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Trace metal concentrations in hair and nails from Alzheimer's disease patients: Relations with clinical severity

Emel Koseoglu (MD) (Professor)[a,*], Rahmi Koseoglu (PhD) (Associate Professor)[b], Murat Kendirci (MD) (Neurology Specialist)[c], Recep Saraymen (PhD)[d], Burak Saraymen (MD)[e]

[a] Erciyes University, Facultyof Medicine, Department of Neurology, Kayseri, Turkey
[b] Erciyes University, Faculty of Science, Department of Physics, Kayseri, Turkey
[c] Nevsehir State Hospital, Department of Neurology, Nevsehir, Turkey
[d] Erciyes University, Faculty of Medicine, Department of Biochemistry, Kayseri, Turkey
[e] Erciyes University, Health Institute, Primary Health Center Doctor, Kayseri, Turkey

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ABSTRACT

Background and objectives: Metals, especially transition metals, seem to be important in the pathogenesis of Alzheimer disease. This study aims to determine the relationship of trace metal elements to the pathogenesis and/or course of Alzheimer Disease in terms of clinical severity.
Methods: The hair and nail trace metal levels of 62 Alzheimer Disease patients at different clinical stages (21 mild, 20 moderate, 21 severe) and 60 healthy control subjects were measured by using inductively coupled plasma-mass spectrometry. The statistical comparisons were performed with regards to the study groups, clinical stages, disease duration and age.
Results: The patient and control groups were significantly different from each other in regards to Mn, Fe, Cu, Cd, Hg ($p < 0.001$), Zn ($p < 0.01$) in nail concentrations and, Na, Al, Pb, Co ($p < 0.001$), Fe, Mn ($p = 0.001$), Hg, Cu, Cd, K in hair concentrations ($p < 0.01$). No difference was detected in the levels of Mg and Ca. Nail Na level showed differences among different clinical stages of the disease ($p < 0.01$). In comparing the mild degree Alzheimer patients to the control group; significant differences were detected in nail Mn, Fe, Cu, Co ($p < 0.001$), Hg, Zn ($p < 0.01$) and, hair Pb, Al ($p < 0.001$), Na, K levels ($p < 0.01$).
Conclusions: Our results have shown that transition and posttransition metals are especially important metals for the disease process. The relation of nail Na level with clinical stages of AD is an interesting new finding, making someone to think that alkali metals may be important in the progression of the disease.

1. Introduction

There are increasing evidences for an imbalance in transition metal homeostasis in AD [1,2]. Several studies have suggested that trace elements may be important in the pathogenesis of AD [3–9]. Dyshomeostasis and concentration of metal ions in senile plaques, neurofibrillary tangles and the cerebrospinal fluid support this notion [10]. Likewise, dissolution or inhibition of amyloid plaque aggregation, which is the main pathological hallmark of the disease with treatments targeting metal ions, metal complexes or metal-protein compounds such as metal chelators is a potential new approach, highlighting the significant role that metals play in the etiology of this disease [11,12].

Among various biological specimens, hair and nail samples serve as the best bio-indicators for the metal analysis. Hair mineral analysis may indicate the mineral composition that has accumulated over a long time span, which is proportional to the level of elements in the body [13]. Nails also indicate metal body burden [14]. The hair and nails, in which trace minerals are sequestered and/or stored, can be used to effectively monitor the highest priority toxic trace metals [15]. The advantage of hair and nail tissue analysis over other diagnostic samples is that trace metal concentrations are not subjected to rapid fluctuation due to diet, air, and water; hence,

Abbreviations: AD, Alzheimer disease; NINCDS-ADRDA, National Institutes of Neurological and Communicative Disorders and Stroke- Alzheimer Disease and Related Disorders.

* Corresponding author.
E-mail addresses: emelk@erciyes.edu.tr (E. Koseoglu), rkoseoglu@erciyes.edu.tr (R. Koseoglu), drmuratkendirci@gmail.com (M. Kendirci), saraymen@erciyes.edu.tr (R. Saraymen), drbur2011@hotmail.com (B. Saraymen).

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Table 1
Demographic properties of the patient and control groups.

	Patient group (n = 62)	Control group (n = 60)
Age (years) Mean ± SD	74.40 ± 8.21	72.27 ± 4.80
Sex (F/M) (n)	34/28	24/36
Education situation		
Absent (n)	25/62	25/60
Elementary School (n)[a]	36/62	34/60
Middle-High School (n)[b]	6/62	1/60
Living environment (Rural/Urban) (n)	20/42	15/45
Disease duration (years) Mean ± SD	4.34 ± 2.23	N/A

Elementary school[a]: 5 years education, middle-high school[b]: 8–11 years education.

there is long term stability over nutritional status [16]. Moreover, these samples remain isolated from other metabolic activities [17].

This study aims to determine the relationship of some transition, posttransition, alkaline earth and alkali trace metals to the pathogenesis and/or course of AD considering clinical severity of the disease, by making comparisons among the nail and hair concentrations of trace elements in the patients with different stages of disease and the healthy control subjects.

