [22] | Case-control | N=177 (53 AD, 124 non AD) | NINCDS-ADRDA | Serum | [Cu]= 945 µg/L (AD); 951 µg/L (non AD) | No significant differences among AD and non AD subjects | Age Gender, Drugintake | 5 out of 12 |
| [23] | Case-control | N=318 (264 AD, 54 non AD) | NINCDS-ADRDA | CSF | [Cu] 18 µg/L (AD); [Cu]=18 µg/L (ADMV); [Cu]=18 µg/L (non AD) | No significant differences among AD and non AD subjects | N.A. | 5 out of 12 |
| [24] | Case-control | N=142 (84 AD, 58 non AD) | NINCDS–ADRDA | Serum | [Cu] 15.46µmol/L (AD); 14.0µmol/L (non AD) [Non-Cp Cu] 2.1µmol/L (AD); 1.5µmol/L (non AD) | Significantly higher [Cu] (d=0.51;p=0.003) in AD subjects compared with healthy controls. | Age | 5 out of 12 |

(continue)

**Table 6.** Summary results on Copper (Cu). *(continued)*

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [25] | Case-control | N= 166 (83 AD, 83 non AD) | NINCDS–ADRDA DSM-IV-TR | Serum | [Cu] 121ug/dL (AD); 102 ug/dL (non AD) | [Cu] (t=-5.832; p < 0.001) is significantly increased copper concentration in AD subjects compared with non AD | N.A. | 4 out of 12 |
| [26] | Case-control | N= 89 (15 AD, 41 MS, 23 healthy controls and 10 healthy elderly controls) | NINCDS-ADRDA | Blood | [Cu] 73.27µg/L (AD); 609.7µg/L (healthy control); 631.6µg/L (healthy elderly control) | [Cu] is significantly decreased in AD subjects (F=1.212; p<0.001) | N.A. | 3 out of 12 |
| [27] | Case-control | N= 186 (93 AD, 45 VaD, 48 non AD) | NINCDS-ADRDA | Serum | [Cu]15.82µmol/L (AD); 12.75µmol/L (non AD) [Free Cu]= 2.72µmol/L (AD); 0.24µmol/L (non AD) | Statistically significant decrease in [Cu] and [Free Cu] in AD subjects (F=6.708; p=0.002 and p<0.01 respectively) | N.A. | 6 out of 12 |
| [28] | Case-Control | N=50 (25 AD, 25 non AD) | DSM IV-TR NINCDS-ADRDA | Serum | [Cu]1.1 mg/L (AD); 0.78 mg/L (non AD) | Statistically significant [Cu] higher in AD subjects (N.R.; p<0.001) | N.A. | 5 out of 12 |
| [29] | Case-Control post-mortem | N= 30 (10 PD, 10 AD, 10 non AD) | NINCDS-ADRDA | Brain (temporal cortex) | [Cu]=4.86 ng/mg (AD); 4.05 ng/mg (non AD) | No statistically significant difference. | N.A. | 6 out of 12 |
| [30] | Case-control | N=87 (36 AD, 18 MCI, 33 non AD) | NINCDS-ADRDA | Serum | [Cu] 1006.6 µg/L (AD); 878.8 µg/L (non AD) | [Cu] is significantly higher in AD subjects (p=0.038) | N.A. | 5 out of 12 |
| [31] | Case-Control | N= 108 (52 ALS, 21 AD, 20 PD, 15 non AD) | DSM IV-TR | CSF | [Cu]= 17.4 ng/mL (Early-onset AD 10.3 ng/mL; Late-onset AD 20.9 ng/mL); [Cu]= 10.2 ng/mL (non AD) | CSF [Cu] is significantly higher (N.R.; p<0.05) in AD subjects. | N.A. | 4 out of 12 |
| [32] | Case-control | N=100 (50 AD, 50 non AD) | NINCDS-ADRDA | Plasma | [Cu] M= 129.75 µg/dL[Cu]F= 132.78 µg/dL (AD) [Cu] M= 140.63 µg/dL [Cu]F= 145.29 µg/dL (non AD) | Statistically significant [Cu] decrease in plasma (F=3.97; p<0.01) in AD subjects | N.A. | 6 out of 12 |
| [33] | Case-control | N= 619 (110 AD MAD:39; IAD36; SAD35. Fist-degree relatives AD (RAD)76; 87 PD. Fist-degree relatives PD (RPD) 46;87 VD First-degree relatives VD (RVD) 56; 134) Non AD 55 Younger control (YCG) and 79 Elderly control (ECG) | NINCDS-ADRDA | Plasma | [Cu] 0.84 mg/L (AD) 0.79 mg/L (non AD) | [Cu] is significantly higher in patients with severe AD (N.R.; p<0.01) | N.A. | 6 out of 12 |
| [34] | Case-control | N=57 (28 AD, 29 non AD) | NINCDS-ADRDA | Serum | [Cu]=108 µg/dL (AD); 117 µg/dL (non AD) Free[Cu] 43.1% (AD); 38.5% (non AD) | [Cu] is significantly lower in AD patients (t=1.98; p=0.05) | N.A. | 6 out of 12 |

