Metals Toxicity Associated with Oxidative Stress and Altered Level of Antioxidants in the Pathogenesis of Alzheimer’s Disease

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Abstract

This review highlights the involvement of metals in inducing oxidative stress and their correlation with disrupted antioxidant levels in Alzheimer’s disease. Our findings indicate that the accumulation of metals plays a crucial role in elevating oxidative stress levels, thereby initiating a pivotal event in the development of Alzheimer’s disease. These pathological alterations could potentially lead to modifications in antioxidant levels aimed at mitigating free radical damage within the body. Notably, nutritional supplements like vitamins E, C, and B12 may also play significant roles in the therapeutically focused approach to Alzheimer’s disease. It is one of the most common forms of dementia in the elderly population. Altered levels of trace metals and heavy metals accumulation such as iron, copper, zinc, lead, cadmium, and mercury have been associated with oxidative stress production in Alzheimer’s disease (AD) pathogenesis. Metals accumulation is also associated with overproduced amyloid-beta may alter the level of antioxidants in AD patients. Dietary intake of essential antioxidants has been suggested to delay or prevent cognitive impairment by many studies. AD stands as one of the prevailing forms of dementia among the elderly. The buildup of metals is additionally intertwined with the excessive production of amyloid-beta, potentially impacting antioxidant levels in individuals with AD. Various studies propose that the consumption of essential antioxidants through dietary means could potentially forestall or mitigate cognitive decline.

INTRODUCTION

Alzheimer’s disease is the most common cause of dementia, making up 60–70% of cases in the world. It affects more than 24 million people globally.1-2 Age is considered as one of the most important risk factors for the disease onset.3 The main pathological feature of this neurodegenerative disease is irreversible neuronal and synaptic loss in the region of the brain responsible for memory and learning. The main pathological hallmark of the disease is gross atrophy of the cortex and hippocampus region with the presence of amyloid-beta plaque and neurofibrillary tangles (NFTs) of hyperphosphorylated tau protein.4-5 AD...
has two subtypes depending on disease onset: early onset of Alzheimer’s disease (EOAD) and late onset of the disease (LOAD). EOAD accounts for only 6% of all the cases, while the remaining are LOAD in nature.6-7 Late-onset or sporadic disease affects the individual more than 65 years of age.8 The disease is also characterized by oxidizing essential biomolecules such as protein, nucleic acid, and lipids.9-10 In the mild cognitive impairment stage, oxidative stress is considered a key factor in the pathogenesis of the disease.9

