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Dyshomeostasis of Serum Oxidant/Antioxidant Status and Copper, Zinc, and Selenium Levels in Elderly Physically Disabled Persons: an AHAP-Based Study

Simin Younesi · Hadi Parsian · Seyed Reza Hosseini · Hajighorban Noreddini · Abbas Mosapour · Ali Bijani · Sohrab Halalkhor

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Abstract The percentage of elderly persons is rapidly growing. Physical disability is one of the main age-related diseases which affect life quality. There are some studies that suggest the oxidative stress and trace elements are involved in physical disability in elderly persons, but the results are inconclusive. Therefore, the aim of this study was to investigate the status of aforementioned parameters in elderly physically disabled patients vs. healthy ones. According to the Katz questionnaire form, 44 subjects with physical disability and 66 age-gender-matched healthy subjects were selected from Amirkola Health and Aging Project (AHAP). The results indicated that patient group had lower serum Zn, Se, and total antioxidant levels than the control group ($p<0.001$), whereas serum total oxidant level and Cu to Zn ratio (CZr) were higher in control group than in healthy one ($p<0.001$). A positive correlation was found between Zn, Se, total antioxidant, and bone mineral density of femur (BMD.F) with activities of daily living (ADL) score ($p<0.01$); meanwhile, a negative correlation between CZr and total oxidant with ADL score was observed ($p<0.01$). Serum total oxidant level and CZr index had the highest area under the curve in receiver operating characteristic (ROC) analysis among the included parameters for discrimination of physically disabled patients than the normal ones. Decrease in serum Zn and Se levels, low BMD, and increase in CZr and oxidative stress were observed in physically disabled patients. It seems that CZr is more reliable parameter than the others to discriminate the physically disabled patients than the healthy persons.

Keywords Aging · Physical disability · Trace element · Oxidative stress · Bone mineral density

S. Younesi · A. Mosapour · S. Halalkhor
Department of Biochemistry and Biophysics,
Babol University of Medical Sciences, Babol, Iran

H. Parsian (✉) · S. R. Hosseini · A. Bijani
Social Determinant of Health Research Center,
Babol University of Medical Sciences, Ganjafrooz Ave, Babol, Iran
e-mail: hadiparsian@yahoo.com

H. Noreddini
Department of Internal Medicine, Ayatollah Rouhani Hospital,
Babol University of Medical Sciences, Babol, Iran

Introduction

The proportion of aged people around the world is growing rapidly due to dramatic increase in life expectancy, which is inevitable and will affect worldwide [1, 2]. Aging is a process that eventually leads to disability and death [3]. Physical function measurements in older subjects are useful to predict the physical disability [4] defined as inability to perform activities of daily living such as bathing, dressing, transferring, etc. [5]. Serum trace element level is important in human metabolism [6]. It has been reported that low serum level of copper and zinc probably leads to physical disability [7]. Zinc and copper are elements in the structure of metalloproteins and metalloenzymes [8]. Superoxide dismutase (SOD) enzyme is able to decrease the free radical levels through cooperating with the other enzymes in a cascade model and can also lead to a delay in the aging process [9]. Superoxide radicals are involved in the metabolism of many toxic agents, and superoxide dismutase converts them into hydrogen peroxide and further degradation of water and oxygen can be catalyzed by catalase [10]. Selenium as a selenocysteine residue is a necessary component in the structure of glutathione peroxidase enzyme (GSH-Px), so Se deficiency may lead to the decrease of the activity of this enzyme as an antioxidant [11]. Zn, Cu, and Se as the components of antioxidants prevent the oxidation of lipids and DNA through oxygen radicals which are generated from aerobic metabolism [9]. Oxidative stress is defined as imbalance of oxidant and antioxidants status [12], and this condition damages to the macromolecules such as DNA and proteins and leads to the decrease of muscle strength [13]. It has been suggested that zinc and selenium as a part of antioxidants defense system are decreased by increasing age [9]. It seems that trace element and antioxidants are necessary to develop the skeleton, and their decline lead to osteoporosis via reducing bone mineral density (BMD) [14–16] which probably increases the physical disability. According to our knowledge, there is no report on the status of oxidant/antioxidant, copper, zinc, and selenium in physically disabled persons in our area. Therefore, we aimed to assess these important biochemical factors in elderly patients with physical disability and compare their levels to those of healthy old control group. In addition, we considered the relationship between BMD and physical disability in these subjects.

