

## Short Communication

# Nutritional status of selenium in Alzheimer's disease patients

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Studies have shown that various antioxidants are decreased in different age-related degenerative diseases and thus, oxidative stress would have a central role in the pathogenesis of many disorders that involve neuronal degeneration, including Alzheimer's disease (AD). The present study aimed to assess the nutritional status of Se in AD patients and to compare with control subjects with normal cognitive function. The case-control study was carried out on a group of elderly with AD ( $n$  28) and compared with a control group ( $n$  29), both aged between 60 and 89 years. Se intake was evaluated by using a 3-d dietary food record. Se was evaluated in plasma, erythrocytes and nails by using the method of hydride generation atomic absorption spectroscopy. Deficient Se intake was largely observed in the AD group. AD patients showed significantly lower Se levels in plasma, erythrocytes and nails (32.59  $\mu\text{g/l}$ , 43.74  $\mu\text{g/l}$  and 0.302  $\mu\text{g/g}$ ) when compared with the control group (50.99  $\mu\text{g/l}$ , 79.16  $\mu\text{g/l}$  and 0.400  $\mu\text{g/g}$ ). The results allowed us to suggest that AD has an important relation with Se deficiency.

### Alzheimer's disease: Selenium status

Alzheimer's disease (AD) is characterised clinically by progressive loss of memory and cognition, pathologically by senile plaques, neurofibrillary tangles and synapse loss<sup>(1)</sup>. Oxidative stress plays a central role in this disease, and it is the first event that precedes the disease, and it is manifested by increase in protein oxidation, lipid peroxidation, DNA and mRNA oxidation and formation of reactive oxygen species (ROS) and reactive nitrogen species in the brain<sup>(2)</sup>.

The nervous system is particularly vulnerable to the deleterious effects of ROS and reactive nitrogen species, since it has the highest amount of oxygen to produce energy, and the brain contains high concentrations of PUFA that are highly susceptible to lipid peroxidation<sup>(3)</sup>. Indeed, senile plaques and neurofibrillary tangles are directly associated with oxidative damage in AD, since this peptides can produce ROS<sup>(4,5)</sup>.

Cells have mechanisms to prevent or repair oxidative damage caused by ROS and reactive nitrogen species. These include antioxidant molecules and enzymes, such as glutathione peroxidase<sup>(5)</sup>. However, the brain has a relatively deficient antioxidant system, which contributes to its susceptibility to oxidative damage<sup>(3)</sup>.

Se is an essential nutrient in the diet due to the requirement for selenocysteine in some selenoproteins. This trace element is known to provide protection from ROS-induced cell damage, and the proposed mechanisms mainly invoke the functions of glutathione peroxidase family and selenoprotein P<sup>(6)</sup>.

Although some studies have shown the increase of oxidative stress in AD patients, there is a lack of information about the importance of Se as part of antioxidant enzymes in this disease. In this context, the present study aimed to evaluate nutritional status of Se in AD patients.

### Methods

#### Subjects

Twenty-eight (eleven male and seventeen female) elderly diagnosed with probable AD (AD group) according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria<sup>(7)</sup> were included in the present

**Abbreviations:** AD, Alzheimer's disease; ROS, reactive oxygen species.

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study. These patients attended at the Geriatric Cognitive Ambulatory or at the Center of Reference in Cognitive Disorders, both at the Hospital das Clínicas of the São Paulo University Medical School (São Paulo, Brazil).

Twenty-nine (ten male and nineteen female) healthy volunteer elderly with normal cognitive function, mini-mental state examination ( $\geq 20$ )<sup>(8,9)</sup> sex-matched with those in the AD group, who attended at the Multidisciplinary Group of Assistance to the Aged Ambulatory at the Hospital das Clínicas of the São Paulo University Medical School (São Paulo, Brazil), were included in the control group (C group).

Participants were selected for the study according to the following inclusion parameters: aged 60 years or older; absence of acute inflammation, infection, fever, diarrhoea, cancer, diabetes, autoimmune disease and vitamin and mineral supplement intake. AD patients should have an active caregiver.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by Ethics Committee of the Faculty of Pharmaceutical Sciences at the University of São Paulo (no. 455) and by the Ethics Committee of the Hospital das Clínicas of the University of São Paulo Medical School (no. 0710/08). Written informed consent was obtained from participants of the C group and caregivers of AD patients.

