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A study of serum copper and ceruloplasmin in Alzheimer's disease in Kerman, Iran

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Abstract

Background and objective: Alzheimer's disease (AD) is the most common and important degenerative brain disease. The cause of AD is unknown and there are several theories including the possible role of trace elements such as copper and the related oxidative stress. The main transporter of copper, α2-globulin ceruloplasmin, is a multifunctional enzyme. Increased brain metal levels are associated with normal aging and a variety of degenerative diseases including AD. Copper and iron levels both show marked increase with age and may lead to the production of neurotoxic hydrogen peroxidase (H_2O_2) that is related to AD pathogenesis. Variations in serum copper concentration occur because of age, sex, hormonal state, diet and geographical factors and therefore investigations on correlation between serum copper level and AD in different populations is useful. *Methods:* We studied serum copper and ceruloplasmin levels in 50 patients with AD, mean age 76.4 years, 26 men and 24 women in a case controlled study. We used MMSE to select the patients and the control group. Other causes of dementia and confounding illness of copper metabolism were excluded. Serum copper level was measured by calorimetric technique and LKB spectrophotometer and ceruloplasmin serum level by ferro-oxidase technique and LKB spectrophotometer. *Results:* There is a positive correlation between serum copper level and age (p=0.018) but there is no difference between case and control groups in serum copper and ceruloplasmin.
Conclusion: There is no correlation between serum copper and ceruloplasmin level and AD in our geographical area.

INTRODUCTION

Dementia is defined as cognitive or intellectual degeneration without impairment of perception or consciousness.[1] Alzheimer's disease (AD) causes 50% of dementia seen in clinics and hospitals.[2] Age is the most powerful risk factor of AD, the prevalence increases dramatically between 65 and 85, doubling every 5 years. With an increasingly aging population, AD will be one of the major health problems in the coming decades.[3] Other risk factors of AD are: APOE4 allele, female sex, low educational level, positive family history, coronary vessel disease and head trauma. Other probably risk factors are: middle age hypertension, raised homocysteine level, and increased fat content in food.[3] Brain atrophy associated with loss of neurons and synapses, with neuritic and amyloidal plaques and neurofibrilary tangle (NFT) form the primary pathology in AD.[3]

Despite extensive studies, the etiology of AD has yet to be completely understood. Recent studies have shown the importance of oxidative reactions in the pathogenesis of AD. Oxidative stress is primarily responsible for the production of neurofibrillary tangles, the histopathology hallmark of the disease.[4,5] Although the origin of oxidative stress is unknown, it may be highly dependent on the presence of trace elements such as iron and copper.[6] Copper is an essential trace element and is one of the substances forming metalo-enzymes and proteins.[7] Iron and copper are seen in senile plaques and NFT. Moreover, E4 allele, an important genetic risk factor for the disease, is associated with higher levels of copper.[4] High levels of copper has pro-oxidant activity. Its free or unbind form may produce radicals such as the hydroxyl radicals, which could change the structure and solubility of proteins and results in tissue damage.[8,9] The main copper transporter in blood is an α2-globulin protein called ceruloplasmin. Ceruloplasmin is a multifunctional enzyme and can convert the toxic "Ferrous" iron to its non-toxic form, "Ferric".[7,10] Copper and iron levels both increase dramatically with aging and may lead to neuro-toxic H_2O_2 production which is associated to the pathogenesis of AD.[5]

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Multhup *et al* found that reduction of copper I I to copper I by APP with electron transferase reaction can intensify the production of hydroxyl radicals and tissue damage, thus explaining the copper dependant toxicity in neuronal degeneration in AD.[11] Squitti *et al* found a higher serum copper and ceruloplasmin levels among patients with AD as compared to control subjects.[12] On the other hand, studies such as those done by Molina *et el*[6] and Ozcankaya *et al*[13] found no difference between serum copper level in AD patients and normal individuals. In view of these conflicting results, we conducted a study to investigate the relationship between serum copper, cereloplasmin levels with AD in Iran.