Materials and Methods

Among 1616 elderly participants in the Amirkola Health and Aging Project (AHAP) cohort [2], according to the Katz activities of daily living (ADL) questionnaire form [17], 44 subjects had physical disability. We randomly selected 66 age-gender-matched subjects without physical disability as the control group; meanwhile, the population had not taken any drugs which affected the studied parameters. In the AHAP [2], fasting blood samples were collected from all participants. Blood samples were immediately centrifuged, and the resulted serum was aliquoted in 0.2-ml microtubes and stored at −80 °C until the final analysis.

ADL Scoring System

ADL scoring system was performed using Katz questionnaire form [17] consisting of six questions with two options. The questions included activities such as walking, bathing, dressing, feeding, transferring, and toileting. The options for each activity and corresponding points were considered independence (1 point) and dependence (0 point). If a subject performed an activity with the help of others, he/she was considered as a physically disabled person.

Analysis of the Biochemical Parameters

Serum antioxidant and oxidant levels were measured using ferric reducing ability of plasma (FRAP) and thiobarbituric acid-reactive substance (TBARS) tests by spectrophotometer (UNICO, USA) [18, 19]. Briefly, in the FRAP test, reduction of ferric to ferrous ions in the presence of antioxidants in low pH causes the formation of colored complex of ferrous-tripyridyl-S-triazine (Fe(III)-TPTZ) and a blue solution is generated with a maximum absorbance at 593 nm [18]. In this method, 1.5 ml of FRAP reagent (include buffer acetate pH 3.6, TPTZ, and $FeCl_3$ by 10:1:1 ratio, respectively) was incubated at 37 °C for 5 min in a water bath and then 50 μl of serum samples was added to the tubes and incubated again at 37 °C for 15 min. For determination of FRAP standard curve as shown in Fig. 1, $FeSO_4$ in various concentrations (125, 250, 500, and 1000 μM) was incubated at 37 °C for 15 min and the absorbance was taken at 593 nm. Using the standard curve, FRAP serum levels were reported as micromoles per liter. For analysis, the oxidant status, the reaction of malondialdehyde (MDA) with two molecules of thiobarbituric acid (TBA), and the elimination of two molecules of water lead to the formation of a pink complex with the maximum absorbance at 532 nm [19]. In this method, 0.375 % TBA (Merck, Germany) and 15 % trichloroacetic acid (TCA, Merck, Germany) were added to 0.25 N HCl (Sigma-Aldrich, USA) to make the solution of TCA-TBA-HCl. Then, 2 ml of TCA-TBA-HCl and 0.5 ml of serum samples were mixed and incubated at 100 °C for 15 min in water bath. The samples were centrifuged at 1500 rpm for 10 min, and the absorbance was read at 532 nm. The TBARS serum levels were reported as micromoles per liter. Serum Zn level was measured using zinc kit (ZishChem Diagnostics, Iran, detection limit 400 μg/dl, sensitivity 4.0 μg/dl, accuracy r=0.970). Available Zn in the serum samples was chelated by 5-Br-PAPS (2-(5-bromo-2-pyridylazo)-5-(*N*-*n*-propyl-*N*-3-sulfopropyl-amino) phenol) and formed colored complex with the color intensity proportional to the amount of zinc. Sample and standard compounds were prepared according to the manufacturer instruction and incubated for 5 min at 37 °C. The absorbance (A) of sample and standard compounds was measured (spectrophotometer, UNICO, USA) at wavelength of 562 nm and corresponding serum Zn levels for the subjects were reported as micrograms per deciliter (Zinc (μg/dl) = $(A_{Sample}/A_{Standard}) \times$ Standard Concentration). The serum Cu and Se levels were measured using atomic absorption spectrophotometer (AAS) (PG-990,

