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An alternative way for the evaluation of zinc status in the elderly; nail zinc levels and relationship with Alzheimer's disease

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Abstract. – BACKGROUND: Zinc is one of the most important elements for human body. Zinc deficiency can occur in any age, if it is seen in elderly its clinical results can be more harmful due to already diminished functions. Some studies showed zinc deficiency has an important role in the pathogenesis of Alzheimer disease. In this study we measured the nail zinc levels and aimed to show its clinical implications in geriatric patients, especially Alzheimer disease.

PATIENTS AND METHODS: 43 patients with Alzheimer disease and 89 patients with normal cognitive function were evaluated. The diagnosis of Alzheimer disease was made according to DSM-IV and NINCDS-ADRDA criteria after cognitive assessment and neuroimaging performed using magnetic resonance. Hand fingernail samples are obtained from the patients.

RESULTS: Mean zinc level from fingernail samples was 117.99 ± 73.44 ppm in Alzheimer Disease patients, 123.86 ± 77.98 ppm in control group (*p*: 0.680).

CONCLUSIONS: This is the first study measuring nail zinc levels in elderly patients with and without Alzheimer disease. Our data reveal no significant difference in nail zinc levels between two groups. However, fingernail zinc may be a useful biomarker in elderly population.

Key Words:
Zinc, Elderly, Alzheimer disease.

Introduction

Zinc is one of the most important elements for human body which plays important roles in many basic physiologic pathways such as immunity, hormonal system and signal transduction[1]. Although zinc deficiency can occur in any age, if it is seen in elderly its clinical results can be more harmful due to already diminished functions. Some studies also showed zinc deficiency has an important role in the pathogenesis of Alzheimer disease (AD)[2-4].

There are some difficulties in determination of body zinc status. Albumin is probably the most important carrier of zinc from the absorption site to its storage place. In the literature, there are several studies about zinc deficiency but most of them deal with albumin and zinc relation and their levels in serum[5-7]. Due to nutritional factors in geriatric population albumin levels in plasma is not stable. There is no known regulatory system in the body related with absorption and storage of zinc. In long term, zinc stored in nails is more stable than the level in plasma[8]. Therefore, nail zinc levels would be more confidential than serum zinc levels. In this study we measured the nail zinc levels and aimed to show its clinical implications in geriatric patients.

Patients and Methods

Population and Setting

Subjects included in this study were patients aged 65 years and over who had presented to the Outpatient Clinics of Division of Geriatric Medicine, Department of Internal Medicine, at Hacettepe University Hospital. A majority of our subjects were from Ankara. However, mainly be-

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cause of the limited number of specialized geriatric centers in Turkey -all of which are located in major cities- many patients were referrals for a comprehensive geriatric assessment from all over Turkey. The inclusion criteria were being of Turkish nationality, 65 years and older, no usage of any drug or supportive product containing zinc, no renal insufficiency, absence of an acute illness preventing comprehensive geriatric assessment, and being cooperable during the assessment. The International Conference on Harmonization, Good Clinical Practice Guideline, and the Declaration of Helsinki were observed. Permission was taken from Ethical Committee of Hacettepe University. After giving informed consent, all patients underwent a complete and comprehensive geriatric assessment.

Measurements

Laboratory tests were evaluated, which consisted of complete blood count (CBC), erythrocyte sedimentation rate (ESR), thyroid stimulating hormone (TSH), plasma low-density lipoproteins (LDL), and high-density lipoproteins (HDL) cholesterol, triglycerides (TG), ferritin albumin and homocysteine.

Co-morbidities were defined using self-report, medication used and after the evaluation of the patient by comprehensive geriatric assessment and laboratory evaluation. For cognitive assessment, Mini-mental State Examination (MMSE)[9] and clock drawing tests[10] were performed. The diagnosis of AD was made according to DSM-IV[11] and NINCDS-ADRDA[12] criteria after cognitive assessment and neuroimaging performed using magnetic resonance (MR). Hand fingernail samples are obtained from the patients. Nail samples are processed and get fluid in Hacettepe University Biochemistry Department. From these nail samples zinc levels are measured in Süleyman Demirel University Biochemistry Department Laboratories with atomic spectrophotometer.