METHODS

We conducted a case-control study on the patients referred to the Neurology Unit of Shafa Hospital and the private neurology clinics in Kerman. We recruited 100 patients including 50 patients with AD, and another 50 with normal cognitive status. The diagnosis of AD was according to the NINCDS/ADRDA criteria, with the MMSE ≤24.[14] The control group consisted of volunteers with the similar age range, with normal cognitive status and has no neurological or psychological disorders. Complete neurological examination was done for the control group and the MMSE was ascertained. For the illiterate individuals in both patient and control groups, Hindi Mental State Examination (HMSE), a modified version of MMSE was used.[14]

Fasting blood serum samples were taken from all subjects and were preserved at -20°C temperature till the end of sampling and then laboratory analysis was done by the colorimetric method (using copperison) using the LKB spectrophotometer (model NOVA) and serum ceruloplasmin level analysis was done by ferro-oxidase method using the same equipment by Razi laboratory in Kerman.

AD cases and the controls were described by sex, age, and mental state. For parametric variables, statistical comparison was performed by using students t test, and X^2 statistics for nonparametric ones. Multivariate analysis was done by using ANCOVA. The relation between copper and ceruloplasmin and MMSE score was analyzed using Pearson-correlation Test. P value of less than 0.05 was considered significant. Statistical analysis was done using SPSS 11.5 software.

RESULTS

There were 50 patients in each group. The average age of the AD patients was 76.4 years consisting of 26 males and 24 males; whereas the average age of the control group was 67.8 years consisting of 25 males and 25 females. The patients in the study group were significantly older than those in the control group, by 8.5 years (p=0.047), and had lower MMSE score (14.3 ± 4.6 versus 25.8 ± 1.5, p < 0.001).

The serum copper levels were not significantly different between the study and the control populations (137.8 ± 19.8 mg/dl versus 132.5 ± 15.7 mg/dl, p=0.14). There was no significant difference after correction for age (136.1 ± 18.9 mg/dl versus 134.2 ± 18.6 mg/dl). However, serum copper levels were significantly correlated with age (r=0.23, p=0.018) and MMSE scores (r=0.498, p=0.001), with higher level of serum copper for older age. Fifty-five subjects had MMSE of less than 20. They had significantly higher serum copper levels than those with MMSE of 20 and above (139.3 ± 19.8 mg/dl versus 131.0 ± 15.8 mg/dl, p=0.039). However the difference was not observed when the effect of age was taken into consideration.

The serum ceruloplasmin levels were not significantly different between the study and the control patients (27.7 ± 9.6 mg/dl versus 31.1 ± 5.4 mg/dl, p=0.62). There were also no correlation between serum ceruloplasmin levels with either age (p=0.73) or MMSE scores (p=0.21). It was, however, significantly though weakly correlated to serum copper levels (r=0.38, p=0.001).

DISCUSSION

The present study was conducted to evaluate the relationship between serum copper and ceruloplasmin levels and AD. Squitti *et al*[4,12,15] found a significant positive relationship between the increased serum copper level and AD, while two other studies found no such difference.[6,13] Our study also did not demonstrate significant difference in serum copper levels between patients and control, although we adopted a similar study design as Squitti *et al*.[4,12,15] Serum copper level is significantly higher with age in our study but not in Squitti *et al*'s.[4,12,15] However, we did find a significant correlation between serum copper level and low MMSE score, which was not significant when it was adjusted for age. Further studies are required, with larger number of subjects and taking into account the effect of age.

Squtti *et al*[4,12,15] also showed a 16% increase in serum ceruloplasmin levels in patients with AD. However, we did not find any significant difference in our AD patients and the control subjects. In fact, our control subjects have higher ceruloplasmin level as compared to AD patients. As with Squtti *et al*[4,12,15], we did not find any relationship between age and serum ceruloplasmin levels, a finding different from many other studies.[16-18] We also did not find significant correlation between serum ceruloplasmin level and MMSE score.