*(continue)*

**Table 6.** Summary results on Copper (Cu). *(continued)*

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [35] | Case-Control | N= 91 (8 AD, 23 HD, 22 PD, 27 ALS 11 non AD) | NINCDS-ADRDA | CSF | Free[Cu]=4 µg/L (AD) 2.5 µg/L (non AD) | No significant difference in free Cu concentration between AD and non AD subjects. | N.A. | 6 out of 12 |
| [36] | Case-control Post mortem | N= 23 (3 Mild AD, 8 Severe AD, 6 DLB, 6 non AD) | BRAAK | Brain tissue (Frontal cortex and hippocampus) | [Cu] 3.9 µg Cu/g (AD); 6.9 µg Cu/g (non AD) | [Cu] is decreased in severe AD close to the statistically significant threshold (N.R.; p=0.057) | Age | 5 out of 12 |
| [37] | Case-control | N= 104 (51 AD, 53 non AD) | NINCDS-ADRDA | Serum | [Cu] 16.1 µmol/L (AD), 13 µmol/L (non AD) | [Cu] is significantly higher (F=17.6; p<0.001) in AD subjects serum | Age Sex | 5 out of 12 |
| [38] | Case- Control | N=78 (45 AD, 33 non AD) | NINCDS-ADRDA | Hair Serum | Hair [Cu] 12.8µg/g (AD); 10.3 µg/g (non AD) Serum [Cu] 0.9µg/mL (AD); 1.01 µg/mL (non AD) | Hair [Cu] is increased (N.R.; p= 0.013 ) in AD subjects No significant difference was found in Serum [Cu] between AD and non AD subjects. | N.A. | 6 out of 12 |
| [39] | Case-control post mortem | N=18 (9 AD, 9 non AD) | BRAAK | Brain tissue (cerebellum, motor cortex, sensory cortex, cingulate gyrus, temporal gyrus, entorhinal cortex, and hippocampus) | Brain-copper levels summed across all seven regions: [Cu]256 µM/dry Kg (AD), 406 µM/dry Kg (non AD) | [Cu] in AD subjects is significantly lower in every brain region: cerebellum (N.R.; p <0.0001); motor cortex (N.R.; p = 0.021); sensory cortex (N.R.; p= 0.027); cingulate gyrus (N.R.; p=0.0079); middle temporal gyrus (N.R.; p = 0.013); entorhinal cortex (N.R.; p= 0.0046); and hippocampus (N.R.; p= 0.070) | N.A. | 5 out of 12 |
| [40] | Case-control | N=702 (399 AD and 303 non AD) | NINCDS–ADRDA | Serum | [Cu]14.98 µmol/L (AD); 13.05 µmol/L (Non-AD) [free Cu] 2.24 µmol/L (AD), 0.28µmol/L (non AD) | [Cu] and Free [Cu] is higher in AD subjects (N.R.; p<0.001) | Age | 6 out of 12 |
| [41] | CohortStudy | N= 957 (186 AD, 76 MCI, 695 non AD) | NINCDS–ADRDA | Serum | [Cu] 13.52 µM (AD); 13.45 µM (non AD) | No significant differences among AD and non-AD subjects | N.A. | 9 out of 14 |
| [42] | Case-Control | N= 207 (89 AD, 118 non AD) | NINCDS-ADRDA | Serum | [Cu] 1146.26 µg/L (AD); 1078.14µg/L (non AD) [Free Cu] 447.57 µg/L (AD); 428.19µg/L (non AD) | [Cu] is significantly higher in the AD subjects (N.R.; p = 0.026). Free [Cu] is not significantly different | Age Education | 7 out of 12 |
| [43] | Case-Control | N= 47 (28 AD, 13 MCI, 6 non AD) | DSM-IV | Serum | [Cu] 1.01 mg/L (AD); 1.04 mg/L (non AD) | No significant differences among AD and non-AD subjects (t=0.587; p=0.560) | N.A. | 7 out of 12 |
| [44] | Case-control | N=104 (50 AD, 24 VaD, 50 non AD) | NINCDS-ADRDA | Serum | [Cu] 156.2 µg/dL (AD); 134.46 µg/dL (non AD) | Statistically significant higher levels in [Cu] in AD subjects (F=6.52;p=0.002) | N.A. | 7 out of 12 |
| [45] | Case-control | N=152 (72 AD, 80 non AD) | NINCDS-ADRDA | Serum | [Cu]=31.49 µmol/L (AD); 23.41 µmol/L (non AD) | No significant differences among AD and non AD subjects (t=1.494; p=0.138) | N.A. | 7 out of 12 |

*(continue)*

**Table 6.** Summary results on Copper (Cu). *(continued)*

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [46] | Case-control | N=57 (30 AD, 27 non AD) | NINCDS-ADRDA | Blood Brain tissue (frontal cortex) | Blood [Cu] 270 µg/L (AD); 260 µg/L (non AD) Brain tissue [Cu] 1.45 µg/g (AD); 1.9 µg/g (non AD) | Brain [Cu] is significantly reduced in AD subjects (N.R.; p<0.0001) No significant difference was found in erythrocytes [Cu] between AD and non AD subjects. | N.A. | 3 out of 12 |
| [47] | Case-control | N=74 (54 AD, 20 non AD) | NINCDS-ADRDA | Serum | [Cu] 15.1µmol/L (AD); 12.9µmol/L(non AD) Free [Cu] 2.1µmol/L (AD); 0.2 µmol/L (non AD) | Free [Cu] is significantly higher in AD subjects (N.R.; p=0.013) [Cu] is increased in AD subjects (N.R.; p=0.021) | N.A. | 5 out of 12 |
| [48] | Case-control | N=100 (50 AD, 50 non AD) | NINCDS-ADRDA | Serum | [Cu] 137.8 mg/dl (AD); 132.5 mg/dl (non AD) | Copper levels were not significantly different between the study and the control populations. | Age | 4 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision);nonCP= Ceruloplasmin; PD= Parkinson's disease; VaD = Vascular Dementia; d=Cohen's d, standardized effect size for mean difference in the biochemical variables (d=0.2 small effect size; d=0.5 medium size; d=0.8 a large effect size; N.R.= Not Reported

**Table 7.** Summary results on Iron (Fe).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [6] | Case-control post mortem | N=68 (42 AD: 14 Moderate and 28 Severe; 26 non AD) | BRAAK | Brain tissue (neocortex, Brodmann area 7) | [Fe]= 360 mg/Kg (Moderate AD); 417mg/Kg (Severe AD); 374.8 mg/Kg (non AD) | [Fe] is significantly increased in severe AD subjects (N.R.; <0.001) | N.A. | 5 out of 12 |
| [13] | Case-control post-mortem | N=31 (16AD, 15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [Fe] 42571 ng/g FC (AD); 34140 ng/g FC (non AD) [Fe] 6994.9 ng/ml VF (AD); 1035.6 ng/ml VF (non AD) | [Fe] is significantly higher in AD subjects, but only in frontal cortex ( N.R.; p=0.018) | N.A. | 4 out of 12 |
| [15] | Case-control | N=85 (44 AD, 41 non AD) | NINCDS-ADRDA | Serum | [Fe] 17,7 nmol/L (AD); 23,8 nmol/L (non AD) | Statistically significant decrease in [Fe] in AD subjects ( N.R.; p<0.05) | Age and sex. | 5 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Fe]= 938.54 µg/L (AD); 1045.07 (non AD) | [Fe] in AD subjects were significantly lower (F=2.891; p=0.039) | Age Sex Education | 6 out 12 |
| [17] | Case-Control | N=65 (25 AD, 15 MCI, 25 non AD) | NINCDS-ADRDA | Serum | [Fe] 879.7µg/L (AD); 988.3µg/L (non AD) | No statistically significant difference | N.A. | 5 out of 12 |
| [18] | Case-Control post-mortem | N= 45 (18 AD, 11 DLB, 16 non AD) | BRAAK | Brain tissue (hippocampus, amygdala) | [Fe] Hippocampus 697 µM of wet tissue (AD); Amygdala 955 µM of wet tissue (AD); [Fe] Hippocampus 669 µM of wet tissue (non AD); Amygdala694 µM of wet tissue (non AD) | Statistically significant increase in amygdala [Fe] in AD subjects (N.R.; p<0.05) | N.A. | 5 out of 12 |