Nail Sampling

Fingernail specimens were taken by using stainless-steel nail clippers on the same day as blood sampling. In order to wipe out contaminating material such as nail polish, chemicals, and dirt, nail specimens were put in an acetone/hexane mixture (3/5, v/v) for 24 hours. Then, they were washed three times with demineralized water and dried in an etuve. After weighing, nail samples were digested in a solution comprising concentrated nitric acid/perchloric acid (5/1, v/v) for 24 hours. For further digestion of the organic matrix within the liquid, the samples were burned by heating mildly until the appearance of the liquid is being clear (with no color). They were diluted by demineralized water in plastic tubes to a defined volume and kept at 4ºC until the analysis. Zinc analysis were performed with a Spestr AA 250 Plus Zeeman atomic absorption spectrometer (Varian, Australia) using a standard additional technique, as stated earlier[1]. Results were expressed in µg/g tissue (ppm) for nail tissue.

Statistical Analysis

Statistical Package for Social Sciences for Windows 15.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All data were entered into a database and were verified by a second independent person. Descriptive statistics were generated for all study variables, including mean and standard deviation (SD) for continuous variables and relative frequencies and percentages for categorical variables. Chi-square method for categorical; Student's *t*-test and ANOVA for continuous data were performed for univariate analysis. Correlation analysis were performed with Pearson's test in numerical and normally distributed parameters, Spearman test in not normally distributed parameters. Two-sided values of $p < 0.05$ were considered as statistically significant.

Results

One hundred and thirty two subjects included in this study. 43 patients with AD [mean age: 73.09 ± 5.42, 18 males (41.9%)] and 89 patients with normal cognitive function [mean age: 71.69 ± 5.55, male 42 (47.2%)] were evaluated. Demographic properties and laboratory profiles of the study population are presented in Table I.

68.2% of patients had hypertension, 32.1% diabetes mellitus, 17% chronic pulmonary lung disease, 24.5% coronary heart disease, 16.8% congestive heart disease, 42% osteoarthritis and 9% of patients had a malignancy. There were no significant difference between the groups regarding comorbidities.

Mean zinc level from fingernail samples was 117.99 ± 73.44 ppm in AD patients, 123.86 ± 77.98 ppm in control group (p : 0.680). Also there were no significant difference between groups regarding age, gender, BMI, hemoglobin,

Table I. Demographic properties and laboratory profiles of AD and control group.

	AD (43)	Control (89)	p
Zinc (ppm)	117.99 ± 73.44	123.86 ± 77.98	0.680
Age (year)	73.09 ± 5.42	71.69 ± 5.55	0.171
Gender (male)	18 (41.9 %)	42 (47.2 %)	0.076
BMI (kg/m^2)	27.43 ± 4.30	27.69 ± 5.12	0.799
Hb (g/dl)	13.83 ± 1.45	14.02 ± 1.29	0.436
Albumin	4.11 ± 0.47	4.13 ± 0.42	0.742
ESR (mm/h)	16.13 ± 14.22	15.21 ± 14.97	0.736
LDL (mg/dl)	116.28 ± 40.57	133.14 ± 62.12	0.971
HDL(mg/dl)	61.10 ± 16.31	56.42 ± 13.92	0.092
TG (mg/dl)	133.14 ± 62.12	161.64 ± 102.59	0.099
TSH (μIU/ml)	1.61 ± 1.11	2.39 ± 4.06	0.248
Ferritin (ng/ml)	95.51 ± 61.04	80.76 ± 80.17	0.288
Homocysteine (μmol/l)	15.41 ± 3.58	15.11 ± 5.23	0.734

BMI: Body Mass Index, ESR: Erythrocyte Sedimentation Rate, Hb: Hemoglobin, LDL: Low-Density Lipoprotein Cholesterol, HDL: High-Density Lipoprotein Cholesterol, TG: Triglycerides, TSH: Thyroid Stimulating Hormone

albumin, ESR, LDL, HDL, TG, TSH, ferritin and homocysteine. Correlation of zinc level of fingernail and other laboratory parameters were shown in Table II.

Discussion

This is the first study measuring nail zinc levels in elderly patients with and without AD. Our data reveals no significant difference in nail zinc levels between two groups.

