Copper, Zinc and Iron Serum Levels in Patients with Alzheimer's disease

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Abstract: Background: Alzheimer's disease is the most common form of dementia in the elderly and it's prevalence is rapidly rising. Oxidative stress plays important role in the pathophysiology of Alzheimer's disease (AD). Metals like copper, iron derived through diet can act as pro-oxidant under oxidative stress. Most studies had investigated single metals in association with AD and the pathogenesis behind their effect. Therefore, in this study we aimed to correlate the levels of the metal ions; iron, zinc and copper with dementia and with each other in the same patient which may indicate a common pathway involving more than one ion. Methods: A case-control study was conducted on 152 elderly selected from the Geriatric outpatient clinics of Ain Shams University Hospitals. They were classified into two groups; 72 AD cases meeting the probable NINCDS ADRDA criteria and 80 elderly sex matched controls. Full medical and personal history was taken from the patient or available caregiver. Cognitive function of all the participants was screened for presence of cognitive impairment using the Mini Mental State Examination (MMSE). Copper, Zinc and Iron levels were measured in serum. Results: The mean age of patients with Alzheimer's disease (AD) was 73.6 ± 9.8 years while the mean age of controls was 65.5 ± 4.75 years with highly statistically significant difference. 48.1% of patients with AD and 62.9% of controls had low copper serum level with no statistical significant difference. 50% of patients with AD and 50% of controls had high Zinc serum level with no statistical significant difference. Conclusion: The mean age of patients with AD was significantly higher than that of the controls and that there is no statistically significant difference between AD and controls as regards serum Copper, Zinc and Iron levels.

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1.Introduction:

Alzheimer's disease (AD) is the most prevalent form of dementia, which affects more than 37 million people worldwide [1].

Alzheimer's disease is a progressive neurodegenerative disorder of unknown etiology characterized by irreversible cognitive and physical deterioration. It has become more common not only in developed nations but also in developing countries as now the population includes more and more old persons. Though the exact cause for the disease isn't known, it is closely related to the formation of protein deposits (amyloid plaques) and tangled bundles of fibres (neurofibrillary tangles) within the cortex [2].

In AD, metabolic imbalance and the resulting oxidative stress are believed to play a major role in disease pathogenesis [3]. Multiple lines of evidence demonstrate that oxidative stress is an early event in Alzheimer's disease, occurring prior to cytopathology, and therefore may play a key pathogenic role in AD [4].

The most prominent lesions in the brain of AD sufferers are the amyloid or "senile" plaques, which predominantly consist of Amyloid β (Aβ) peptides derived from the proteolytic processing of the amyloid precursor protein (APP) [5]. Various studies have implicated that the toxicity of Aβ is due to abnormal interaction of trace metals zinc, copper and iron in neocortex [6,7,8].

Metal ions in the brain are handled by a number of proteins and transport systems. Studies show disturbances in handling of metal ions in the brain due to the deficiency of these proteins in AD patients. Failure of incorporation of metal ions could contribute to pooling of metal ions in the extra cellular space and contributes to the Aβ precipitation. Further, numerous abnormalities of blood brain barrier reported in individuals with AD may further contribute to fluctuating levels of plasma ions [9,10].

In vivo studies demonstrate that elevated serum copper levels differentiate patients with AD from age matched normal individuals with good selectivity and specificity [11]. Copper homeostasis imbalance and excessive oxidative stress in brain tissue leading to its accumulation in senile plaques and neurofibrillary tangles are documented in AD [12]. According to Rachna *et al.,* [13] serum copper concentration may be a potential marker for AD. Hence, studies of the systemic levels of copper in patients of AD which are reported rarely in literature are of increasing interest. It is still debatable, whether raised copper levels contribute to pathogenesis or is an effect of the disease [14].

As the most abundant trace metal in the brain, zinc is found tightly associated with numerous proteins conferring either structural or catalytic properties upon them [15]. It is hypothesized that zinc could have a role in sustaining the adhesiveness of APP during cell-cell and cell-matrix interactions [16,17]. It is has also been linked to dementia severity in AD [18].

Although zinc dyshomeostasis may contribute to the development of AD, further work is required to clarify the molecular and cellular mechanisms affected by zinc under both normal and disease situations [5].

It has also become apparent that iron progressively accumulates in the brain with age. There is increasing evidence that iron accumulation in the brain can cause a vast range of disorders of the central nervous system [19,20]

In AD, iron is an important cause of oxidative stress because of its over-accumulation in the brain [21]. It is significantly concentrated in and around amyloid senile plaques, and neurofibrillary tangles (NFTs), leading to alterations in the pattern of the interaction between iron regulatory proteins and their iron responsive elements (IREs), and disruption in the sequestration and storage of iron [22; 23]

Chelation has the potential to prevent iron-induced ROS, OS, and Aβ aggregation and, therefore, chelation therapy may be considered a valuable therapeutic strategy for AD [24].