*(continue)*

**Table 7.** Summary results on Iron (Fe). *(continued)*

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Fe]1,6 mg/L (AD); 1.5 mg/L (AD+vasc); 1,8 mg/L(non AD) CSF [Fe]0.19 mg/L (AD); 0.17 mg/L (AD+vasc); 0.23 mg/L(non AD) | Non statistically significant variation | Age | 5 out of 12 |
| [20] | Case-control | N= 122 (62 AD, 60 non AD) | NINCDS-ADRDA | Nail Hair | Nail [Fe]163.97 µg/g (AD), 384.62 µg/g (non AD) Hair [Fe] 229.31 (AD), 332.98 µg/g (non AD) | AD subject showed statistically significant difference in [Fe] (N.R.; $p < 0.001$) nail concentrations. AD subjects showed statistically significant lower concentrations of [Fe] ( N.R.; $p = 0.001$) in hair | N.A | 7 out of 12 |
| [21] | Case-control | N= 39 (24 AD, 15 non AD) | NINCDS-ADRDA | Serum | [Fe] 1.717 (AD), 0.651 µg/dL (non AD) | [Fe] in AD subjects were significantly higher ( N.R.; p=0.001) | N.A | 5 out of 12 |
| [22] | Case-control | N=177 (53 AD, 124 non AD) | NINCDS-ADRDA | Serum | [Fe]= 858 µg/L (AD); 1.610 µg/L (non AD) | Statistically significant decrease in [Fe] in AD subjects ( N.R.; p<0.05) | Age Gender Drugintake | 5 out of 12 |
| [23] | Case-control | N=318 (264 AD, 54 non AD) | NINCDS-ADRDA | CSF | [Fe]=0.19 mg/L (AD); 0.17 mg/L (ADMV); 0.23 mg/L (non AD) | No statistically significant variation | N.A. | 5 out of 12 |
| [24] | Case-control | N=142 (84 AD, 58 non AD) | NINCDS–ADRDA | Serum | [Fe] 82.4 µg/dL (AD);80 µg/dL (non AD) [Transferrin] 2.5g/L (AD); 2.7g/L (non AD) | No statistically significant difference | Age | 5 out of 12 |
| [25] | Case-control (Replication study) | N= 166 (83 AD, 83 non AD) | NINCDS–ADRDA | Serum | [Fe] 119ug/dL (AD); 137ug/dL (non AD) | [Fe] (t=3.277; $p < 0.05$) concentration is significantly decreased in AD subjects | N.A. | 4 out of 12 |
| [27] | Case-control | N= 186 (93 AD, 45 VaD, 48 non AD) | NINCDS-ADRDA | Serum | [Fe] 72.77ng/dL (AD); 83.89 ng/dL (non AD) [Transferrin] 2.50g/L (AD); 2.71g/L (non AD) | No statistically significant difference | N.A. | 6 out of 12 |
| [29] | Case-Control post-mortem | N= 30 (10 PD, 10 AD, 10 non AD) | NINCDS-ADRDA | Brain (temporalcortex) | [Fe] 123.07 ng/mg (AD); 131.25 ng/mg (non AD) | No statistically significant difference | N.A. | 6 out of 12 |
| [31] | Case-Control | N= 108 (52 ALS, 21 AD, 20 PD,15 non AD) | DSM IV-TR | CSF | [Fe] 238.6 ng/mL (Early-onset AD 221.6 ng/mL; Late-onset AD 247.2 ng/mL) [Fe] 238.0 ng/mL (non AD) | No statistically significant variation | N.A. | 4 out of 12 |
| [32] | Case-control | N=100 (50 AD, 50 non AD) | NINCDS-ADRDA | Plasma | [Fe] M=70.33 µg/dL [Fe]F= 64.52µg/dL (AD) [Fe] M=83.83µg/dL [Fe]F=79.15 µg/dL (Non-AD) | Statistically significant decrease in [Fe] (F=2.6; p<0.01) in AD subjects | N.A. | 6 out of 12 |
| [37] | Case-control | N= 104 (51 AD, 53 non AD) | NINCDS-ADRDA | Serum | [Fe] 73 µg/dL (AD), 85 µg/dL (non AD) | No statistically significant variation | Age Sex | 5 out of 12 |
| [43] | Case-Control | N= 47 (28 AD, 19 non AD) | DSM-IV-TR | Serum | [Fe]= 1.06 mg/L (AD); 1.14 mg/L (non AD) | No statistically significant variation | N.A. | 5 out of 12 |
| [45] | Case-control | N=152 (72 AD, 80 non AD) | NINCDS-ADRDA | Serum | [Fe]=558 µg/dL (AD); [Fe]=610 µg/dL (non AD) | No statistically significant difference in [Fe] in AD subjects | N.A. | 7 out of 12 |

*(continue)*

**Table 7.** Summary results on Iron (Fe). *(continued)*

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [49] | Case-control | N=70 (34 AD, 36 non AD) | NINCDS-ADRDA | Plasma | [Ferritin]=187.4 g/L (AD); 153.9 g/L (non AD) | A statistically significant increase in [Fe] ( N.R.; p<0.001) in AD subjects | N.A. | 4 out of 12 |
| [50] | Community-based, cross-sectional cohort | N=1112 (211 AD, 133 MCI, 768 non AD) | NINCDS-ADRDA | Blood | [Fe] 20.76 µmol/L (AD); 21.93 µmol/L (non AD) [Transferrin] 33.16 µmol/L (AD); 32.96 µmol/L (non AD) [Ferritin]114.40 µg/L (AD); 120.25 µg/L (non AD) TransferrinSaturation 25.66% (AD); 27.02% (non AD) | No statistically significant difference | Age Gender APOE-ε4 | 5 out of 12 |
| [51] | Case-Control | N= 159 (107 AD, 52 non AD) | NINCDS-ADRDA | Serum | [Fe]83.2 µg/dL (AD); 87.1 µg/dL (non AD) | [Fe] in AD subjects is statistically significant lower (N.R.; p=0.001) | N.A. | 4 out of 12 |
| [52] | Case-control | N= 82 (41 AD, 41 non AD) | NINCS-ADRDA | Serum | [Fe] 15 µmol/L (AD);18 µmol/L ( non AD) | No statistically significant variation. | Age Gender | 4 out of 12 |
| [53] | Case-control | N=95 (49 AD, 46 non AD) | NINCDS-ADRDA | Serum | [Fe] 69.3 ng/dL (AD); 76.7 ng/dL (non AD) | No statistically significant difference in [Fe] between AD and non-AD subjects. | Age Sex | 6 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.= Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision); PD= Parkinson's disease; VaD = Vascular Dementia; M=Male; F=Female; ALS= Amyotrophic Lateral Sclerosis; d=Cohen's d, standardized effect size for mean difference in the biochemical variables (d=0.2 small effect size; d=0.5 medium size; d=0.8 a large effect size; N.R.= Not Reported

0.001 for nails and p=0.001 for hair) among cases when compared to non-AD subjects.

Three further articles investigated cerebrospinal fluid metal concentrations, but with no statistically significant variation between the two groups.