Zinc is an element, important in many aspects of physiology of human being. It acts as a catalyst and as a cofactor for many enzymes. Zinc also has a critical role in the structure of cell membranes and its deficiency leads to increased susceptibility of the phospholipid cell membrane to free-radical damage and oxidative changes[13].

Its deficiency has shown to be associated with some problems commonly seen in the elderly, like frequent infection episodes, difficulty in tasting, loss of appetite, defect in bone mineralization, hair loss, depression, difficulty in concentration, mental lethargy, growth retardation, male hypogonadism, diarrhea, anemia, and difficulty in night seeing[14]. Zinc which rises to high micromolar concentrations at the synapse during activity is believed to play a key role in learning and memory via its function as a neuronal mesenger[15,16] and a modulator of synapse transmission and plasticity. An anti-atherogenic effect of zinc has been hypothesized due to its antioxidant and membrane-stabilizing properties[17,18].

The elderly has some functional deterioration due to age related physiological changes. Significant changes occur in dietary habits in elderly period, and oral intake of zinc may became inadequate due to alterations in their functional and cognitive status, disease related anorexia, change in physical factors such as loss of teeth and change in taste sensitivity[14]. As a consequence, geriatric population is under risk of zinc deficiency. Zinc deficiency in elderly is not a well studied issue. Pepersack et al[19] tried to determine the prevalence of zinc deficiency in hospitalized elderly, and found 28% of the patients zinc deficient. It is easy to estimate that, zinc deficiency is also common in community-dwelling elderly and, therefore, zinc status should be carefully assessed in daily clinical practice.

Table II. Relationship between nail zinc levels and laboratory investigations.

	r	p
Hb	-0.08	0.16
Hematocrit	0.07	0.21
Leukocyte	0.07	0.20
MCV	0.17	0.02*
Albumin	-0.20	0.01*
ESR	0.15	0.04*
Ferritin	0.20	0.01*
Triglycerides	-0.07	0.38
LDL	0.16	0.03*
Homocysteine	-0.051	0.48

Hb: Hemoglobin, MCV: Mean corpuscular volume, ESR: Erythrocyte Sedimentation Rate, TG: Triglycerides, LDL: Low-Density Lipoprotein

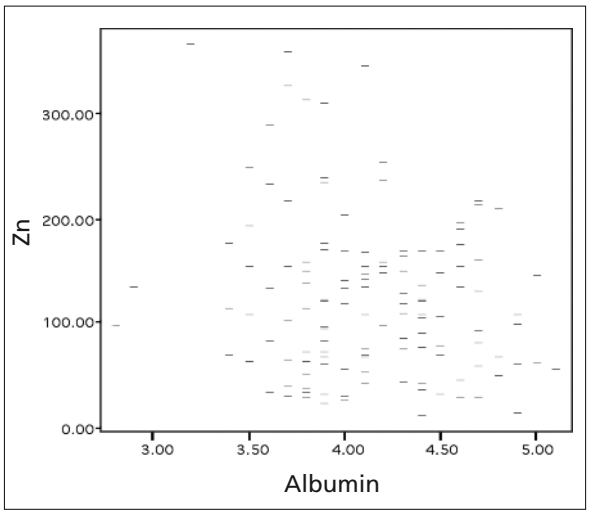

Figure 1. Relationship between nail zinc levels and serum albumin level.

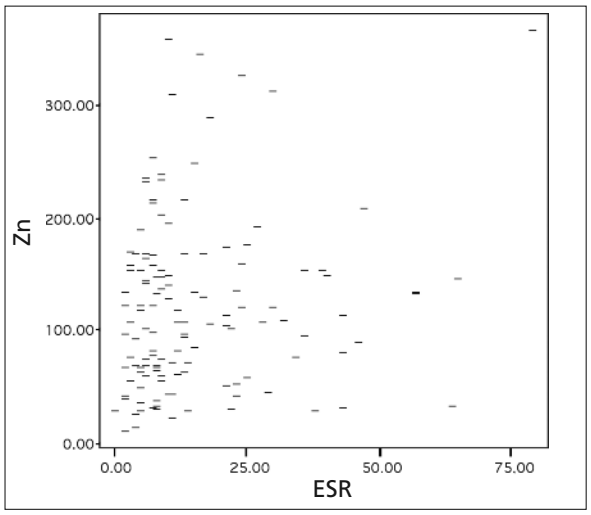

Figure 2. Relationship between serum zinc level and erythrocyte sedimentation rate.