Most studies have investigated single metals in association with AD and the pathogenesis behind their effect. Therefore, in this study we aimed to correlate the levels of the metal ions, iron, zinc and copper with dementia and with each other in the same patient which may indicate a common pathway involving more than one ion.

2.Methodology:

Participants and study design

This is a case-control study enrolling 72 AD patients meeting the probable NINCDS ADRDA criteria [25] and 80 elderly sex matched controls, all being recruited from outpatient clinics of the Geriatrics and Gerontology department of Ain Shams University Hospitals.

Full medical and personal history was taken from the patient or available caregiver. Cognitive function of all the participants was screened for presence of cognitive impairment using the Mini Mental State Examination (MMSE) then using the modified Hachinski ischemic index (HII) included patients with scores less than 5.

Exclusion criteria included: the presence of any neurological disorders (as cerebrovascular stroke and Parkinson's disease) or psychiatric conditions (as depression, schizophrenia or delirium) or patients who score 5 or more in the HII or those who refused to participate.

The control group composed of 80 community-dwelling elderly individuals was recruited from the catchment's area of the same hospital. The inclusion criteria were age higher than 60 years, MMSE scores within normal for age and educational level. Controls were excluded if they presented with chronic renal disease, history of significant head injury or stroke; other psychiatric conditions such as major affective disorder or evidence of current depression; uncorrectable vision or hearing loss or other conditions such as substance abuse or use of medications that could impair cognitive function.

Lab methodology

Blood samples were collected for analysis of serum zinc, copper, Iron. 5 mL of venous blood was placed into vacationer tubes and metal-free tubes.

We used

- Stanbio Iron, Procedure No.0370 (Quantitative colorimetric determination of Iron in serum).
- Zinc (colorimetric method) for in vitro determination of Zinc in serum.
- Copper in serum (colorimetric method).

Ethical considerations

The study was approved by the Ethical Committee of the Faculty of Medicine, Ain Shams University. Informed written consent was obtained from participants, their nearest relatives, or both depending on the patient's cognition.

Statistical analysis:

The collected data was revised, coded, tabulated and analyzed using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, USA 2006). Data was expressed as Mean ± SD for quantitative parametric measures. Comparison between two independent mean groups for parametric data was done using Student t test. The probability of error at 0.05 was considered significant, while at 0.01 and 0.001 were highly significant.

3.Results:

The studied group included 152 subjects. The mean age of the studied group was 69.34 ± 8.6 years . The studied group included 70 males and 82 females.

In the studied group 44% of males and 55.6% of females had AD. The mean age of patients with AD was 73.6 ± 9.8 years and mean age of controls was 65.5 ± 4.75 years with highly statistically significant difference (Table 1).

Table 1: Demographic data of the studied group

		Cases (N=72)	Controls (N=80)
Sex*	Male	32 (44%)	38 (47.5%)
	Female	40 (55.6%)	42 (52.5%)
Age in years**		73.6 (±9.8)	65.5 (±4.75)

* Expressed as No. (%) ** Expressed as mean (SD)

As regard serum copper level, 48.1% of patients with AD and 62.9% of controls had low serum copper level with no statistical significant difference.

As regard Zinc level 50% of patients with AD and 50 % of controls had high serum Zinc level with no statistical significant difference, while as regards serum iron level 76.1% of patients with AD and 63.2% of controls had high serum iron level with no statistical significant difference (Table 2).

Table 2: Serum levels of Copper, Zinc and Iron

Minerals	Serum level	AD	Controls	X^2	*P*
Copper* (12-25 umol/L)	Low	26 (48.1%)	44 (62.9%)	2.715	0.257
	Normal	8 (14.8%)	8 (11.4%)		
	High	20 (37%)	18 25.7%)		
Zinc* (60-110 ug/dl)	Low	26 (36.1%)	22 (28.9%)	1.665	0.435
	Normal	10 (13.9%)	16(21.1%)		
	High	36 (50%)	38 (50%)		
Iron* (65-170 ug/dl)	Low	17 (23.9%)	26 (34.2%)	4.071	0.131
	Normal	0 (0.0%)	2 (2.6%)		
	High	54 (76.1%)	48 (63.2%)		

* Expressed as No. (%).

In the current study the mean serum Cu level was higher in AD than controls (31.49 ± 31.21 versus 23.41 ± 28.4 umol/L respectively) with no statistical significant difference.