Finally, four post mortem case control studies were performed on different regions of brain tissue and reported discordant results. Szabo et al., Graham et al. and Akatsu et al. found significantly higher iron levels in patients with a diagnosis of Alzheimer's disease when compared with subjects without dementia. Graham et al. found higher iron levels only in severe AD patients. In Szabo et al.'s work there was an increase in iron concentration in the frontal cortex (p=0.018), but not in the ventricular fluid. The study of Akatsu et al. showed a similar increase in metal but only in the amygdala. The article by Yu et al. showed no statistically significant difference in the temporal cortex region.

**Lead (Pb)**

Eight articles concerning lead concentrations in AD and non-AD subjects met the eligibility criteria, as shown in Table 8. Six case control studies, carried out on serum or plasma samples, showed discordant results. Giacoppo et al., in their research on 89 AD and non-AD subjects, found a statistically significant decrease in blood lead levels (p<0.001) in patients with Alzheimer's disease. The study by Lee et al., carried out in 210 subjects: 80 with a diagnosis of Alzheimer's disease and 130 without, identified a significant reduction in blood lead concentrations between AD and non-AD subjects (p=0.035). Conversely, the same study also reported an increase in lead levels of patients with Alzheimer's disease (0.20µg/dL compared with 0.17 µg/dL in the control group) when the evaluation of metal concentration was determined on serum samples.

Furthermore, five articles[13,14,16,22,23] didn't report any statistically significant variations in Pb levels between the two groups.

Concerning studies evaluating cerebrospinal fluid samples, similar discordant results were obtained, although on a smaller number of studies.[19,23] In their article published in 2009, Gerhardsson et al, evaluating CSF samples in a population of 318 subjects (264 with AD and 54 controls) found no statistically significant difference in metal concentration between AD and non-AD patients. Conversely, the year before another case-control study,[18] testing cerebrospinal fluid lead levels in 227 patients, reported a significant reduction (p<0.010) of metal in patients with Alzheimer's disease compared with healthy controls.

Finally, a recent post mortem case control study, carried out in 2016 by Szabo et al. on brain tissue samples (frontal cortex and ventricular fluid) of 31 subjects, didn't show any statistically significant differences in Pb levels among AD (N=16) and non-AD subjects (N=15).

**Manganese (Mn)**

As shown in Table 9, twelve articles concerning manganese concentration in biological matrices met the eligibility criteria. Six case-controls studies tested differences in blood metal levels, reporting discordant results. Baum et al. and Alimonti et al. didn't report any significant variations in serum between AD subjects and healthy controls.

Gonzalez-Dominguez et al., compared 25 AD, 15 MCI and 25 non-AD, found significantly lower manganese serum levels (p<0.05) in AD patients when compared with healthy controls. Similar findings were reported by Arslan et al., in a study performed on 39 subjects (24 AD and 15 non-AD), (p=0.001), and by Paglia et al. on 118 subjects (34 AD, 20 MCI, 24 SMC and 40 non-AD) (p <0.001).

**Table 8.** Summary results on Lead (Pb).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [13] | Case-control post-mortem | N=31 (16 AD,15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [Pb] 27 ng/g FC (AD); 27 ng/g FC (non AD) [Pb] 34 ng/ml VF (AD); 27.5 ng/ml VF (non AD) | No statistically significant variations. | N.A. | 4 out of 12 |
| [14] | Case-control | N=207 (89 AD, 118 non AD) | NINCDS-ADRDA | Serum | [Pb]1.52µg/L (AD); 1.96µg/L (non AD) | No significant difference. | Age | 6 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Pb] 0.13 µg/L (AD); 0.16 (non AD) | No statistically significant variations (F=1.026;p=0.384) | Age Sex Education | 6 out of 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Pb] 0.72 µg/L (AD); 0.59 µg/L (AD+vasc); 0.61 µg/L (non AD) CSF [Pb] 0.23 µg/L(AD); 0.24 µg/L (AD+vasc) 0.32 µg/L (non AD) | In CSF [Pb] is significantly lower in AD subjects (N.R.; p ≤0.010) | N.A. | 5 out of 12 |
| [22] | Case-control | N=177 (53 AD, 124 non AD) | NINCDS-ADRDA | Serum | [Pb]= 0.38 µg/L (AD); 0.53 µg/L (non AD) | No statistically significant variations. | Age Gender Drugintake | 5 out of 12 |
| [23] | Case-control | N=318 (264 AD, 54 non AD) | NINCDS-ADRDA | CSF | [Pb] 0.23 µg/L (AD); 0.24 µg/L (ADMV); [Pb] 0.32 µg/L (non AD) | No statistically significant variations | N.A. | 5 out of 12 |
| [26] | Case-control | N= 89 (15 AD, 41 MS, 23 healthy controls and 10 healthy elderly controls) | NINCDS-ADRDA | Blood | [Pb] 14.04µg/L (AD); 23.15µg/L (healthy control); 24.60µg/L (healthy elderly control) | [Pb] is decreased in AD subjects (F=1.482; p<0.001) | N.A. | 3 out of12 |
| [54] | Case-control | N=210 (80 AD, 130 non AD) | NINCDS-ADRDA | Blood Serum | Blood [Pb] 1.90 µg/dL (AD); 2.19 µg/dL (non AD) Serum [Pb] 0.20 µg/dL (AD); 0.17 µg/dL (non AD) | Statistically significant difference in blood [Pb] (N.R.; p=0.035) in AD subjects | N.A. | 4 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision); PD= Parkinson's disease.

By contrast, one study[19] showed a significant (p≤0.001) increase in mean plasma concentrations in 173 patients with AD, compared with 54 healthy controls.

Koseoglu et al., in their study on hair and nails samples of 122 subjects, found a significant decrease in metal concentration in both biological matrices among patients with diagnosis of Alzheimer's disease when compared with non-AD subjects.

Concerning studies on cerebrospinal fluid matrices, Gerhardsson et al. in two articles, published in 2008 and 2009 respectively, reported significantly lower concentrations of manganese in subjects with AD (p ≤0.010 and p=0.004, respectively) than in controls.

Finally, three post-mortem case control study reported no statistically significant difference in Mn brain levels between AD and non-AD subjects, irrespectively from which brain region was investigated (frontal cortex, temporal cortex, hippocampus and amygdala).

**Mercury (Hg)**

Eight studies evaluated the difference in mercury concentration between subjects diagnosed with Alzheimer's disease and healthy patients, as shown in Table 10. Among the five articles assessing blood levels,[14,16,19,26,52] only one[19] showed statistically significant variations (an increase) in metal levels, while the others found no substantial difference between cases and controls.

Concerning cerebrospinal fluid, Gerhardsson et al. described similar concentrations in cases and controls <0.21 µg/L whereas, a significant decrease in metal concentration in 62 AD patients was reported by Koseoglu et al in comparison to 60 controls.

Finally, a post mortem case control study showed no statistically significant difference in metal concentration of frontal cortex and ventricular fluid between 16 AD and 15 non-AD subjects.