#### Selenium intake

Se intake was evaluated by using a 3-d (2 weekdays and 1 weekend day) dietary food record, up to 7 d before the blood sample was drawn. AD patients and their caregivers were requested to register what they had eaten. Se intake was measured by using NutWin software (version 2.5; EPM-UNIFESP, São Paulo, Brazil).

Software database was supplied with Se data from the study of Ferreira *et al.*<sup>(10)</sup>. Items consumed by the participants, which were not originally listed in this database, were included in the software as data obtained from food composition tables<sup>(11)</sup> or food labels provided by participants.

Se intake was adjusted by the energy, according to Willet<sup>(12)</sup>, using linear regression (linear regression of nutrient intake on total energy intake) and addition of a constant (mean energetic intake of the group). Se intake was compared with dietary reference intake recommendations for the particular age and sex group<sup>(13)</sup>.

#### Biochemical assays

Se concentration was determinate in plasma, erythrocyte and nail samples by using hydride generation atomic absorption spectroscopy<sup>(14)</sup>.

Plasma and erythrocytes were obtained from a fasting, morning blood sample. Toenails and fingernails samples were collected by the participants after 20 d of nails growth without nail polish, up to 20 d before the blood sample was drawn.

All reagents had analytical grade or higher purity from Merck. Nanopure water was used to prepare all solutions and to dilute the samples.

The standard reference material Seronorm<sup>®</sup> was analysed for testing the accuracy and precision of the analytical technique.

#### Statistical analysis

All statistical analyses were carried out using the Statistical Package for the Social Sciences software, version 17.0, for Windows (SPSS, Chicago, IL, USA).

The results were showed as means and standard deviations.

Variables distribution was evaluated by using Kolmogorov–Smirnov test. Differences between AD and C groups were analysed with Student's *t* test. Pearson's correlation coefficients were used to estimate correlations between nutritional parameters and between cognitive evaluation and nutritional status.

A *P* value of 0.05 was considered statistically significant.

#### Results

In the present study, twenty-eight elderly (39.3% men and 60.7% women) in the AD group and twenty-nine healthy elderly (34.5% men and 65.5% women) in the C group were evaluated. There was no sex difference between the two groups; however, the mean age of the AD group was 80.6 (SD 5.7) years, statistically different from the C group, which showed a mean age of 71.2 (SD 6.2) years ( $P < 0.05$ ). Therefore, given that age might influence Se concentrations, correlation analysis between Se parameters and age was performed, but correlations were not observed ( $P > 0.05$ ).

Se levels in plasma and erythrocyte were significantly higher in the C group, although in both groups some participants showed values below the normal range<sup>(15)</sup>. There is no normal values range for Se level in nails, but higher values in the C group were observed when compared with the AD group (Table 1).

**Table 1.** Selenium concentrations in plasma ( $\mu\text{g/l}$ ), erythrocytes ( $\mu\text{g/l}$ ) and nails ( $\mu\text{g/g}$ ), and selenium content in diet ( $\mu\text{g/d}$ ) of elderly with Alzheimer's disease (AD) and those in the control (C) group

	Groups							
	AD (n 28)				C (n 29)			
	Plasma	Erythrocyte	Nails	Diet	Plasma	Erythrocyte	Nails	Diet
Recommendation <sup>(18)</sup>	60.00–120.00	90.00–90.00	–	45	60.00–120.00	90.00–190.00	–	45
Mean	32.59	43.74	0.30	40.99	50.99*	79.16*	0.40*	48.91*
SD	21.99	23.02	0.14	11.23	21.07	46.38	0.13	15.50
Below normality (%)	89.3	96.3	–	61.5	75.9	67.9	–	37.0
Normality (%)	10.7	3.7	–	38.5	24.1	25.0	–	63.0
Above normality (%)	0	0	–	0	0	7.1	–	0

\*  $P < 0.005$ .

Higher Se intake was observed in the C group, and it was adequate in 38.5% of the AD group and in 63% of the C group (Table 1).

