Serum concentrations of zinc and selenium in elderly people: results in healthy nonagenarians/centenarians

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Abstract

Trace elements such as zinc (Zn) and selenium (Se) play an important role in maintaining the metabolic homeostasis in elderly people and the risk of deficiency seems to increase in proportion to the age. Zn and Se concentrations, as indices of the micronutrient status in healthy subjects over 90 years, are scarcely analyzed and could represent a model for studying the physiology of successful aging. Our aim was to investigate Zn and Se concentrations in the healthy persons over the age of 90 years. One hundred and fifty two subjects volunteered for the study. They were divided into two groups: 90 non-institutionalized nonagenarians/centenarians (91–110 years) (group A) and 62 elderly subjects (60–90 years) used for comparison (group B). Serum concentrations of Zn and Se were determined, respectively, by flame atomic absorption spectrophotometry (FAAS) and electrothermal atomic absorption spectrophotometry (ETAAS). The effect of age and sex on ion concentrations was investigated. Mean values ± standard deviation of Zn and Se concentrations in the group A were 11.97 ± 2.00 and 0.87 ± 0.28 μmol/l, respectively. A significant decrease of Se and Zn values was demonstrated in group A, when compared with group B, in both males and females. However, 84.4% of the ‘healthy’ nonagenarians/centenarians had both Zn and Se concentrations equal to or greater than the lowest values of the elderly group and only 3.3% of cases showed both Zn and Se deficiencies. Consequently, a prospective and follow-up evaluation of Zn and Se could be proposed as a good index for a correct monitoring of the micronutrient deficiencies, that could represent an early sign of disease. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Zinc; Selenium; Aging; Nonagenarians; Centenarians; Antioxidant

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1. Introduction

Vitamins, minerals, and trace elements such as Zn and Se, do play an important role in maintaining the metabolic homeostasis in elderly people as well as in the prevention of many age-associated diseases (Navarro-Alarcon et al., 1998; Schmidt, 1991) and in maintenance of normal immune function, too (Pike and Chandra, 1995; Shankar and Prasad, 1998). Zn and Se are potent antioxidants involved in cellular defense against oxygen free radicals and the risk of deficiency seems to increase in proportion to the age. Evidence is accumulating that most of the degenerative diseases have their origin in deleterious free radical reactions. These diseases include atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia and degenerative eye disease (Florence, 1995; Preziosi et al., 1998; Galan et al., 1997; Bonithon-Kopp et al., 1997; Magalova et al., 1997).

The process of biological aging also might have a free radical basis. Most free radical damage to cells involves oxygen radicals or, more generally, activated oxygen species (AOS) which include non-radical species such as singlet oxygen and hydrogen peroxide as well as free radicals. The AOS can damage genetic material, cause lipid peroxidation in cell membranes, and inactivate membrane-bound enzymes. Humans are well endowed with antioxidant defenses against AOS. The antioxidants, including trace elements, as Zn, Cu and Se, inhibit the oxidation of membrane fat polyunsaturated acids and DNA by oxygen radicals produced during aerobic metabolism (Florence, 1995).

Selenium is an important trace element; as a selenocysteine residue, the best known biochemical role of Se is to be a part of the active site of the glutathione peroxidase antioxidant enzyme (GSH-Px). It has been demonstrated that Se is a modulator of the response to oxidative stress and a Se supplementation induced a faster restoration of the endogenous antioxidative defense system against the production of reactive oxygen species (Jozanov-Stankov et al., 1998).

Zinc is considered a major trace element for the correct functioning of an organism; it is a structural constituent of many proteins, hormones and hormone receptors, and it has a fundamental role in biomembrane function, many enzymatic activities, cell division and differentiation, programmed cell death and gene transcription. Zn also functions as an antioxidant: it is a constituent of the antioxidant enzyme superoxide dismutase (Shankar and Prasad, 1998) and maintains the physiological values of metallothioneins, which also are antioxidants. Furthermore, Zn prevents the reactions between thiols and iron, which give rise to free radicals, and is also an essential constituent of the nucleic acid-repairing enzymes and a stabilizing factor for biomembranes.

The correlation between Zn, Se and aging is still poorly investigated; in particular, the subjects with age superior to 90 years have been scarcely analyzed and often the protocols do not classify patients according to the pathologies and socio-environmental conditions. The aim of our study was to estimate serum concentrations of Zn and Se in a population of ‘healthy’ nonagenarians/centenarians; following the identification of the normal reference ranges in these subjects, better assessment of the aging process and correct monitoring of the micronutrient deficiencies in this age group become easier.
2. Materials and methods

All testing procedures used in our Laboratory are validated according to the quality assurance criteria in the EU accepted standard for the operation of testing laboratories\(^1\) and in conformity with Good Laboratory Practices.\(^2\) The study was performed on coded samples, so that the examiner did not know the source of the sample.