**Oxidative Stress**

Oxidative stress is a condition of an imbalance in redox state, a higher generation of reactive oxygen species (ROS), or failure of the antioxidant mechanism in the brain.12 The brain is rich in biometals and peroxidation-susceptible lipids with high consumption of oxygen than any other organ of the body. So, it is considered more prone to oxidative stress with aging.13 Several in-vivo and in-vitro studies suggested a direct relationship between oxidative stress and synaptic dysfunction in AD.14,15 Aβ, oligomer peptide is neurotoxic, impairing synaptic plasticity, neuronal loss, neuroinflammation, cholinergic denervation, dendritic alterations, neurotransmitter imbalance, and substantial synaptic loss.16,17 Mitochondrial DNA has been found to be damaged in different parts of the brain.18 Increased oxidized products of DNA have been observed in the CSF sample of the patients.19 The level of lipid peroxidation has been found to be elevated in the brain tissue in AD and other neurodegenerative diseases.20 The human body has an antioxidant mechanism to defend the excess reactive oxygen species (ROS) level. Some antioxidants like superoxide dismutase, glutathione, and peroxidase scavenge the excess radical produced.21 Markers for lipid peroxidation were found higher, including hydroxynonenal (HNE) and malondialdehyde (MDA) in patients.22,23 In familial AD some genes were found to be mutated such as APP, PS1, and PS2 involved in the amyloid cascade hypothesis, tend to overproduction of Aβ.24 Several non-genetic risk factors have been identified for LOAD, including stroke, head trauma, smoking, diabetes, obesity, hypertension, and depression.25-31 Some protective factors have also been found that may reduce the chances of disease onset or can delay the onset, including mental activity, social engagement, physical activity, education, coffee consumption, and past vaccination etc.32-33 Aβ has the property to make insoluble fibrils that accumulate in the patients’ extracellular brain. There are two forms of Aβ: soluble and the fibrillar form.39 After proteolytic cleavage of membrane-associated amyloid precursor protein (APP), Aβ is produced.40 In the amyloidogenic pathway, amyloid-beta is processed in two major ways, cleavage by β secretase at N-terminus of the amyloid-beta region of APP and by γ secretase at the C-terminus.41,42 Furthermore, α secretase can also process APP.44 Genetic risk factors involve any mutation in some important genes such as APP, PSEN1, and PSEN2 that can over-produce more amyloid beta-peptide.45,46 Overproduced extracellular amyloid-beta has been associated with oxidative stress.47,48 Recent studies have shown that metals get accumulate in amyloid plaques that may be associated with oxidative stress in the brain of AD. Copper-reducing ability has been observed between APP and Aβ.49 ApoE4 is one of the most common genetic risk-factors in late-onset of disease. ApoE has been found in three isoforms of ApoE2, ApoE3, and ApoE4.50 ApoE is made up of 299 amino acids with a molecular mass of ~36 kDa acting as a cholesterol transporter.51 The main function of ApoE4 is to regulate cholesterol levels and lipid metabolism. It transports neuronal cholesterol through the blood to the brain which maintains the synaptic plasticity of neurons.52 ApoE4 carriers have more chances to accumulate amyloid beta in the brain than non-carriers.53 A lower level of brain-derived neurotrophic factor (BDNF) in the hippocampus of AD patients has been associated with excess ApoE4.54 Several studies have shown that the ApoE2 allele has a protective role against the onset of AD. It has been reported that ApoE2 homozygote can reduce the risk of developing AD by 40%.55,56 ApoE4 has three folds of more risk factors for the development of cerebral ischemia over ApoE3.57 Although the association between ApoE4 genotype and severity of NFT pathology have not been found from human data.58,59 ApoE4 is the most pathogenic for causing inflammation, shown by using different models.60 PSEN1, PSEN2, and APP were
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mostly found associated with the autosomal dominant form of the early onset of AD disease. There are more than 200 mutations in PSEN1 throughout the world; only less than 40 rare ones have been identified in PSEN2. In 1995, after the identification of APP and PSEN1 gene, PSEN2 was reported as a causative gene was for AD. PSEN2 gene is located on chromosome 1 that is encoded for PSEN2 protein. It is a transmembrane protein made up of 448 amino acids with a molecular weight of 55 Da. PSEN2 variant protein was found accumulated in the cerebral cortex and hippocampus region of the brain in sporadic AD. Cell culture studies have reported the up-regulation of the PSEN2 variant under hypoxic conditions. Presenilin mutation may alter intracellular calcium signaling, leading to amyloid-beta deposition to form neuronal apoptosis plaques. Several cell culture-based studies and mouse models have suggested that mutation in presenilin can increase the production of amyloid-beta 42, which is a characteristic feature of AD pathology. Furthermore, researchers have suggested a metal hypothesis, in which evidence of essential biometals such as calcium, manganese, zinc, iron, copper, and magnesium were disrupted. These metals have been found to play an important role in amyloid-beta accumulation and tau metabolism in AD. Different metal ions such as iron, zinc, and copper have been seen to bind with amyloid-beta and influence the aggregation pathway present in nearby extracellular senile plaques. Moreover, various toxic heavy metals such as mercury, lead, and cadmium are abundant in nature for its industrial applications. Heavy metals have no biological function in the body and its low doses can harm health. Metal ions may play an essential role in brain functioning and its altered homeostasis has been reported in aggregates of amyloid-beta in neurodegenerative disease. Trace metals like Zn, Cu, and Fe were found to be excessively bound with amyloid-beta and nearby extracellular senile plaques. Some heavy metals (As, Pb, Cd, and Hg) also have been reported to induce cognition impairment in the brain. Furthermore, the ApoE4 gene has been found to mutate from multiple risk factors in late onset of AD. A complex mechanistic pathway may be a cause of AD.

Biometals role in AD

Iron (Fe)

Studies have reported that iron levels increase with aging, but excessive iron levels lead to the Fenton reaction’s overproduction of reactive oxygen species, which can cause neuronal cell damage. Redox-active iron complexes in the form of Fe²⁺ or Fe³⁺ has been deposited around Aβ plaque and neurofibrillary tangles in the cortex of the AD brain. Several studies have supported the co-localization of iron and Aβ together. One study used transmission electron microscopy (TEM) to understand the form of iron in plaques and confirmed the presence of iron in the core of senile plaques in iron oxide (Fe₃O₄) form by using magnetite nanoparticles. It is suggested that iron loading regulated alpha -cleavage of APP in-vitro, elucidating that iron-mediated oxidative stress results in APP misregulation. Iron cannot only promote amyloid-beta but also impact amyloidogenic processing of APP. The brain iron metabolism is regulated by proteins including iron regulatory proteins (IRPs), Tf, TfRI, ferritin, FPN1 and DMT1, etc. Several studies have found that iron homeostasis disruption may be a possible hallmark for neurodegenerative disorders. A higher level of iron has been loaded near senile plaques that increase the production of amyloid-beta by increasing the expression of the APP gene. Iron has a role in the synthesis of myelin and neurotransmitters synthesis, but also in maintaining the high metabolic rate of neurons. Interestingly, studies have also observed the progressive accumulation of iron in the brain region such as substantia nigra, globus pallidus, caudate nucleus, and cortex during normal aging of the brain, but similarly associated with neurodegenerative disease. Nevertheless, a low concentration of iron was found in the cerebral cortex, the brainstem, and the cerebellum of the brain. Thus, these ROS enhance or potentiat the neurodeneration in the AD brain.