## Discussion

Deficient Se intake was observed in the most of the AD patients, although the dietary reference intake recommendation was only partially achieved by the C group. Since Se content of foods is largely related to the Se content of soil, it is important to reveal that in São Paulo state there is low Se concentration in the soil, and thus high deficiency of this mineral is observed in this population<sup>(16)</sup>.

Assessment of Se intake from the diet has many difficulties, namely the absence of specific food composition tables for this trace element and Se variation in different regions<sup>(17–19)</sup>. This is probably the main reason for the lack of studies describing in detail the Se intake in AD patients.

Assessment of Se levels in blood is a useful biomarker of Se status. Plasma is a marker of current exposure, while erythrocytes reflect longer-term nutritional status, due to its incorporation in erythrocyte synthesis, which have a half-life of 120d<sup>(20,21)</sup>. Nail clippings are considered a superior marker of Se status because they provide a time-integrated measure of exposure of up to a year<sup>(22)</sup>. Some authors suggest that Se status should be assessed by determination of two or more biomarkers, in order to avoid possible misunderstandings arising from the hierarchy of importance of selenoproteins<sup>(20,23)</sup>. According to these authors, our assessment of Se status covered different periods of exposure, allowing us to verify Se intake in different periods.

There is a lack of studies correlating Se status and AD, and most of them only assessed Se levels in plasma or whole blood. The studies about Se in AD patients are inconclusive. Ceballos-Picot *et al.*<sup>(24)</sup> observed higher Se plasma and erythrocyte levels in AD patients when compared with the control group. However, Smorgon *et al.*<sup>(25)</sup> assessed the association between micronutrients and cognitive function, and observed that AD patients showed lower plasma Se when compared with control subjects. Other studies noticed that Se levels were positively correlated with cognitive function in elderly subjects, and thus Se deficiency could be a risk factor for AD<sup>(26–28)</sup>. Although the average plasma and erythrocyte Se concentrations in the AD and C groups were below the recommended levels, individual values were significantly different in each group, revealing that AD has an important role in Se deficiency. Confirming these data, Se level in nails was significantly lower in the AD patients when compared with control subjects.

Some studies have shown that Se status decreases slightly in elderly compared with younger adults<sup>(29–31)</sup>. It can reflect lower bioavailability, increased requirements, metabolic changes, or a diet limited in energy which may not be sufficiently nutrient dense to provide adequate levels of micronutrients<sup>(30,32)</sup>. In the present study, no significant correlation between Se parameters and age was observed. However, the age difference between the AD and C groups could be a bias in the present study, since nutritional status of Se can decrease with age. Indeed, the present study has a small sample size, and this can limit the interpretation of our data.

The brain is the last organ to be depleted in Se deficiency and, in repletion, it is the first one to establish adequate levels of Se. This preferential treatment suggests the importance of this antioxidant mineral in brain functioning, since it is a main constituent of selenoproteins<sup>(33)</sup>. Glutathione peroxidase is an essential line of defence against free radicals acting against hydrogen peroxide and lipid peroxidation, protecting the brain against oxidative stress, which has a central role in AD. Besides, selenoprotein P, which is synthesised at the cerebral level and protects the brain against oxidative damage, is the most important selenoprotein for cerebral functions<sup>(34,35)</sup>. Thus, Se may affect the rate of disease via protection against ROS, directly as an antioxidant or indirectly by improving metabolism<sup>(36)</sup>.

Oxidative stress has an important role in AD aetiology, and it is the earliest event preceding this disease, although some structures formed in AD are related to formation of ROS<sup>(37)</sup>. Thus, the relationship between Se and AD could have two meanings: Se could be depleted owing to the oxidation that accompanies ageing and AD progression; and change in Se levels could be the first event in relation to dietary intake<sup>(38)</sup>. However, as we did not evaluate oxidative stress in the present patients, we cannot establish direct association between Se deficiency and oxidative stress in AD patients. In this way, more studies with larger population are needed in order to find the exactly relationship between Se and AD, since a limitation of the present study was the small number of participants.

## Conclusions

AD patients showed lower Se levels, although a Se deficiency was also observed in the C group. Thus, we can suggest that the oxidative stress present in AD has an association with Se deficiency observed in the present AD patients. In order to overcome the problem of Se deficiency in AD patients, new strategies should be developed.

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