2.1. Subjects

Samples from 90 subjects (53 females and 37 males) aged 91–110 years were collected (group A). For comparison, 62 subjects (16 females and 46 males) aged 60–90 were enrolled as controls (group B). The age distribution within these groups ranged respectively from 91 to 110 years (mean ± SD = 96.3 ± 5.4 years, median value = 97.0 years) and from 60 to 90 (mean ± SD = 71.2 ± 9.2 years, median value = 70.0 years). Table 1 gives more detailed information about elderly and old-oldest populations, split by 10 years intervals.

All clinical investigations have been conducted in accordance with the Declaration of Helsinki. Subjects were selected as reported in a previous research (Ravaglia et al., 1997) following the suggestions of the SENIEUR protocol admission criteria (Ligthart et al., 1984). This protocol, usually applied to immuno-gerontological studies, gives a series of exclusion criteria concerning clinical, laboratory and pharmacological data: patients suffering from severe chronic or acute diseases, or the alteration of hematological and renal function parameters were not enrolled.

Clinical criteria included no current infection (from six weeks), no current inflammation (from six weeks), no malignancy (either past or present), no vaccination in the six weeks preceding the examination and no conditions influencing the immune system, such as malnutrition (Body Mass Index), alcoholism and drug abuse.

Laboratory data performed following Senieur protocol are reported in Table 2, as well

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\(^1\) European Committee for Standardization. General Criteria for the operation of testing laboratories. EN 45001, 1989.

<table>
<thead>
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<th></th>
<th>Females</th>
<th>Males</th>
<th>Normal values (units)</th>
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<td>Leucocytes</td>
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<td>Alkaline phosphatase</td>
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<td>Albumin</td>
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Table 2
Haematology and serum values performed following the Senieur protocol, as well as albumin serum levels. All the values are expressed as mean ± SD (median value). Results concern the oldest old subjects ranging from 91 to 110 years (group A) and the elderly subjects ranging from 60 to 90 years (group B), divided by gender.
as serum values that allowed to exclude malnutrition. Urine analysis was negative for glucose and proteins, with rare leucocytes and casts.

A screening was performed to detect possible alterations which might interfere with trace element status: participants were asked to report all prescription and non-prescription drugs currently used (receiving or non-receiving), including vitamins and oligoelement supplements, alcohol and smoking history (ex-smokers, non-smokers, or current smoker). Concerning alcohol intake, because of the peculiar features of this very old population, the scale was dichotomized as ‘drinking’ or ‘not drinking wine at meals’, no subject being a current or ex heavy drinker.

Subjects receiving corticosteroids, non-steroidal antiinflammatory drugs (NSAIDs) or anticoagulants were excluded. Subjects that used Zn and Se supplements were also excluded.

2.2. Metal ion analysis

Whole peripheral blood was collected and centrifuged at 400 g to separate the serum. Polystyrene, polypropylene or polyethylene disposable material was used after rinsing with 2% HNO₃ and bidistilled/deionized water; moreover, a careful examination of the impurity in the labwares and reagents was performed by cession tests.

Zn content was investigated by employing a flame atomic absorption spectrometer (Pye Unicam, PU9400) with an air/acetylene flame (fuel flow rate of 0.9 l/min) and a hollow cathode lamp. Lamp current: 5.0 mA, bandpass: 0.5 nm and wavelength (λ): 213.9 nm were employed as spectrometer parameters.

A linear calibration curve was performed by using certified standard solutions (NIST) at three concentrations (1.5, 3.0 and 4.6 μmol/l). Specimens were diluted 1:5 with 5% glycerol ultrapure in double distilled and deionized water and analyzed in duplicate. Seronorm Trace Elements (Nycomed) and Serum Trace Elements Control Toxicology, Normal Range, (Utak Laboratories, Inc.), were used as controls for validating method accuracy and precision. All the results were expressed as μmol/l. The analytical Detection Limit was 0.15 μmol/l.

Serum samples were analyzed for Se content using a Graphite Furnace Atomic Absorption Spectrometer (GFAAS) with Zeeman background correction, autosampler and pyrolytic carbon-coated graphite tubes (Unicam Model Solaar 939 QZ, Cambridge, UK). Slit: 0.5 and wavelength (λ): 196.0 nm were used as spectrometer parameters.