**Molybdenum (Mo)**

Among the three evaluated articles, only one[16] reported an increase (p=0.008) in molybdenum blood concentration in patients with di-

**Table 9.** Summary results on Manganese (Mn).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [13] | Case-control post-mortem | N=31 (16 AD,15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [Mn] 151 ng/g FC (AD); 182 ng/g; FC (non AD) [Mn] 25.1 ng/ml VF (AD); 23.5 ng/ml VF (non AD) | No statistically significant variations. | N.A. | 4 out of 12 |
| [15] | Case-control | N=85 (44 AD,41 non AD) | NINCDS-ADRDA | Serum | [Mn] 21.4 nmol/L (AD) 13.3 nmol/L (non AD) | No statistically significant variations | Age Sex | 5 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Mn]= 0.59 µg/L (AD); 1.24 (non AD) | [Mn] in AD subjects were significantly lower (F=14.783; p<0.001) | Age Sex Education | 6 out 12 |
| [17] | Case-Control | N=65 (25 AD, 15 MCI, 25 healthy control) | NINCDS-ADRDA | Serum | [Mn] 0.665µg/L (AD);1.09µg/L (non AD) | In AD subjects total serum [Mn] is significantly reduced (N.R.; p<0.05) | N.A. | 5 out of 12 |
| [18] | Case-Control post-mortem | N= 45 (18 AD, 11 DLB, 16 non AD) | BRAAK | Brain tissue (hippocampus, amygdala) | AD [Mn] Hippocampus= 7.4µM of wet tissue [Mn] Amygdala= 8.8 µM of wet tissue Non AD [Mn] Hippocampus= 8.7µM of wet tissue [Mn] Amygdala= 7.8µM of wet tissue | No statistically significant variations. | N.A. | 5 out of 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Mn] 1.5 µg/L(AD); 1.4 µg/L(AD+vasc); 0.94 µg/L(non AD) CSF [Mn] 0.61 µg/L(AD); 0.63 µg/L(AD+vasc); 0.73 µg/L(non AD) | Plasma [Mn] is significantly higher in AD subjects (N.R.; p ≤0.001) CSF [Mn] is significantly lower in AD subjects (N.R.; p ≤0.010) | N.A | 5 out of 12 |
| [20] | Case-control | N= 122 (62 AD, 60 non AD) | NINCDS-ADRDA | Nail Hair | Nail [Mn] 2.09 µg/g (AD); 6.76 µg/g (non AD) Hair [Mn] 0.49 µg/g (AD); 1.15 µg/g (AD) | Statistically significant difference in nail [Mn] (N.R.; p < 0.001) AD subjects showed statistically significant lower [Mn] (N.R.; p = 0.001) in hair | N.A | 7 out of 12 |
| [21] | Case-control | N= 39 (24 AD, 15 non AD) | NINCDS-ADRDA | Serum | [Mn] 0.012 µg/dL (AD), 0.089 µg/dL (non AD) | [Mn] is lower in AD subjects (N.R.; p=0.001) | N.A. | 5 out of 12 |
| [22] | Case-control | N=177 (53 AD, 124 non AD) | NINCDS-ADRDA | Serum | [Mn] 0.60 µg/L (AD); 0.60 µg/L (non AD) | No statistically significant variations. | Age Gender Drugintake | 5 out of 12 |
| [23] | Case-control | N=318 (264 AD, 54 non AD) | NINCDS-ADRDA | CSF | [Mn] 0.61 µg/L (AD); 0.63 µg/L (ADMV); 0.73 µg/L (non AD) | Statistically significant difference in [Mn] in AD subjects (N.R.; p=0.004) and in ADMV subjects (N.R.; p=0.048) | N.A. | 5 out of 12 |
| [29] | Case-Control post-mortem | N= 30 (10 PD, 10 AD, 10 non AD) | NINCDS-ADRDA | Brain (temporalcortex) | [Mn]=0.84 ng/mg (AD); 1.23 ng/mg (non AD) | No statistically significant difference. | N.A. | 5 out of 12 |
| [31] | Case-Control | N= 108 (52 ALS, 21 AD, 20 PD, 15 non AD) | DSM IV-TR | CSF | [Mn] 1.8 ng/mL (AD);1.2 ng/mL (early-onset AD); 2.1 ng/mL (late-onset AD); 1.9 ng/mL (non AD) | No statistically significant variations. | N.A. | 4 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.= Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision); PD= Parkinson's disease; VaD = Vascular Dementia; ALS= Amyotrophic Lateral Sclerosis; DLB= Dementia with Lewy bodies.

**Table 10.** Summary results on Mercury (Hg).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [13] | Case-control post-mortem | N=31 (16 AD, 15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [Hg]10 ng/g FC (AD); 9 ng/g FC (non AD) [Hg]<LOQ VF(AD); <LOQ VF (non AD) | No statistically significant variation. | N.A. | 4 out of 12 |
| [14] | Case-control | N=207 (89 AD, 118 non AD) | NINCDS-ADRDA | Serum | [Hg]= 1.46µg/L (AD);1.54µg/L (non AD) | No statistically significant difference | Age | 6 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Hg] 0.32 µg/L (AD); 0.62 (non AD) | [Hg] in AD subjects were significantly lower (F=8.732; p<0.001) | Age Sex Education | 6 out 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Hg] 0.28 (AD); 0.23 (AD+vasc); <0.21 µg/L(non AD) CSF [Hg] <0,21 µg/L(AD);<0.21 (AD+vasc);<0,21 µg/L (non AD) | [Hg] is significantly higher in subjects with AD (N.R.; p ≤0.001) | Age | 5 out of 12 |
| [20] | Case-control | N= 122 (62 AD, 60 non AD) | NINCDS-ADRDA | Nail Hair | Nails [Hg]0.19 µg/g (AD); 0.28 µg/g (non AD) Hair [Hg]0.12 µg/g (AD); 0.19 µg/g (non AD) | AD subjects showed statistically significant difference [Hg] (N.R.; p < 0.001) AD subjects showed statistically significant lower [Hg] (N.R.; p < 0.01) in hair | N.A. | 7 out of 12 |
| [23] | Case-control | N=318 (264 AD, 54 non AD) | NINCDS-ADRDA | CSF | AD [Hg]<0.21 µg/L ADMV [Hg] <0.21 µg/L HC [Hg] <0.21 µg/L | No statistically significant variations. | N.A. | 5 out of 12 |
| [26] | Case-control | N= 89 (15 AD, 41 MS, 23 healthy controls and 10 healthy elderly controls) | NINCDS-ADRDA | Blood | [Hg] 5.415µg/L (AD); 6.111µg/L (healthy control); 3.326µg/L (healthy elderly control) | No statistically significant difference. (F=11.68; p=0.0008) | N.A. | 3 out of 12 |
| [54] | Case-control | N=210 (80 AD, 130 non AD) | NINCDS-ADRDA | Blood Serum | Blood [Hg] 2.93 µg/g (AD);3.23 µg/g (non AD) Serum [Hg]1.45 µg/g (AD);1.43 µg/g (non AD) | No statistically significant variations. | N.A. | 4 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision); PD= Parkinson's disease; LOQ = Limit of Quantification.

agnosis of Alzheimer's disease compared to healthy controls. No statistically significant changes in metal levels were found between cases and controls in the other two reports, irrespectively from the biological matrix investigated (blood, cerebrospinal fluid) (Table 11).