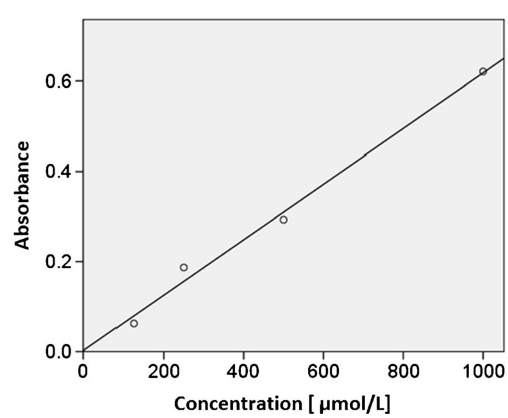

Fig. 1 FRAP standard curve for $FeSO_4$ concentration (μmol/l) (r= 0.991)

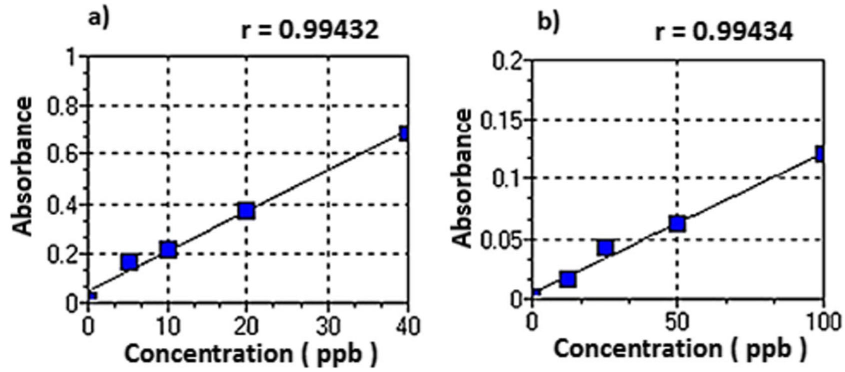

Fig. 2 Standard curves for **a** Cu and **b** Se using AAS (ppb)

China) equipped with a graphite furnace. The operating parameters were set for measuring serum Cu and Se levels as recommended by manufacturer (wavelength 324.7 and 196 nm, bandwidth 0.4 and 0.4 nm, lamp current 3 and 5 mA, respectively for Cu and Se). $CuSO_4$ (Merck, Germany) and SeO_2 (Merck, Germany) were used to prepare Cu and Se standard solutions. By using 0.1 M HNO_3 (Merck, Germany), various concentrations of the Cu and Se were prepared and the related standard curves were plotted (Fig. 2). For measuring the Cu and Se levels in samples, serums were diluted with 0.1 M HNO_3 by 1:20 and 1:2 ratios (respectively for Cu and Se). Then, 10 μl of the diluted samples was injected into the graphite furnace and concentration of the samples was measured as ppm.

Determination of BMD

BMD of the patient and control groups was measured using dual energy X-ray absorptiometry (DEXA) with the Lexxos densitometer. The diagnosis of osteopenia and osteoporosis was performed using spine and femur BMD measurements according to the WHO criteria [20] in which T-score≤−2.5 SD was considered as osteoporosis, −2.5<T-score≤−1.0 SD as osteopenia, and T-score>−1.0 SD as normal.

Statistical Analysis

All statistical analyses were conducted using SPSS version 18.0. Descriptive analyses were done to provide information on general characteristics of the studied population. Independent-samples *t* test or Mann–Whitney *U* test was used to compare mean of all variables between control and patient groups. The data were presented as mean±SD, and statistical significance was defined as $p<0.05$. The correlation between the variables was evaluated by Pearson's correlation test, and corresponding graphs were plotted using matrix scatter plot. We used the previous published index, i.e., Cu to Zn ratio (CZr) for discrimination of the physically disabled patients than the healthy ones. Receiver operating characteristic (ROC) analysis was used to compare the diagnostic power of the parameters in probable discrimination of the elderly patients than the healthy ones, and the area under the curve (AUC) of each parameter was determined.