In the current study the mean serum Zn and Iron levels were higher in the control group than AD with no statistically significant difference (Table 3).

Table 3: Comparison between AD and controls as regard mean serum minerals levels

Minerals	AD	Controls	t	*p*
Copper ** (12-25 umol/L)	31.49 ± 31.21	23.41 ± 28.4	1.494	0.138
Zinc ** (60-110 ug/dl)	111.3 ± 78.34	123. ± 97.2	0.810	0.419
Iron** (65-170 ug/dl)	558. ± 429	610.7 ± 587	0.618	0.538

** Expressed as mean (SD)

In the current study 11 patients with AD had the serum level of all the 3 minerals elevated, while 33 patients with AD had the serum level of only 2 minerals elevated and 26 patients with AD had the serum level of only 1 mineral elevated ((Table 4).

Table 4: Number of elevated minerals in the AD subjects

No. of minerals with high serum level	No. of AD subjects
3 minerals	11
2 minerals	33
1 mineral	26
None mineral	2

4.Discussion:

Aiming to compare the levels of the metal ions; iron, zinc and copper in subjects with AD and non-demented subjects, we construct this study. The studied group included 152 subjects, 70 males and 82 females with a mean age of 69.34 ± 8.6 years.

When comparing serum copper level in the group suffering from AD and the controls, it was found that there were more subjects in the control group with low serum level of copper (44 controls versus 26 cases). Yet, the results show a statistically non-significant difference in the distribution of subjects in the low, normal and high serum copper

level groups between the cases and the controls. Moreover, there was no significant difference between the mean level of copper in both groups. This disagrees with the most recent study which found that in two AD mouse models that exhibit neurodegeneration, the plaques contained about 25% more copper than an AD mouse model that shows little neurodegeneration **[26]**. On the other hand and in the same year of 2013 another research was done stating that "Researchers in The Birchall Centre at Keele University, Staffordshire, UK, have provided unequivocal evidence that under conditions which are approximately similar to those found in the brain, copper can only protect against beta amyloid forming beta sheets and as such it is highly unlikely that copper is directly involved in the formation of senile plaques in Alzheimer's disease." [27]

The authors of the current study believe that it is probably not the absolute serum level rather than the metabolic handling, both in the body and brain, that may lead to AD. The study also proposes that the interaction of copper with other metal ions may be the pathogenesis of AD changes in the brain rather than the pathogenesis triggered by one metal ion as studied by previous research.

Similar to the findings involving the copper in this study, there was no significant difference in the serum levels of zinc and iron between the cases and controls. There was also no statistically significant difference in the distribution of subjects in the low, normal and high serum iron and zinc level groups between the cases and the controls.

Bourassa *et al.,* [26] found that none of the AD mouse models that they studied had significant increases in iron and very little increases in zinc. Metal content was not related to the age of the plaque. Yet, the researchers measured iron content in the cortex of all three mouse models. They found that iron content was doubled in all AD mouse model cortices compared to controls, whether or not the models showed neurodegeneration [26].

Agreeing with Bourassa *et al.,* [26] was Raven *et al.,* 2013 who found that using the MRI technology allowed them to determine that the increase in iron is occurring together with the tissue damage. They found that the amount of iron is increased in the hippocampus and is associated with tissue damage in patients with Alzheimer's but not in the healthy older individuals or in the thalamus. So the results suggest that iron accumulation may indeed contribute to the cause of Alzheimer's disease.[28]

The study by Watts *et al.,* [29] mentions that low zinc with ageing affects learning and cognition but the same study also describes high zinc levels to cause neurodegeneration. Lead researcher, Dr Nicole Watts, says: "Zinc is thought to aid signaling in the brain as it's released into the space between brain cells. However, when there's too much zinc between the brain cells it can become toxic. High levels of zinc in this area between the brain cells are known to be a factor in neurodegenerative diseases, so regulating the amount of absorption by the cells is crucial."

Since our findings didn't agree with the most recent studies we aimed to prove the hypothesis that elevated metal ions may have an intricate network of interactions that leads in the end to AD.

The results didn't verify the hypothesis as there were two subjects with none of the minerals elevated in the serum, and only eleven subjects had all three ions elevated. Still not many studies correlated serum levels with brain tissue levels.

Conclusion:

Despite the agreeing of many studies that metal ions are higher in AD patients than normal subjects some patients with AD had normal or low levels of all or some of the three ions studied. This makes it difficult to ascertain if elevated metal ions work separately or there is still a link between their effect on the human brain that yet has to be investigated.

Conflicts of interest: None

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