**Nickel (Ni)**

As shown in Table 12, six studies assessing differences in nickel concentration between AD and non-AD subjects met the eligibility criteria.[13,16,19,22,23,29] Independently from the biological matrix evaluated (plasma, serum, cerebrospinal fluid, brain tissue) no significant variation between patients with diagnosis of Alzheimer's disease and healthy controls was found.

**Selenium (Se)**

Six articles evaluated selenium levels in patients with diagnosis of Alzheimer's disease and healthy controls (Table13). Differences in metal concentrations were assessed in two biological matrices: blood and cerebrospinal fluid.

**Table 11.** Summary results on Molybdenum (Mo).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Mo]= 1.20 µg/L (AD); 0.83 (non AD) | [Mo] is increased in AD subjects (F=4.199; p=0.008) | Age Sex Education | 6 out of 12 |
| [17] | Case-Control | N=65 (25 AD, 15 MCI, 25 non AD) | NINCDS-ADRDA | Serum | [Mo] 1.02µg/L (AD); 1.06µg/L (non AD) | No statistically significant differences. | N.A. | 5 out of 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Mo] 1 µg/L(AD);1.1 µg/L(AD+vasc); 1 µg/L(non AD) CSF [Mo] 0.23 µg/L(AD); 0.26 µg/L(AD+vasc); 0.24 µg/L(non AD) | No statistically significant variation. | N.A. | 5 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision).

The five studies on blood samples[15,16,17,26,32] showed non univocal results: three studies showed a decrease in selenium, whereas no substantial differences were reported in two studies. In detail, Giacoppo et al. reported a significant (p<0.001) decrease in selenium blood levels of 15 AD patients compared with the healthy control group (N=33). A study by Vural et al. found similar results, with blood Se levels that were significantly lower (p<0.001) in patients with Alzheimer's disease compared to non-AD subjects ([Se]=58.15µg/dl in male and 58.43 µg/dl in female with AD; [Se]=67.84 µg/dl in male and 68.70 µg/dl in female without AD). A study by Paglia et al. found a significant decrease (p= 0.026) of selenium blood levels in AD subjects.

Another two case control studies on selenium blood concentration[15,17] showed no substantial variation in metal levels between case and control.

**Table 12.** Summary results on Nickel (Ni).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [13] | Case-control post-mortem | N=31 (16 AD,15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [Ni] 52 ng/g FC (AD); 36 ng/g FC (non AD) [Ni] 19.6 ng/ml VF (AD); 12 ng/ml VF (non AD) | No statistically significant variations. | N.A. | 4 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Ni] 1.10 µg/L (AD); 1.08 (non AD) | No statistically significant variations. | Age Sex Education | 6 out 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Ni] 2.9 µg/L (AD); 2.8 µg/L (AD+vasc); 2,8 µg/L(non AD) CSF [Ni] 0.36 µg/L (AD); 0.43 µg/L (AD+vasc); <0.28 µg/L(non AD) | No statistically significant variations. | N.A. | 5 out of 12 |
| [22] | Case-control | N=177 (53 AD, 124 non AD) | NINCDS-ADRDA | Serum | [Ni] 0.53 µg/L (AD); 0.39 µg/L (non AD) | No statistically significant variations. | Age Gender Drug intake | 5 out of 12 |
| [23] | Case-control | N=318 (264 AD, 54 non AD) | NINCDS-ADRDA | CSF | [Ni] 0.36 µg/L (AD); 0.43 µg/L (ADMV);<0.28 µg/L (non AD) | No statistically significant variations. | N.A. | 5 out of 12 |
| [29] | Case-Control post-mortem | N= 30 (10 PD, 10 AD, 10 non AD) | NINCDS-ADRDA | Brain (temporalcortex) | [Ni] 1.09 ng/mg (AD); 1.51 ng/mg (non AD) | No statistically significant difference. | N.A. | 5 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision); PD= Parkinson's disease.

**Table 13.** Summary results on Selenium (Se).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [15] | Case-control | N=85 (44 AD,41 non AD) | NINCDS-ADRDA | Serum | [Se]1.42 nmol/L (AD); 1.39 nmol/L (non AD) | No statistically significant variation. | Age Sex | 5 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Se]= 70.36 µg/L (AD); 82.62 (non AD) | [Se] is decreased in AD subjects (F=3.199; p=0.026) | Age Sex Education | 6 out of 12 |
| [17] | Case-Control | N=65 (25 AD, 15 MCI, 25 non AD) | NINCDS-ADRDA | Serum | [Se] 120.5µg/L (AD); 122.9µg/L (non AD) | No statistically significant variation. | N.A. | 5 out of 12 |
| [23] | Case-control | N=318 (264 AD, 54 non AD) | NINCDS-ADRDA | CSF | [Se]1.8 µg/L (AD); 1.8 µg/L (ADMV);1.9 µg/L (non AD) | No statistically significant variation. | N.A. | 5 out of 12 |
| [26] | Case-control | N= 89 (15 AD, 41 MS, 23 healthy controls and 10 healthy elderly controls) | NINCDS-ADRDA | Blood | [Se] 42.78µg/L (AD); 71.10µg/L (healthy control); 73.27µg/L (healthy elderly control) | [Se] is decreased in AD subjects (F=2.301; p<0.001) | N.A. | 3 out of 12 |
| [32] | Case-control | N=100 (50 AD, 50 non AD) | NINCDS-ADRDA | Plasma | [Se]M= 58.15µg/dl [Se]F= 58.43 µg/dl (AD) [Se]M=67.84 µg/dl [Se]F= 68.70 µg/dl (non AD) | Statistically significant decrease [Se] (F=6.77; p<0.001) in AD subjects | N.A | 6 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision); M=Male; F=Female; MS= Multiple Sclerosis; DLB= Dementia with Lewy bodies.

The only available study performed on different biological matrices (Gerhardsson L et al., 2009) found no statistically significant difference between cases and controls in the cerebrospinal fluid samples of 318 subjects (264 AD and 54 healthy controls).

**Tin (Sn)**

As shown in Table 14, among the four articles that met eligibility criteria, no statistically significant variation in metal levels were found between case and control, irrespective of the biological matrix investigated (blood, brain tissue).