Results

The demographic and clinical characteristics of the control and patient group are shown in Table 1. The mean age of the control and patient groups was approximately the same, and there were no significant differences between the groups ($p>0.05$). Mean±SD of serum trace element and oxidative stress levels of studied persons are presented in Table 2. As it is clear, the CZr index and serum oxidants levels in patient group were statistically higher than those in control ($p<0.001$), whereas serum Zn, Se, and total antioxidant levels were statistically lower in the patient than in the control group ($p<0.001$). Serum Cu level in crude data was higher in the patient group in comparison with the healthy one, but the

Table 1 The demographic and clinical characteristics of the study population

	Control group (*n*=66)	Patient group (*n*=44)	*p* values
Age (year)	77.1±8.6	76.6±9.2	0.932
Gender F/M	36/30	29/15	0.235
Married	48 (18)	35 (9)	0.687
Educated	17 (49)	5 (39)	0.180
ADL score	14±0	9.9±3.8	<0.001
Femur BMD (T-score)	0.74±0.11	0.65±0.14	0.032
Spine BMD (T-score)	0.78±0.16	0.80±0.20	0.189
BMI (kg/m^2)	45.3±6.1	26.1±4.2	0.143
Smoker	8 (58)	4 (40)	0.760
Hypertension	51 (15)	30 (14)	0.289
Diabetes	26 (40)	15 (29)	0.573

BMD bone mineral density, *BMI* body mass index

Table 2 Comparison of the analyzed biochemical parameters in physically disabled patients and healthy person

	Control group (n=66)	Patient group (n=44)	Mean difference (%)	p values
Cu (μg/dl)	151.9±54.1	168.2±64.1	10.8	0.277
Zn (μg/dl)	128.1±42.8	67.2±18.6	−47.5	<0.001
Se (μg/l)	70.6±23.2	52.6±12.8	−25.5	<0.001
CZr	1.3±0.6	2.8±1.5	112.5	<0.001
TBARS (μmol/l)	4.8±1.7	10.4±2.7	117.3	<0.001
FRAP (μmol/l)×10^3	1.7±0.3	0.5±0.2	−69.9	<0.001

TBARS status of total oxidant, *CZr* Cu to Zn ratio, *FRAP* status of total antioxidant

difference was not statistically significant (p>0.05). The results of correlation analysis between parameters and ADL score are shown in Figs. 3 and 4. There was a positive correlation between Zn, Se, total antioxidant, and bone mineral density of femur (BMD.F) with ADL score (p<0.01) and a negative correlation between total oxidant and ADL score (p<0.01). ROC curve analysis is shown in Fig. 5, and related data are summarized in Table 3. Total oxidant and CZr had the highest AUC for discrimination of patient with physical disability than control group (AUC=0.979 and 0.832, respectively).

Discussion

We found that serum Zn level was 47.5 % lower in patient group compared to control group. Serum Zn level deficiency is often associated with the increase of the serum Cu level and with aging [8]. Modulators and compensatory mechanisms in the body affect the serum level of Cu and Zn, and these compensatory factors lead to a decrease in serum Zn level and an increase in serum Cu level [7]. Serum Cu level was 10.8 % higher in patient group in comparison with control group, but this difference was not statistically significant. The results also indicated that CZr level was 112.5 % higher in patient group compared to control group (p<0.001). It was reported in another study that the increase of CZr was mostly owing to serum Zn level deficiency and not due to the increase of the serum Cu level [8]. Also, CZr indicated more significant relationships with physical performances in comparison with serum Cu and Zn level, alone [7]. According to the ROC curve, although total oxidant status has the maximum AUC among the included parameters, this factor is easily affected with many of the other factors such as lifestyle and nutrition. We observed an AUC of equal to 0.832 for CZr, and it seems that CZr is a more reliable parameter for discriminating the patient with physical disability against the control group, as reported by the others [7, 8, 11]. Serum Se level was 25.5 % lower in patient group than in the control one, and there was a positive correlation between serum Se level and ADL score. It was also reported an association between serum Se level deficiency and elevated IL-6 level, which led to the sarcopenia and finally the development of physical disability [13]; meanwhile, low serum Se level could be considered as an independent predictor