The association between zinc deficiency and AD was firstly studied by Burnet[2]. Since that time multiple studies of zinc in AD and control brain have been paradoxical. Some studies demostrated reduced zinc in hippocampus, inferior parietal lobule and occipital cortex of AD patients[3,20,21], but later studies showed high level of zinc in hippocampus, amygdala[22-27].

The results of the studies evaluating the serum zinc levels were also conflicting in AD and control serum. Haines et al[28], Molina et al[29], Maylor et al[30] revealed no significant differences between AD and control serum. Rulon et al[31], Gonzalez et al[32] showed significant elevations of zinc in AD patients serum.

Unfortunately there is not a commonly used standardized test to measure zinc level and estimate zinc stores in tissue. Human body is a dynamic organism changing in every aspect due to external factors. Especially elderly people are more vulnerable. So, we think that parameters associated with inflamation could lead to misdiagnosis of hyper- or hypozincemia[19]. Therefore, we want to study the nail zinc levels as a indicator of tissue zinc status. Nail is sampled easily and can be stored at room temperature. In contrast to serum, fingernail clippings may provide a more stable measure of zinc status[33].

The association between acute phase reactants such as ferritin and ESR are clinically significant and positively correlated. Albumin levels as a negative acute phase reactant are negatively correlated with nail zinc levels. But the relationship between nail zinc level and these acute phase reactants were weak. This finding may let us to think that inflamation is a condition which leads to increase zinc storage.

Our study has a few limitations since environmental factors can affect nail zinc storage; evaluating these factors is impossible.

Conclusions

Fingernail zinc may be a useful biomarker in studies including elderly population. Whereas some results are still conflicting, further research is warranted.

Conflict of Interest

None to declare.

References

1) Ma J, Betts NM. Zinc and copper intakes and their major food sources for older adults in the 1994-96 continuing survey of food intakes by individuals (CSFII). J Nutr 2000; 130: 2838-2843.
2) Burnet FM. A possible role of zinc in the pathology of dementia. Lancet 1981; 1: 186-188.
3) Andrási E, Suhajda M, Sáray I, Bezúr L, Ernyei L, Réffy A. Concentration of elements in human brain: glioblastoma multiforme. Sci Total Environ 1993; 139-140: 399-402.
4) Shcherbatykh I, Carpenter DO. The role of metals in the etiology of Alzheimer's disease. J Alzheimers Dis 2007; 11: 191-205.

5) Marcellini F, Giuli C, Papa R, Gagliardi C, Malavolta M, Mocchegiani E. Psychosocial and biochemical interactions in aging: preliminary results from an Italian old sample of "Zincage" project. Arch Gerontol Geriatr 2007; 44: 259-269.

6) McClain C, Humphries L, Hill K, Nickl NJ. Gastrointestinal and nutritional aspects of eating disorders. J Am Coll Nutr 1993; 12: 466-474.

7) Cousins R. Toward a molecular understanding of zinc metabolism. Clin Physiol Biochem 1986; 4: 20-30.

8) Garland M, Morris JS, Rosner BA, Stampfer MJ, Spate VL, Baskett CJ, Willett WC, Hunter DJ. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer Epidemiol Biomarkers Prev 1993; 2: 493-497.

9) 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: 189-198.

10) Stähelin HB, Monsch AU, Spiegel R. Early diagnosis of dementia via a two-step screening and diagnostic procedure. Int Psychogeriatr 1997; 9: 123-130.

11) Rabe-Jabło ska J, Bie kiewicz W. Anxiety disorders in the fourth edition of the classification of mental disorders prepared by the American Psychiatric Association: diagnostic and statistical manual of mental disorders (DMS-IV – options book). Psychiatr Pol 1994; 28: 255-268.

12) McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984; 34: 939-944.

13) Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. Physiol Rev 1993; 73: 79-118.

14) Prasad A. Clinical manifestations of zinc deficiency. Annu Rev Nutr 1985; 5: 341-363.