**Table 14.** Summary results on Tin (Sn).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [13] | Case-control post-mortem | N=31 (16 AD,15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [Sn] 26 ng/g FC (AD); 32 ng/g FC (non AD) [Sn]7 ng/ml VF (AD): 5 ng/ml VF non AD) | No statistically significant variations. | N.A. | 4 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Sn] 0.22 µg/L (AD); 0.14 (non AD) | No statistically significant variations (F=0.380; p=0.768) | Age Sex Education | 6 out 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [Sn] 0.9 µg/L(AD); 0.81 µg/L (AD+vasc); 0.85 µg/L(non AD) CSF [Sn] 0.21µg/L(AD); 0.16 µg/L (AD+vasc); 0.25 µg/L(non AD) | No statistically significant variations. | N.A. | 5 out of 12 |
| [22] | Case-control | N=177 (53 AD, 124 non AD) | NINCDS-ADRDA | Serum | [Sn] 1.18 µg/L (AD); 0.53 µg/L (non AD) | No statistically significant variations. | Age Gender, Drugintake | 5 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision).

**Vanadium (V)**

As shown in Table 15, four articles met the eligibility criteria.[13,16,17,19] The study by Paglia et al., carried out on 118 subjects (34 AD, 20 MCI, 24 SMC and 40 non-AD), showed a significant decrease ($p< 0.001$) in vanadium blood levels in AD subjects.

By contrast, Gonzalez-Dominguez et al. (studied population: 25 AD, 15 MCI and 25 Healthy Control), and Gerhardsson L et al. (studied population: 173 AD and 54 non-AD patients) found no significant differences among different groups. Conversely from plasma concentrations, Gerhardsson L et al. reported significantly decreased V concentrations in AD patients when compared with healthy controls ([V] =2.9 µg/L in AD versus [V] =3.2 µg/L in non-AD). Furthermore, Szabo ST et al. in their study carried out on brain tissue of 31 subjects (16 AD and 15 non-AD) detected no significant changes in frontal cortex and ventricular fluid vanadium levels.

**Zinc (Zn)**

As shown in Table 16, an overall number of 13 studies assessing zinc concentration in different biological matrices (blood, cerebrospinal fluid, hair, and brain tissue) were eligible. Many of the studies (N=9) evaluated metals levels in serum samples, with not univocal results: 5 studies out of 9[16,17,26,44,53] showed a significant decrease in zinc blood levels in patients with Alzheimer's disease; no statistically significant variation in metal concentration emerged from a comparable number of articles.[25,38,45] Only the research carried out by Alimonti et al. on 177 subjects resulted in increased levels of zinc among AD subjects when compared with healthy controls. Three further studies were post mortem case controls, undertaken on brain tissue samples, and all showed no significant variations in zinc concentrations between cases and healthy controls. One study[19] was carried out on 318 subjects (264 AD and 54 healthy controls) and showed almost identical levels of CSF zinc in cases and controls.

Finally, Koç et al., 2015, in their case control study on a population of 78 subjects found a statistically significant (p=0,02) increase in hair zinc concentration among AD patients compared with healthy controls.

## Discussion

*Strenghts and limits of the study*

Worldwide high incidence and prevalence of Alzheimer's disease make its prevention and early diagnosis one of the most promising Public Health challenges. Nevertheless, to the best of our knowledge, this is the first systematic study focused on metals as environmental risk factors for Alzheimer's disease.

Albeit the plethora of researches achieved, demonstrating the clear interest in the topic, mainly poor quality studies are available at the moment. Small sample sizes, no clear choice of exposed and unexposed subjects, high variability of results, lack of adjustment techniques for the most important confounding factors make it often difficult to draw conclusions. Indeed, as reported in Tables 2-16, overall quality of the included studies is quite poor, neither reaching the sufficiency threshold: according to the NIH tool score,[12] the median score of the selected studies is 5.5 out of 12.

Furthermore, it is surprising not to find researches investigating interactions among metals, although a tight intercommunication among some of them is well known to influence their absorption, their bond to circulating transporters and regulatory proteins.[56]

*Main findings*

The most part of the 44 eligible studies investigating difference in metals concentration reported no univocal outcomes.[57] Only some elements gave a clear tendency of results. Chrome, molybdenum, nickel and tin showed no real difference between AD and non-AD subjects, irrespectively to the biological matrix evaluated.

Conversely, a defined tendency to significant variation is available in blood and post-mortem studies investigating copper: blood levels were significantly increased in AD subjects while a decrease in brain tissue concentrations was observed in 70% of

**Table 15.** Summary results on Vanadium (V).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [13] | Case-control post-mortem | N=31 (16AD,15 non-AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [V] 3 ng/g FC (AD); 5 ng/g FC (Non-AD) [V] <LOQ VF (AD); <LOQ VF (Non-AD) | No statistically significant variations. | N.A. | 4 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC,40 non AD) | NINCDS-ADRDA | Blood | [V]= 0.08 µg/L (AD); 0.04 (non AD) | [V] is increased in AD subjects (N.R.; p<0.001) | Age Sex Education | 6 out 12 |
| [17] | Case-Control | N=65 (25 AD, 15 MCI, 25 non AD) | NINCDS-ADRDA | Serum | [V] 0.0599µg/L (AD); 0.0598µg/L (non AD) | No statistically significant difference. | N.A. | 5 out of 12 |
| [19] | Case-control | N= 314 (173 AD, 54 non AD, 87AD + vasc., ) | DSM-IV; NINCDS-ADRDA | Plasma CSF | Plasma [V] <2.2 µg/L(AD);<2.2 µg/L(AD+vasc); <2.2 µg/L(non AD) CSF [V] 2.9 µg/L(AD); 2.9 µg/L(AD+vasc); 3.2 µg/L(non AD) | In CSF [V] is significantly lower in AD subjects (N.R.; p ≤0.010) | N.A. | 5 out of 12 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision); LOQ = Limit of Quantification.