Other factors, such as sex, hormonal state, diet and geographical differences are known to affect serum copper levels.[19] Socio-cultural and genetic factors may also affect serum copper and ceruloplasmin levels indirectly. For example, in communities where the elderly are taken care of at home rather than in institutions, better diet in the former may affect the serum levels of copper and ceruloplasmin. In view of this, future study examining the correlation between serum copper and ceruloplasmin levels and Alzheimer disease will have to take socio-cultural and lifestyle factors into consideration.

REFERENCES

1. Victor M, Ropper AH. Adam and Victor's principles of neurology. 7th ed. Mc Graw Hill. 2001: 444-63.
2. Small SA, Mayeux R. Alzheimer's disease and related dementias. In: Rowland LP, ed: Merrit's neurology. 10th ed., Lippincott Williams & Wilkins, 2000: 633-41.
3. Dekosky ST, Kafer DI, Lopes OI. Dementias. In: Bradley WG, Daroff RB, Fenichel GM, Jankovic J, eds: Neurology in clinical practice. 4th ed. Butterworth Heinemann, 2004: 1901-51.
4. Squitti R, Lupoi D, Pasqualetti P, *et al*. Elevation of serum copper level in Alzheimer's disease. *Neurology* 2002; 59: 1153-61.
5. Maynard CJ, Cappai R, Volitakis I, *et al*. Overexpression of Alzheimer's disease amyloid-beta opposes the age-dependent elevation of brain copper and iron. *J Biol Chem* 2002; 277(47): 44670-6.
6. Molina JA, Jimenez-Jimenez FJ, Aguilar MV, *et al*. Cerebrospinal fluid level of transitional metals in patients with Alzheimer's disease. *J Neural Transm* 1998; 105: 479-88.
7. Hassan BS, Reza BS. Clinical chemistry. 1st ed. Moavenate Pajouheshi, 1993: 46-8.
8. Kowalik-Jankowska T, Ruta-Dolejsz M, Wisniewska K, Lankiewicz L, Kozlowski H. Possible involvement of copper (II) in Alzheimer's disease. *Environ Health Perspect* 2002; 110 (Suppls): 869-70.
9. Lianos RM, mercer JF. The molecular basis of copper homeostasis copper related disorders. *DNA cell Biol* 2002; 21(4): 259-70.
10. Patel BN, Dunn RJ, Jeong SY, Zhu Q, Julien JP, David S. Ceruloplasmin regulate iron level in the CNS and prevents free radical injury. *J Neurosci* 2002; 22(15): 6578-86.
11. Multhaup G, Schlicksupp A, Hesse L, *et al*. The amyloid precursor protein of Alzheimer's disease in reduction of copper (II) to copper (I). *Science* 1996, 271(5254): 1406-9.
12. Sqitti R, Cesaretti S. Ceruloplasmin implication in copper mediated toxicity of Alzheimer's disease. The 8th International conference of Alzheirmer's disease and related disorders, Stockholm, Sweden, 2002: 1458.
13. Ozcankaya R, Delibas N. Malondialdehyde, superoxid dismutase, melaton, iron, copper and zinc blood concentration in patients with Alzheimer's disease: cross-sectional study. *Croat Med J* 2002; 43: 28-32.
14. Folstein MF, Folstein SE, McHugh PR. Mini-Mental state: A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189-98.
15. Squitti R. Reply to letter to the editor <hpp://www. *Neurology.* Org / cgi / eletter-submit / 59 / 8/ 1153.
16. Madaric A, Ginter E, Kadrabova J. Serum copper, Zinc and copper / zinc ratio in males: influence of aging. *Physiol Res* 1994; 43(2): 107-11.
17. Mezzetti A, Pierdomenico SD, Costantini F, *et al*. Copper / Zinc ratio and systemic oxidant load: effect of aging and aging-related degenerative disease. *Free Radic Biol Med* 1998; 25(6): 676-81.
18. Milne DB, Johnson PE. Assessment of copper status: effect of age and gender on reference ranging in healthy adults. *Clin Chem* 1993; 39(5): 883-7.
19. Johnson PE, Milne DB, Lykken GI. Effects of age and sex on copper absorption, biological half-life, and status in human. *Am J Clin Nutr* 1992; 56: 917-25.