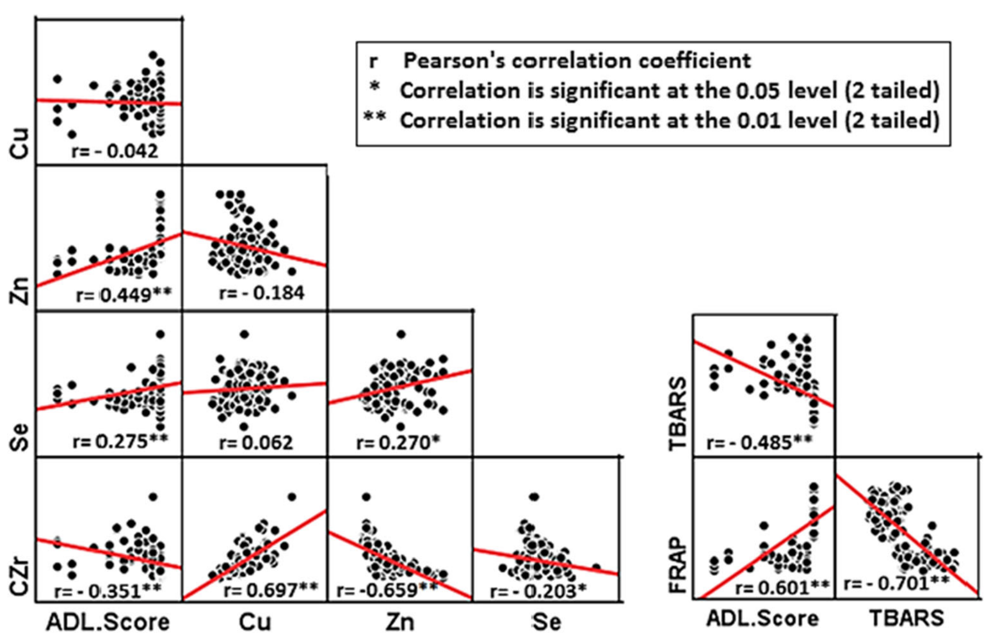

Fig. 3 Correlation analysis between trace elements and oxidative stress parameters with ADL score. *CZr* Cu to Zn ratio, *TBARS* total oxidant status, *FRAP* total antioxidant status

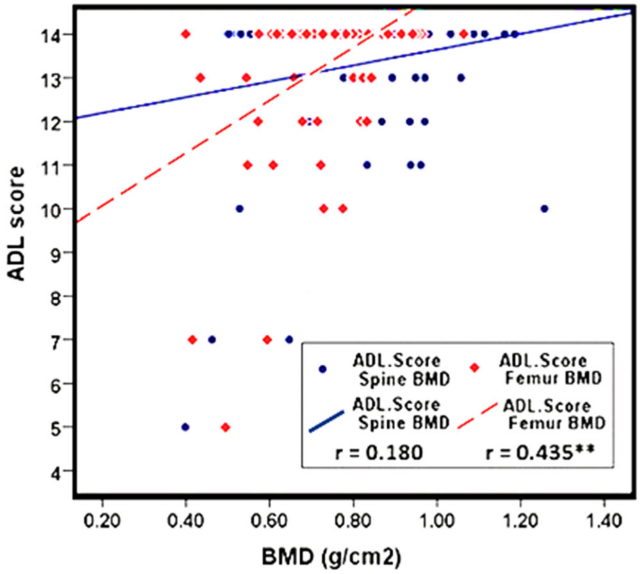

Fig. 4 Correlation analysis between BMD of femur and spine with ADL score in included persons

of physical disability [21]. Zn and Se showed a positive correlation with total antioxidant status while a negative correlation was observed between those elements and total oxidant status. It was suggested that Zn and Se serum levels were decreased by aging [22]. These elements are involved in biochemical mechanisms of the human body such as DNA and RNA reproduction, cell membrane integrity, and the destruction of free radicals. These elements play important role in cellular defense because they are strong antioxidants against free radicals. Decrease of the antioxidant level causes imbalance between antioxidants and free radicals, and the oxidative stress is increased through dysregulation of cellular function and muscle damage, which is led to disability [21]. Reactive oxygen species (ROS) are generated during physiological