**Table 16.** Summary results on Zinc (Zn).

| Author | Study Design | Population | Diagnosis of Alzheimer Disease | Matrix | Mean Metals Concentrations | Results | Adjustment Factors | Quality Assessment (Score) |
|---|---|---|---|---|---|---|---|---|
| [6] | Case-control post mortem | N=68 (42 AD: 14 Moderate and 28 Severe; 26 non AD) | BRAAK | Brain tissue (neocortex, Brodmann area 7) | [Zn] 42.5 mg/Kg (Moderate AD); 45.7 mg/Kg (Severe AD); 45.8 mg/Kg (non AD) | No significantly differences. | N.A. | 4 out of 12 |
| [13] | Case-control post-mortem | N=31 (16 AD,15 non AD) | BRAAK | Brain tissue: Frontal Cortex (FC) and Ventricular Fluid (VF) | [Zn] 12099 ng/g FC (AD); 12073 ng/g FC (non AD) [Zn]=845.2 ng/ml VF (AD); 732.4 ng/ml VF (non AD) | No statistically significant variations. | N.A. | 4 out of 12 |
| [16] | Case-control | N=118 (34 AD, 20 MCI, 24 SMC, 40 non AD) | NINCDS-ADRDA | Blood | [Zn]= 609.40 µg/L (AD); 697.87 (non AD) | [Zn] is decreased in AD subjects (N.R.; p=0.020) | Age Sex Education | 6 out of 12 |
| [17] | Case-Control | N=65 (25 AD, 15 MCI, 25 non AD) | NINCDS-ADRDA | Serum | [Zn] 815 µg/L (AD); 910.5µg/L (non AD) | In AD subjects [Zn] is significantly reduced (N.R.; p<0.05) | N.A. | 5 out of 12 |
| [18] | Case-Control post-mortem | N= 45 (18 AD, 11 DLB, 16 no AD) | BRAAK | Brain tissue (hippocampus, amygdala) | AD [Zn]Hippocampus= 293 µM of wet tissue [Zn]Amygdala= 273 µM of wet tissue Non AD [Zn] Hippocampus= 297 µM of wet tissue [Zn] Amygdala= 264 µM of wet tissue | No significantly differences. | N.A. | 5 out of 12 |
| [22] | Case-control | N=177 (53 AD, 124 non AD) | NINCDS-ADRDA | Serum | [Zn] 691 µg/L (AD); 795 µg/L (non AD) | Statistically significant [Zn] increase in AD subjects (N.R.; p<0.05) | Age Gender Drugintake | 5 out of 12 |
| [23] | Case-control | N=318 (264 AD, 54 non AD) | NINCDS-ADRDA | CSF | [Zn]17 µg/L (AD); 17 µg/L (ADMV); 17 µg/L (non AD) | No statistically significant variation. | N.A. | 5 out of 12 |
| [25] | Case-control | N= 166 (83 AD, 83 non AD) | NINCDS–ADRDA DSM-IV-TR | Serum | [Zn] 69ug/dl (AD); 72ug/dl (non AD) | No significantly differences. | N.A. | 4 out of 12 |
| [26] | Case-control | N= 89 (15 AD, 41 MS, 23 healthy controls and 10 healthy elderly controls) | NINCDS-ADRDA | Blood | [Zn] 1.788µg/L (AD); 2.810µg/L (healthy control); 3.080µg/L (healthy elderly control) | [Zn] is decreased in AD subjects (F=1.364; p<0.001). | N.A. | 3 out of 12 |
| [38] | Case- Control | N=78 (45 AD, 33 non AD) | NINCDS-ADRDA | Hair Serum | Hair [Zn] 75µg/g (AD); 98 µg/g (non AD) Serum [Zn] 0.47µg/mL (AD); 0.52 µg/mL (non AD) | Statistically significant decrease in hair [Zn] in AD subjects (N.R.; p=0.02) No statistically significant difference in serum [Zn] | N.A. | 6 out of 12 |
| [43] | Case-Control | N= 47 (28 AD, 19 non AD) | DSM-IV-TR | Serum | [Zn] 0.62 mg/L (AD); 0.69 mg/L (non AD) | [Zn] in AD group is significantly lower (t=2.086; p=0.043) | N.A. | 5 out of 12 |
| [45] | Case-control | N=152 (72 AD, 80 non AD) | NINCDS-ADRDA | Serum | [Zn]111.3 µg/dL (AD); 123 µg/dL (non AD) | No statistically significant differences (t=0.810; p=0.419) | N.A. | 7 out of 12 |
| [55] | Cross-sectional | N=958 (205AD, 753 nonAD) | NINCDS-ADRDA | Serum | [Zn] 12.206 µM (AD); 12.730 µM (non AD) | Decreased [Zn] in AD is an effect of ageing. | Age Sex ApoEε4 | 7 out of 14 |

AD = Alzheimer Disease; Non-AD = Non Alzheimer Disease; NINCDS = National Institute of Neurological and Communicative Disorders and Stroke; ADRDA = Alzheimer's Disease and Related Disorders Association; MCI= Mild cognitive impairment; SMC= subjective Memory Complaint; N.A.=Non Available; CSF = Cerebrospinal Fluid; DSMIV-TR= Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision); MS= Multiple Sclerosis; DLB= Dementia with Lewy bodies.

the studies, substantially agreeing with results of the metanalysis by Schrag et al.,[58] that underlined heterogeneity of the study results on iron, copper and zinc except for brain copper levels, that were significantly depleted in patients with AD. Less robust results were recorded for nail copper levels, that were significantly decreased in patients with Alzheimer's disease, although this data comes from only one study.

Differently from copper, higher levels of iron were found in the brain tissue of AD subjects, in particular in the Brodmann area, frontal cortex, and amygdala, while there was no significant difference in metal concentration in other biological matrices.

## Conclusions

Although much is known about pathogenesis of Alzheimer's disease, available evidence doesn't explain all the factors involved in the pathologic process and further models, as "the brain trace metal dyshomeostasis" hypothesis, take place in order to explain those residual part of disease risk. Lifetime absorption of low-moderate metal concentrations, as those recorded in environmental exposure, make it difficult to clearly distinguish between exposed and unexposed subjects.

Besides aluminium, widely recognized as a risk factor for Alzheimer's disease onset, it has to be pointed out that there is also some evidence of different metal concentrations between AD patients and healthy people, in particular for copper and iron. Concerning the first metal, blood levels are significantly increased in AD subjects, as there is a higher transport towards blood circulation to the detriment of noble organs, such as brain tissue, where copper is decreased, in particular in the hippocampus, amygdala, frontal cortex, cerebellum, motor and sensory cortex, cingulate gyrus, temporal gyrus, and entorhinal cortex. Conversely, in patients with Alzheimer's disease, higher levels of iron were found in the brain tissue of AD subjects, in particular in the Brodmann area, frontal cortex, and amygdala, while there was no significant difference in metal concentration in other biological matrices. Moreover, because of the complex interaction among iron, copper and zinc, it could be effective to focus on studies investigating Cu/Zn, Cu/Fe, and Zn/Fe ratios as possible biomarkers of effect, instead of concentrating on measuring single metal levels in biological matrices.

Limited evidence was also found on manganese and vanadium: significantly lower blood and cerebrospinal fluid levels were recorded respectively.

Although these hypotheses are fascinating, with the aim to identify groups at risk and to begin to understand Alzheimer's disease in terms of prevention, interpretation of this study should proceed with caution, in order to avoid speculation.

Indeed, in the environmental field, statistically significant associations do not provide direct evidence of a causal relationship between exposure to metals and Alzheimer's disease. They trace a direction for future research, reinforcing support to the hypothesis that a dyshomeostasis of metals could be behind the pathogenesis of this disease, also without being its main causal factor.

Therefore, focusing further studies on a limited group of metals, excluding those with a clear tendency to non significant variations in AD subjects (e.g. chrome, molybdenum, nickel and tin), would be a useful approach.

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