Copper (Cu)

Copper is an essential biometal that play an important role in different cellular functions such as enzyme cofactor for energy metabolism, cellular respiration, neurotransmitter synthesis, and anti-
oxidant defense against free radical. Recently, a meta-analysis showed an increased copper level in serum and plasma samples of AD patients. Although, CSF copper level was not significant in AD patients as compared to healthy subjects. In-vivo and in-vitro studies showed that copper can bind with tau protein and can induce its aggregation as hyper-phosphorylation or generation of reactive oxygen species. Copper also promotes the APP distribution at the cell membrane by inducing exocytosis and reducing endocytosis.

**Zinc (Zn)**

Zinc is second the most abundant biometal in the body and a vital component of over a hundred enzymes and protein. Zinc has been found to play an essential role in cell signaling through neurotransmitters. Zinc is transported into the brain via the blood-brain barriers and cerebrospinal fluid. The level of zinc metal in the brain is balanced by three families of proteins such as metallothioneins (MTs), Zrt-Irt like (ZIP) proteins, and Zn transporter (ZnT) proteins, but very less has been studied about ZnTs in the disease progression. Znt-1 protein has located at the plasma membrane and expressed in the brain and other organs. Synthetic zinc deficiency leads to the development of depression symptoms, improper development of the brain, and impaired brain cognition. Additionally, the concentration of zinc has been found higher in the brain hippocampus, amygdala, neocortex region, and comparatively low in the cerebellum. Zinc has also been found concentrated within amyloid plaques, which is supposed to be released from glutamatergic synapses. Moreover, it has also been elucidated that zinc can make intramolecular and intermolecular bonding between two amyloid-beta-peptide to promote the aggregation of Aβ. Furthermore, a low zinc level can cause an overload of other metals like copper, nickel, and many other toxic heavy metals by upregulating zinc transporters. It is suggested that zinc is associated with APP adhesiveness that leads to cell-cell and cell-matrix interaction. Besides, zinc metal can increase the expression level of PSEN1 to facilitate zinc uptake. Zinc may inhibit the activity of the γ-secretase enzyme responsible for an amyloid-beta generation. Many studies have found an increased level of zinc in the brain and CSF samples of AD. Zinc has a more binding ability in iron and copper upon a wide range of pH. Altered biometals level are associated with with increased amyloid-beta synthesis and mutated genes.

**Heavy Metals Accumulation in AD Patients**

Literature has supported the role of aluminum exposure behind the etiology of AD onset. An in-vivo study supported that chronic Al exposed rats have increased aggregation of amyloid-beta in the hippocampus and cortical regions of the brain. Similar findings were also supported by cell culture studies of neurons, in which chronic exposure of Al lead amyloid-beta and fibrillar accumulation on the cell surface. Moreover, a high level of Al metal can alter the expression level of three important genes such as APP, PSEN1, and PSEN2 that are responsible for AD pathology. One study demonstrated that Al might cause conformational changes in amyloid-beta-protein by CD spectroscopy, and several factors may affect the oligomerization process such as pH, temperature, solvent composition, and peptide concentration. Similarly, Iron and zinc also have been found to induce the aggregation of Aβ protein in AD. Furthermore, Al has been found associated with many other AD-related proteins such as APP, tau protein, paired helical filament tau protein, and α-synuclein protein associated with another neurodegenerative disease like Parkinson’s disease. Most of the neurodegenerative pathways were associated with Al including depletion of neurotrophic factor, generation of free radicals, dephosphorylation of tau, and neuronal cell death. Several epidemiological studies had reported an association between the exposure of aluminum from drinking water and dementia. The postmortem studies have confirmed that the environmental or occupational exposure of Al in an early onset of AD patients without any genetic predispositions had been positively correlated with a high level of Al in their brain tissue also.