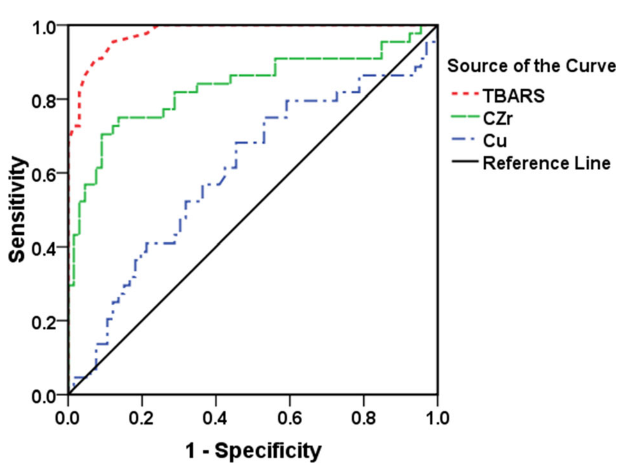

Fig. 5 The results of ROC curve analysis for determination of the diagnostic accuracy of the parameters. *CZr* Cu to Zn ratio, *TBARS* total oxidant status

Table 3 The results of ROC curve for discrimination of the patients group than the healthy person

	AUC	Std. error	Asymptotic 95 % confidence interval		*p* values
			Lower bound	Upper bound	
TBARS	0.979	0.010	0.959	0.999	<0.001
CZr	0.832	0.044	0.745	0.918	<0.001
Cu	0.603	0.057	0.492	0.714	0.068

AUC area under the curve, *TBARS* status of total oxidant, *CZr* Cu to Zn ratio

functions and metabolisms [23]. Uncontrolled production of ROS increases with age, which leads to oxidative stress and consequently damages to essential macromolecules such as protein and lipids [24]. Measuring of ROS is difficult due to its short half time, so the damage of oxidative stress to protein, lipid, and DNA is measured instead of it [15]. A positive correlation was observed between total antioxidant status and ADL score; meanwhile, there was a negative correlation between oxidant status and ADL score. It probably means that increasing of oxidative stress and decreasing of total antioxidant cause physical disability in patient group. It was also shown that elevated serum oxidative stress level was associated with severe walking disability among older women through oxidative damage to DNA, protein, and lipids which led to muscle weakness [25]. Similarly, in another study, it was stated that oxidative stress increased with age may lead to poor grip strength in elderly subjects [13]. A positive correlation was seen between BMD.F and BMD.S with ADL score. As previously published, there was a higher incidence rate of osteopenia and osteoporosis among physically disabled subjects [26, 27]; therefore, we may be able to conclude that disabled patients probably have lower BMD. Trace elements such as Se, Zn, and Cu are necessary to develop the skeleton and the decrease of any of them and may increase the risk of osteoporosis. It seems that among the various included parameters in this study, the CZr index is a more reliable parameter than the others for discriminating of patient with physical disability than the control group. Surely, for reaching to a conclusive statement in this area, a comprehensive evaluation of all important biochemical parameters of oxidative stress (including cytosolic ROS production of erythrocytes, nitric oxide, glutathione peroxidase, catalase …) and a larger patient sample size are needed.

Conclusion

The results of this study show that decrease of serum Zn and Se levels, increase of CZr and oxidative stress, and low BMD are common findings in physically disabled patients. Among the studied parameters, it seems that CZr is more reliable parameter than the others to discriminate the physically disabled patients against the control group.

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Conflict of Interest There is no conflict of interest.

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