1.1. Material and methods

The study was carried on in the Department of Neurology and in The Technology Investigation Center of Erciyes University, between March 2011 and August 2013. The study protocol was approved by the Institutional Ethical Committee. All procedures were in accordance with the ethical standarts of the responsible committee on human experimentation (institutional and national) and with the Helsinki Decleration of 1975, as revised in 2000. The study involved 62 AD patients on different clinical stages (21 mild, 20 moderate, 21 severe) and 60 healthy control subjects. All subjects and/or their relatives gave written informed consent for their involvement into the study. The patient participants were selected from the Erciyes University Department of Neurology Outpatient Dementia Clinic and, the healthy control subjects from primary health centers in the city of Kayseri by using covariate adaptive randomization technique considering age, sex, living environment and health status as covariates. All participants have been living in Kayseri at least for the last five years and all were either retired or housewife. The diagnosis of possible AD was put on the criteria of NINCDS-ADRDA (National Institutes of Neurological and Communicative Disorders and Stroke- Alzheimer Disease and Related Disorders) [18].The patients with Hatchinski Ischemic Score [19] higher than 5 were not involved into the study. The clinical ratings of the patients were done according to the Clinical Dementia Rating [20] and Turkish version of Mini Mental State Examination (MMSE) [21]. The healthy control subjects did not differ from the patient group in respect to the distributions of age, gender and living environment (rural or urban) ($p > 0.01$) (Table 1).

Natural hair (not dyed, bleached or straightened) and nail samples were taken from all of the subjects and kept in plastic bags in a dark place. Hair samples were cut from the sub-occipital zone of the head with a stainless steel scissors and nail samples were cut from finger nails with a stainless steel clipper. Samples of 5 mg of nail and 5 mg of hair for each subject were collected for the analysis; samples were washed thorough stirring with different solvents in sequence: acetone, water, water, water, acetone [22]. The samples were then subjected to acid digestion procedure using % 65 HNO_3. The sample solutions were then diluted to exactly 10 ml with double distilled water and so became ready for the measurement process. The measurements of some metals, namely Mn, Fe, Cu, Cd, Hg, Na, Al, Pb, Co, K, Mg, Ca, were performed in the samples with the method of Inductively Coupled Plasma Mass Spectrometry using Agilent 7500 A device. The values of metal concentrations were expressed in μg/g dry tissue.

The level comparisons of the trace metal elements in healthy control subjects to all AD patients and to mild degree patients were performed by using Mann Whitney *U* test. The comparisons among different AD clinical stages were achieved with Kruskal-Wallis Variance Analysis. The relationships of trace metal element levels with age and disease duration were investigated by using Spearman Rho correlation or Pearson correlation analysis test depending on the distribution properties of the groups. Significance level in the statistical tests was accepted as $p < 0.01$.

2. Results

The comparison of metal levels between all AD patients and healthy control subjects, shared similar distribution of gender, age and living environment distribution showed significant differences in nail levels of Mn, Fe, Cu, Cd, Hg ($p < 0.001$), Zn ($p < 0.01$) and hair levels of Na, Al, Pb, Co ($p < 0.001$), Fe, Mn ($p = 0.001$), Hg, Cu, Cd, K ($p < 0.01$) (Tables 2 and 3). It was found that levels of Na and K were significantly higher, while the others' levels were significantly lower in the patient group. No difference was detected in the levels of Mg and Ca, which are alkaline earth metals.

Nail Na level showed a difference between different clinical stages of the disease ($p < 0.01$) increasing with clinical severity (Table 2). In comparing the mild degree AD patients to the control group; nail Mn, Fe, Cu, Co ($p < 0.001$), Hg, Zn ($p < 0.01$) and hair Pb, Al ($p < 0.001$), Na, K levels ($p < 0.01$) have been found to show significant changes. Metal levels have been found not to be related to age or disease duration in any of the study groups ($p > 0.01$).

2.1. Discussion and conclusion

This study investigates the relationship of hair and nail concentrations of trace elements in AD in regards to the disease severity. Our results have shown that there is existence of metal dyshomeostasis in all clinical severities of AD. The levels of transition-posttransition metals, which are redox active, are found to be changed starting from the early stages of the disease. The relation of nail Na level with the clinical stages is interesting as it concludes a new finding that alkali metals may be important in the progression of the disease. In fact, the studies investigating the levels of alkali metals in AD are fewer in number compared to the transition metals.

We have found that the levels of transition (Cr, Mn, Fe, Co, Cu, Zn, Cd, Hg) and posttransition metals (Al, Pb) in AD patients are different from those in the healthy control subjects. Both of the studied alkaline earth metals, Mg and Ca, haven't showed any significant differences between the patient group and the healthy control group. These results have demonstrated that the redox-active metals are probably more harmful than the others, in conformity with the metal hypothesis of AD [23], a complementary mechanism to amyloid plaque deposition. This hypothesis proposes that transition metals interact with amyloid β by forming an oxidative stress causing neurotoxicity.

In this study, it has been observed that the levels of transition and posttransition metals are decreased in hair and nail samples of AD patients. Upon consideration of the current literature, some studies have found that the levels decreased likely, while some studies have reported the opposite or partialy opposite results [24–31]. The levels of metals in biological samples, other than brain, of AD patients, with respect to healthy control subjects are inconsistent [32]. However, Cu, Zn and Fe ions are shown to be sequestered in amyloid plaques, modulating β amyloid aggregation [33,34] and,