**Lead (Pb)**

Lead is heavy metal considered a potent neurotoxin and able to substitute calcium uptake in the body.
It is believed that lead may cross the blood-brain barrier through calcium channels and enter inside neurons and glial cells of the brain.\textsuperscript{142} Lead accumulation can promote neuronal death through glutamate-signaling, disruption of neurons and glial migration, excitotoxicity, lipid peroxidation, and loss of synapses.\textsuperscript{143} Several studies in the elderly suggested an association between bone lead level and reduced brain cognition.\textsuperscript{144,145} Other cross-sectional studies conducted on blood samples did not find this association.\textsuperscript{146,147} Studies conducted on mice model revealed the accumulation of lead in the hippocampus and cerebral cortex, a similar pattern also has been reported in humans.\textsuperscript{148,149} Additionally, one postmortem study conducted in dementia patients confirmed the presence of diffused neurofibrillary tangles with calcification in the brain of lead-exposed workers from Japan.\textsuperscript{150} Interestingly, about 150 genes were found to alter differentially expressed in the early lead-exposed mice model.\textsuperscript{151} Some affected genes such as neprilysin that plays a role in removing amyloid-beta from the brain were found underexpressed in old age compared to controls. However, a gene coding for amyloid-beta precursor protein was overexpressed in AD.\textsuperscript{152} Lead enters the human body in the form of dust and is absorbed through the gastrointestinal tract or lung epithelia. Divalent metal transporters uptake lead that binds firmly to heme molecules and circulates via blood throughout the body. It is considered that a small percentage of circulating lead is toxic in its free form that is bioavailable in the plasma.\textsuperscript{153} Whole blood is the most commonly used biomarker of lead toxicity exposure. It was assumed that the half-life of lead is relatively short, approximately 35 days in the blood.\textsuperscript{154} Spongy trabecular bones have a comparatively intermediate half-life of Pb about 5 to 15 years, while dense bones such as tibia have a longer half-life nearly 10 to 30 years in adults.\textsuperscript{155,156}

The lead level can also be quantified in postmortem bone samples by acid digestion technique using inductively coupled plasma mass spectrometry (ICP-MS).\textsuperscript{157} Epidemiological studies supported the consistent result that early life Pb exposure can dysregulate the APP pathway in the animal model and showed increased expression of APP mRNA. Amyloid-beta accumulation was also elevated without affecting $\alpha$, $\beta$, or $\gamma$-secretases at age of 20 months.\textsuperscript{158,159}

**Arsenic**

Arsenic is available in the form of inorganic arsenic (iAs), a naturally occurring metalloid on the earth’s crust. Human is getting exposed to arsenic mostly through drinking water.\textsuperscript{160} Notably, about 100 million people around the world had exposed to a high concentration of arsenic, which has been observed as a global health problem.\textsuperscript{161} Arsenic has been identified as an important environmental risk factor that can induce neurodegenerative diseases such as AD.\textsuperscript{162} Arsenic can accumulate in different parts of the brain mainly in the pituitary gland in inorganic and methylated arsenicals forms.\textsuperscript{163} The maximum acceptable level of arsenic in drinking water was 50 mg/L from 1942 to 2006 by the US environment protection agency. At this level, cancer risk increases by 100 folds.\textsuperscript{164} Interestingly, selenium metal is considered an essential biometal that plays a key role in arsenic detoxification.\textsuperscript{165} Animal model studies revealed that exposure to as causes reduced memory and learning ability.\textsuperscript{166} An epidemiological study found that humans exposed to a high level of arsenic have an increased risk of type-2 diabetes.\textsuperscript{167} It has been supported that occupational exposure to arsenic can lead to memory loss.\textsuperscript{168} Arsenite may reduce the expression level of brain-derived neurotrophic factor, which has a critical role in learning and memory in cell line model.\textsuperscript{169} Ultrastructural changes in hippocampal neurons, altered pathological changes of neurons, and endothelial cells have been observed.\textsuperscript{170} The low level of exposure might induce APP expression, leading to increased amyloid-beta cleaved by BACE1 enzymatic activity.\textsuperscript{171}