15) Smart TG, Hosie AM, Miller PS. Zn2+ ions: modulators of excitatory and inhibitory synaptic activity. Neuroscientist 2004; 10: 432-442.

16) Paoletti P, Vergnano AM, Barbour B, Casado M. Zinc at glutamatergic synapses. Neuroscience 2009; 158: 126-136.

17) Henning U, Wolf WP, Holtzhauer M. Primary cultures of cardiac muscle cells as models for investigation of protein glycosylation. Mol Cell Biochem 1996; 160-161: 41-46.

18) Henning U, Krieger K, Klimke A. Specific binding of 3H-spiperone to peripheral blood cells: relevance for the interpretation of binding studies in psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry 1999; 23: 225-241.

19) Pepersack T, Rotsaert P, Benoit F, Willems D, Fuss M, Bourdoux P, Duchateau J. Prevalence of zinc deficiency and its clinical relevance among hospitalised elderly. Arch Gerontol Geriatr 2001; 33: 243-253.

20) Andrási E, Nádasdi J, Molnar Z, Bezur L, Ernyei L. Determination of main and trace element contents in human brain by NAA and ICP-AES methods. Biol Trace Elem Res 1990; 26-27: 691-698.

21) Corrigan FM, Reynolds GP, Ward NI. Hippocampal tin, aluminum and zinc in Alzheimer's disease. Biometals 1993; 6: 149-154.

22) Cornett CR, Markesbery WR, Ehmann WD. Imbalances of trace elements related to oxidative damage in Alzheimer's disease brain. Neurotoxicology 1998; 19: 339-345.

23) Danscher G, Jensen KB, Frederickson CJ, Kemp K, Andreasen A, Juhl S, Stoltenberg M, Ravid R. Increased amount of zinc in the hippocampus and amygdala of Alzheimer's diseased brains: a proton-induced X-ray emission spectroscopic analysis of cryostat sections from autopsy material. J Neurosci Methods 1997; 76: 53-59.

24) Deibel MA, Ehmann WD, Markesbery WR. Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. J Neurol Sci 1996; 143: 137-142.

25) Ehmann WD, Markesbery WR, Alauddin M, Hossain TI, Brubaker EH. Brain trace elements in Alzheimer's disease. Neurotoxicology 1986; 7: 195-206.

26) Samudralwar DL, Diprete CC, Ni BF, Ehmann WD, Markesbery WR. Elemental imbalances in the olfactory pathway in Alzheimer's disease. J Neurol Sci 1995; 130: 139-145.

27) Wenstrup D, Ehmann WD, Markesbery WR. Trace element imbalances in isolated subcellular fractions of Alzheimer's disease brains. Brain Res 1990; 533: 125-131.

28) Haines A, Iliffe S, Morgan P, Dormandy T, Wood B. Serum aluminium and zinc and other variables in patients with and without cognitive impairment in the community. Clin Chim Acta 1991; 198: 261-266.

29) Molina JA, Jiménez-Jiménez FJ, Vargas C, Gómez P, de Bustos F, Ortí-Pareja M, Tallón-Barranco A, Benito-León J, Arenas J, Enríquez-de-Salamanca R. Cerebrospinal fluid levels of non-neurotransmitter amino acids in patients with Alzheimer's disease. J Neural Transm 1998; 105: 279-286.

30) Maylor EA, Simpson EE, Secker DL, Meunier N, Andriollo-Sanchez M, Polito A, Stewart-Knox B, McConville C, O'Connor JM, Coudray C. Effects of zinc supplementation on cognitive function in healthy middle-aged and older adults: the ZENITH study. Br J Nutr 2006; 96: 752-760.

31) Rulon LL, Robertson JD, Lovell MA, Deibel MA, Ehmann WD, Markesber WR. Serum zinc levels and Alzheimer's disease. Biol Trace Elem Res 2000; 75: 79-85.

32) González C, Martín T, Cacho J, Breñas MT, Arroyo T, García-Berrocal B, Navajo JA, González-Buitrago JM. Serum zinc, copper, insulin and lipids in Alzheimer's disease epsilon 4 apolipoprotein E allele carriers. Eur J Clin Invest 1999; 29: 637-642.

33) He K. Trace elements in nails as biomarkers in clinical research. Eur J Clin Invest 2011; 41: 98-102.