Furnace thermal program comprised two drying stages, respectively of 20 s at 80°C, without ramp, and 30 s at 120°C, with a 10 s ramp; an ashing stage, an atomization stage and a tube clean step. 30 s at 1200°C were chosen for ashing and 3 s at 2100°C for atomization; read and temperature control were performed at this time; the last stage consisted of tube clean of 3 s at 2600°C.

Specimens were diluted 1:10 with 0.1% HNO₃ and 0.05% Triton X100 (diluent) and analyzed as 15 μl aliquots in triplicate. Palladium nitrate (2 g/l of diluent) as matrix modifier was added. A calibration curve type ‘Standard curve, linear least square fit’ was performed by employing a bovine serum at low Se concentration and certified standard solutions at concentrations of 0.63 and 1.27 μmol/l (NIST).

Certified bovine serum SRM 1598 (Office of Reference Materials-Lab., Government
Chemist, UK) and Serum trace elements toxicology controls, Normal and High Range, (Utak Laboratories, Inc.) were used in order to validate method accuracy and precision. The test repeatability was assured by rejecting the results if the percentage of Related Deviation Standard (RDS%) was more than 10%. Dixon test was used to eliminate aberrant values. All the results were expressed as $\text{mmol/l}$. The analytical Detection Limit was 0.09 $\text{mmol/l}$ and was used to establish the sensitivity of the method for sample matrix.

2.3. Statistics

Ion concentration was expressed as arithmetic mean plus and minus standard deviation of the mean ($\text{mean} \pm \text{SD}$). Specific differences between the groups were analyzed by applying the Student’s t test. The effect of drugs, alcohol consumption and smoke on ion concentrations was evaluated by using the analysis of variance (ANOVA). The Bonferroni–Dunn’s multiple comparison test was applied to detect specific differences groups.

The correlation between ion concentration and age was calculated using the Fisher’s $r$ coefficient. In all analyses $p < 0.05$ was considered as statistically significant.

Data were analyzed employing the StatView 4.5™ software for Macintosh (Abacus Concepts).

3. Results

Average serum Zn and Se concentrations plus and minus standard deviations ($\text{mean} \pm \text{SD}$) and median values are reported in Table 3.

Serum samples of group A had a mean Zn concentration of 11.97 ± 2.00 μmol/l (median value 12.08 μmol/l), while group B had a mean concentration of 14.51 ± 2.07 μmol/l (median value 14.53 μmol/l).

<table>
<thead>
<tr>
<th>Zn concentration ($\mu$mol/l)</th>
<th>Group A (#90) mean ± SD (median value)</th>
<th>Group B (#62) mean ± SD (median value)</th>
<th>$P$ value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative results</td>
<td>11.97 ± 2.00 (12.08)</td>
<td>14.51 ± 2.07 (14.53)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Females</td>
<td>12.07 ± 1.50 (12.16)</td>
<td>14.61 ± 2.49 (14.61)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Males</td>
<td>11.81 ± 2.58 (11.93)</td>
<td>14.47 ± 1.93 (14.07)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

$^a$ $P$ value $= \text{groups A vs B}$. The $P$ value $<0.05$ was considered statistically significant.
Table 4
Descriptive statistics (mean ± SD and median value) of Zn and Se values: subjects of group A (91–110 years) and group B (60–90 years) have been split by 10 years intervals

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
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<tbody>
<tr>
<td></td>
<td>91-100 years</td>
<td>101-110 years</td>
</tr>
<tr>
<td><strong>Zn concentration (μmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative results</td>
<td>11.8 ± 1.9 (12.1)</td>
<td>12.1 ± 2.0 (11.9)</td>
</tr>
<tr>
<td>Females</td>
<td>11.9 ± 1.7 (12.1)</td>
<td>12.2 ± 1.3 (12.5)</td>
</tr>
<tr>
<td>Males</td>
<td>11.6 ± 2.4 (12.1)</td>
<td>12.1 ± 2.8 (11.8)</td>
</tr>
<tr>
<td><strong>Se concentration (μmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative results</td>
<td>0.90 ± 0.29 (0.89)</td>
<td>0.84 ± 0.27 (0.83)</td>
</tr>
<tr>
<td>Females</td>
<td>0.93 ± 0.33 (0.90)</td>
<td>0.86 ± 0.22 (0.84)</td>
</tr>
<tr>
<td>Males</td>
<td>0.87 ± 0.23 (0.83)</td>
<td>0.82 ± 0.34 (0.83)</td>
</tr>
</tbody>
</table>
Se concentration of nonagenarians/centenarians was 0.87 ± 0.28 μmol/l (median value 0.87 μmol/l), while elderly subjects had 1.14 ± 0.22 μmol/l (median value 1.12 μmol/l).