**Cadmium**

Cadmium is a nonessential, toxic heavy metal classified as a human carcinogen by the National Toxicology Program.\textsuperscript{172} The nervous system of humans has been found severely affected by exposure of cadmium (Cd).\textsuperscript{173} Many studies have reported occupational exposure of cadmium, it can cross the blood-brain barrier and pass through olfactory neurons to their olfactory bulbs.\textsuperscript{174} It has been illustrated that Cd can produce free radicals in the brain.
that may potentially damage the neurons, central nervous system, white matter, and oligodendrocytes (OLG).175 Experimental studies have demonstrated that Cd is also a potential neurotoxicant for the peripheral nervous system. Additionally, the half-life of cadmium is more than 15 years in the human body. Increased burden of Cd has been shown in elderly workers and are more susceptible to develop a peripheral polyneuropathy (PNP) in advanced age.176 Many evidence supports the role of cadmium as a possible etiological factor of Alzheimer's disease177 cerebral cortical neurons have been observed to directly affected with Cd-mediated toxicity and cell apoptosis.178 Subsequently, Cd has associated with increased lipid peroxidation in the striatum, parietal cortex, and cerebellum in AD cases.179 Reactive oxygen species (ROS) production has been observed in time and concentration-dependent by Cd exposure in PC12 and SH-SY5Y cells.180 Cadmium can imbalance the intracellular calcium level, which is crucial in intracellular signaling.181 Occupational or environmental exposure of Cd has been associated with many diseases such as urinary bladder, lung, breast, prostate, pancreas, and nasopharynx cancers.182 Furthermore, it has been illustrated that Cd arsenite in yeast cells may cause misfolding of nascent proteins, which results in decreased cellular viability. It has been supposed that possible causes of different pathological conditions similar in Alzheimer's and Parkinson's disease.183 Several studies have found that Cd can generate reactive oxygen species, deposition of calcium ion, upregulation of caspase-3, down-regulated Bcl-2, decreased p-53 lead Cd-induced apoptosis.184 Moreover, this heavy metal can adversely affect the differentiation and proliferation of cells, which contributes to apoptosis and necrosis of cells.185

**Mercury**

Mercury (Hg) exposure in the environment may occur due to mining leakage or pollution.186

It has been reported that inorganic mercury (iHg) can lead to some specific pathological conditions in AD pathogenesis.187 At the same time, dental amalgam is also a primary source of Hg accumulation in the central nervous system.188 Subsequently, AD patients have been characterized by an increased level of plasma mercury. However, results of cerebrospinal fluid (CSF) levels were unaffected.189 Conversely, several studies have demonstrated an insignificant level of Hg in blood samples.190 Fish consumption has been positively related to mercury levels in human blood samples.191 Furthermore, Hg can also induce oxidative stress and cell cytotoxicity.192 It has been highlighted that methylmercury can easily cross the blood-brain barrier and affect the neurons in a particular area of the nervous system such as the visual cortex, cerebellum, and dorsal root of ganglia.193 Postmortem studies have confirmed the concentration of mercury in the brain of AD patients.194

**Antioxidants level in AD**

The most dietary antioxidants are vitamins C, E, and β-carotene, which have been studied widely. It has been found that vitamin E levels in serum samples of 4,809 elderly persons were lower than young ones. Vitamin E is considered a powerful antioxidant thus may be a beneficial effect on the progression of the disease.195 A study conducted on 5,395 participants for dietary assessment in dementia patients had tested a mixture of antioxidants to reduce the oxidative stress associated with AD and concluded that high dietary intake of vitamins E and C may lower the risk of Alzheimer's onset.196 Vitamin E is essential to preventing lipid peroxidation, while tocotrienol may protect against cell viability induced by peroxyl-radical-induced.197 Glutathione has also a role in maintaining α-tocopherol (Vitamin E) and ascorbate (vitamin C) in its reduced form that may function as antioxidants to defend free radicals.198-200

The antioxidant parameters such as catalase, superoxide dismutase, glutathione peroxidase, total glutathione, and total antioxidant capacity (TAC) levels have found to decrease in cases than controls. Although, the markers for ROS like lipid peroxidation and protein carbonyl groups were higher in AD cases than controls.201 The World Health Organization (WHO) recommends a daily intake of five to eight portions (400–600 g) of vegetables and fruits to lower the risk of micronutrient deficiencies such as cardiovascular disease, cancer, cognitive impairment, and other diet-related health problems.202 In mouse model studies, vitamin E has proven to
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Conclusion

In this comprehensive review, our conclusions highlight the significant role of metals accumulation in driving heightened oxidative stress, which serves as a pivotal factor in the onset of AD. These pathological shifts potentially trigger modifications in antioxidant levels aimed at mitigating the impact of free radicals within the body. Heavy metals are associated with a range of detrimental consequences, including cognitive impairment, disruptions in enzymatic pathways, and neuronal transmission abnormalities, potentially fostering the overproduction of amyloid-beta. As a counter measure, the adoption of an adequate dietary intake of antioxidants emerges as a promising strategy to potentially thwart or delay the onset of dementia-related symptoms.

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Conflict of interest

I have no conflicts of interest to disclose. All authors have read and approved the full manuscript.

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