In our study a significant decrease of Se and Zn serum values in nonagenarians/centenarians (group A), if compared with values of the elderly group (B) was observed. The lowest concentration of ions measured in the serum of elderly subjects (group B) was designated as the limit in the elderly population (Zn value: 9.63 μmol/l; Se value: 0.5 μmol/l): deficiency was defined as serum nutrient concentration below such lower limit.

Six out of subjects (6.7%) showed Zn deficiency and normal Se concentration, five subjects (5.5%) showed Se deficiency and normal Zn concentration, and only three (3.3%) had deficiency of both ions. The Zn and Se values of the other subjects (84.5%) were included in the elderly subject range.

When the subjects were split by gender and tested for differences using the Student’s t test, no significant difference was found, both in group A (Zn P value = 0.54; Se P value = 0.39) or group B (Zn P value = 0.81; Se P value = 0.14).

Consequently, the statistical difference concerning Zn and Se values between the groups A and B were maintained in both male and female subpopulations.

Table 4 shows the descriptive statistics (mean ± standard deviation and median value) of the investigated subjects divided by 10 year intervals.

The Bonferroni–Dunn’s multiple comparison test applied to such groups indicated no significant difference between 60–70, 71–80, 81–90 year groups concerning Zn and Se values, while significant differences were found between these groups and the groups of nonagenarians and centenarians (p < 0.005).

As indicated in Table 5, by applying the Fisher test, a highly significant positive correlation between the Zn and Se values was observed, in groups A and B.

Table 5
Fisher’s to z coefficient: the correlation was performed between Zn and Se values (cumulative, split by age and by age, gender); Zn values and years (cumulative and split by gender) Se values and years (cumulative and split by gender). P value <0.05 was considered statistically significant (group A: 91–110 years; group B: 60–90 years).

| Zn, Se (cumulative correlation) (#152) | r = 0.51 | P value <0.001 |
| Zn, Se (females) (#69) | r = 0.42 | P value <0.001 |
| Zn, Se (males) (#83) | r = 0.56 | P value <0.001 |
| Zn, Se (group A) (#90) | r = 0.42 | P value <0.001 |
| Zn, Se (group A: females) (#53) | r = 0.37 | P value <0.01 |
| Zn, Se (group A: males) (#37) | r = 0.48 | P value <0.01 |
| Zn, Se (group B) (#62) | r = 0.27 | P value <0.05 |
| Zn, Se (group B: females) (#16) | r = 0.33 | P value n.s. |
| Zn, Se (group B: males) (#46) | r = 0.26 | P value n.s. |
| Zinc, Age (#152) | r = −0.50 | P value <0.001 |
| Zinc, Age (females) (#69) | r = −0.43 | P value <0.001 |
| Zinc, Age (males) (#83) | r = −0.52 | P value <0.001 |
| Selenium, Age (#152) | r = −0.46 | P value <0.001 |
| Selenium, Age (females) (#69) | r = −0.26 | P value <0.05 |
| Selenium, Age (males) (#83) | r = −0.55 | P value <0.001 |

As indicated in Table 5, by applying the Fisher test, a highly significant positive correlation between the Zn and Se values was observed, in groups A and B.
The cumulative correlation was also significant ($r = 0.51; \ P$ value <0.001), while the correlation was not present if the group B was split by gender (Table 5), probably due to the number of subjects. Using the Fisher test, also a highly significant inverse correlation between both Zn and Se values and the age of the subjects was demonstrated; this correlation remained if the subjects were divided by gender.

Fig. 1 shows the corresponding regression plots; moreover, the single data points (subjects) are shown.

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Drugs, alcohol use and smoking history did not significantly affect these findings ($p$ value >0.05), when the analysis of variance (ANOVA) and the Bonferroni–Dunn’s multiple comparison test were applied.
4. Discussion

Aging process is widely debated. One of the prevailing hypotheses states that senescence may result from the accumulation of oxidative damage and that dietary antioxidants may slow the degenerative process because certain age-related degenerative changes were found to be reversed by antioxidant treatment (Hack et al., 1998; Harman, 1956; Stadtman, 1992; Shigenaga et al., 1994; Gilchrest and Bohr, 1997; Manton et al., 1997).

In particular, there is growing interest in the possible relationship between Zn and Se metabolism, oxidative stress and cellular aging. Zinc and selenium are involved in many biochemical processes supporting life. The most important of these processes are cellular respiration, DNA and RNA reproduction, maintenance of cell membrane integrity, and sequestration of free radicals. Zinc and selenium are involved in destruction of free radicals through cascading enzyme systems. Superoxide radicals are reduced to hydrogen peroxide by superoxide dismutases in the presence of zinc cofactors. Hydrogen peroxide is then reduced to water by the selenium–glutathione peroxidase couple. Efficient removal of these superoxide free radicals maintains the integrity of membranes, reduces the risk of cancer, and slows the aging process. Trace element-deficient patients usually present with common symptoms such as malaise, anemia, infection, skin lesions, and low-grade neuropathy (Chan et al., 1998).

Theoretically, zinc and selenium can exert a number of indirect antioxidant functions. A lot of researchers searched evidence to support this concept by studying Zn and Se deficiency in relation to disease and some studies showed that they might have a preventive role in some degenerative diseases (Di Silvestro, 2000; Huang et al., 2000; Powell, 2000; Simonoff et al., 1992).

Moreover, the efficacy of an intake of zinc and selenium on markers of the antioxidant system has been demonstrated (Preziosi et al., 1998), as well as the crucial role of a supplementation with micronutrients in the maintenance of normal immune function in the elderly (Pike and Chandra, 1995).

Nevertheless, information on the influence of zinc and selenium on cell functions in aging is contradictory and the most of the experimental studies have not been able to decisively determine how such influence is established in the degenerative diseases associated with old age (Gamez et al., 1997).

For this reason more research is needed in this area and the healthy elderly subjects over 90 years could represent an experimental model quite good for studying the physiology of aging: these subjects have reached the old-oldest age free from pathologies and are an example of successful aging. Yet, the few data available in the oldest old subjects are not sufficient to quantify micronutrient requirements needed to protect against oxidative damage and, consequently, to reduce the incidence of chronic diseases. So, aim of our research was to analyze the trace element status in the oldest old subjects in order to obtain a better knowledge of the aging process.

We performed the evaluation of Zn and Se serum levels in a large population of healthy non-institutionalized nonagenarians/centenarians (group A) and in elderly subjects of inferior age (group B).

We found a significant difference between the concentrations of both ions in the populations A and B and a significant inverse correlation of these values with the age,
both in men and in women. Moreover, a positive significant correlation was found between the serum concentrations of Zn and Se.

To our knowledge, serum reference values of the essential trace element status in the healthy nonagenarians and centenarians have not been reported, and other studies have analyzed only institutionalized or younger subjects (Madaric et al., 1994; Monget et al., 1996; Ortuno et al., 1997; Rukgauer et al., 1997; Artacho et al., 1997; Malvy et al., 1993; Coudray et al., 1997; Licastro et al., 1995; Rink and Seyfarth, 1997); consequently a comparison to the literature is not possible. However, our findings, though in different populations, are in agreement with other results: some researchers found that Zn and Se concentrations were significantly negatively correlated with age in institutionalized subjects (Monget et al., 1996), or in men aged ≤89 years (Madaric et al., 1994). Some studies reported the serum concentrations of Zn and Se in 93 institutionalized elderly people 63–97 years and did not show any significant difference in the ion serum levels as regards the sex of the subjects (Gamez et al., 1997; Artacho et al., 1997).

The results obtained in the present study lead us to suggest an observation: if we considered the lower limits of Zn and Se ranges in the 60–90 years subjects as deficiency values for the aged population, 84.5% of the patients had values equal or superior to such values, whereas only 6.7% of cases had Zn deficiency, 5.5% Se deficiency and 3.3% had both Zn and Se deficiency. Bearing in mind that the nonagenarians/centenarians were healthy subjects and the proportion of cases with ion deficiency was relatively scarce, we hypothesized that the normal ion concentrations that we found in a high number of subjects could contribute to their longevity. This assumption could support the statement of Paolisso et al. (1998), who demonstrated a lower degree of oxidative stress in healthy centenarians than in aged subjects.

In conclusion, a prospective and follow-up evaluation of Zn and Se levels could be proposed as a good index in the monitoring of aging pathologies, allowing to detect significant micronutrient deficiencies that could represent an early sign of disease. On the contrary, a normal micronutrient status could represent a biological characteristic favoring an healthy aging.

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References


