CORRELATION BETWEEN SERUM ZINC LEVEL AND ERYTHROCYTE SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN NON-FRAIL AND FRAIL GERIATRIC PATIENTS

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Abstract—**Introduction:** Frailty syndrome (FS), as a health problem in elderly, can interfere with the quality of life. Many factors play roles in its occurrence, one of which is oxidative stress. Superoxide dismutase (SOD), in which one of its components is zinc (Zn), is one of the endogenous antioxidants that plays a role in preventing oxidative stress. This study was aimed to know the correlation between serum zinc level (sZn) and erythrocyte SOD (eSOD) activity in non-frail and frail geriatric patients.

Methods: This was cross-sectional study with consecutive sampling method conducted in the Geriatric Clinic Cipto Mangunkusumo hospital, from August through September 2014, on 60 patients (30 patients for each group of non-frail and frail). Data were collected from interviews, physical measurements, and venous blood sampling.

Results: Among 90% of the subjects had low Zn intake, at the same time, the mean of sZn was 8.41 ± 1.36mol/l and 93.3% of the subjects had experienced Zn deficiency. The mean of eSOD activity was 1526 ± 508U/gHb, and there was no significant difference between the groups. There were no significant correlation between sZn and eSOD activity both in the overall subject (r = -0.04; p = 0.076), as well as the non-frail group (r = -0.099; p=0.604) and frail group (r = 0.01; p = 0.957).

Conclusion: Serum zinc level did not have any significant correlation with the eSOD activity, neither on overall or both non-frail and frail groups.

Keywords: geriatric patient, serum zinc level, erythrocyte SOD activity, frailty syndrome

INTRODUCTION

Aging process is a natural process with the downward trend in the functional capacity, which occurs continuously at the molecular, cellular, and organ level. Lowering in the body functional capacity affects the occurrence of certain health problems, such as the frailty syndrome.[1,2]

Frailty syndrome is a clinical syndrome caused by the accumulation of the aging process, physical inactivity, weight loss, inadequate chronic intake, lifestyle, and unhealthy environment.[1] These factors are interrelated and endwith decreasein muscle and bone mass.[3,4] Sarcopenia plays an important role in the occurrence of frailty syndrome; which is marked with a condition of loss of skeletal muscle mass and strength due to the aging process.[3,5] Pathogenesis of sarcopenia is multifactorial, including malnutrition, nutritional oxidative stress, inflammation, hormonal changes, physical inactivity, and genetic factors.[5]

Naturally, the human body produces reactive oxygen species (ROS) which are prooxidants and on the other hand has a defense mechanism to cope with the damage that is caused by free radicals through the work of antioxidants, either endogenous or exogenous.[6] In the aging process, the ability of antioxidant defense mechanism is decreased, caused by a decrease in endogenous antioxidants, one of which is the enzyme superoxide dismutase (SOD); and contributes to the occurrence of sarcopenia.[5,6]

Zinc is one of the minerals that plays an important role in maintaining metabolic homeostasis and antioxidant mechanisms,[7,8] which is also an important part in a variety of protein structures of the body, including the role of zinc in the SOD to maintain the stability of the structure, especially isoform CuZnSOD.[9]

Various studies concerning the correlation between serum zinc level and eSOD activity showed no consistent results. A weak negative correlation between serum zinc level and eSOD activity was shown in few healthy elderly subjects. Zinc supplementation increases eSOD activity.[10] Nonetheless, other studies in healthy elderly subjects showed no correlation between zinc level and SOD activity.[7]

Most studies were done in healthy elderly subjects. There are still no data concerning the correlation between zinc level and eSOD activity in elderly patients with frailty syndrome, especially in Indonesia. This study aimed to determine the correlation between serum zinc level (sZn) and erythrocyte SOD (eSOD) activity in non-frail and frail geriatric patients.

METHODS

Design and Subjects

This is a cross-sectional study aimed to determine the correlation between serum zinc level and erythrocyte SOD activity in non-frail and frail geriatric patients, which was conducted at Cipto Mangunkusumo Geriatric Clinic from mid-August 2014 through mid-September 2014. The Ethics Committee from the Faculty of Medicine, Universitas Indonesia, had approved this study. The number of subjects, (this study used r = 0.5; β = 0.1; and α = 0.05), including 10% chance of drop-out was 30 for each non-frail and frail group.[11] A total of 60 geriatric patients, who participated in this study, signed an informed consent, and completed the study.

Frailty syndrome status is determined based on the index Frailty 40 items by research assistants of the Geriatric Division of Internal Medicine Department of Faculty of Medicine of Universitas Indonesia. The inclusion criteria were all elderly patients in the geriatric clinic who were given an informed consent and agreed to participate in this study. The exclusion criteria were as follows: having acute infection, intestinal disorder, and cognitive status disorder by Mini Mental State Examination scores <24.

Study Measurements

The design was cross sectional study, which was done in the geriatric clinic in Cipto Mangunkusumo General Hospital, Jakarta, Indonesia. Data were collected consecutively from August to September 2014, by trained research assistants. Data werecollected from interviews for demographic characteristics, anthropometric measurements including body weight and knee height, evaluation of food intake with 3 x 24 hours estimated food record method for macronutrient assessment, and semiquantitative food frequency questionnaire (FFQ) for zinc intake assessment. Volumes and portion sizes for 3 x 24 hours food record and semiquantitative FFQ were estimated using photographs and food models. Intake data were analyzed using Nutrisurvey 2007 program for Windows operating system.

Nutritional status was evaluated based on body mass index (BMI) for Asia-Pacific population.[12] Body mass index was calculated as weight (kg) divided by height (m²). Weight was measured with minimum clothing using platform SECA, meanwhile height was determined by knee height using knee height measuring instrument.[13]

Venous blood sampling was performed for serum zinc level and erythrocyte SOD activity. Serum zinc levels was determined by atomic absoprtion spectrophotometry method, while erythrocyte SOD activity was determined by using colorimetry method.[14] This study used cut-off values of 10.1 μmol/l serum zinc deficiency level for females and 10.7 μmol/l for males. While erythrocyte SOD activity normal range was 1102–1601 U/g Hb.

Statistical Analysis

All data were analyzed using Statistical Package for Social Science (SPSS) version 20 for Windows operating system. Kolmogorov-Smirnov normality test was done to the numeric data of the total subjects, while Shapiro Wilk test was done to numeric data of each group (frail and non-frail group). For data which were normally distributed according to normality test, Pearson bivariate analysis was done to determine the correlation between serum zinc level and eSOD activity. If not normally distributed, Spearman rank test was used. Significance level used was p<0.05.

RESULTS

All subjects completed all procedures used in the study, the characteristics of the demographic and nutrition status data is shown in Table 1. The mean age was 72.6 ± 6.5 years with the highest percentage in the age range 70–79 years (55.0%). Mean BMI was 24.41 ± 4.69 kg/m², with only 3.3% of the subjects classified as underweight and most of the subjects (61.7%) were in the overweight category, meanwhile obese II subjects (10%) were only found in the frail group. There were significant differences in BMI between the groups, which was 2.791 kg/m² (CI95% = -5.132 – (-0.450)) higher in the frail group.

Tabel 1. Demographic, Nutrition, Macronutrient and Zinc Intake Status of the Subjects

Characteristic	Frequency (n)	Percentage (%)
Age (year)		
60–69	19	31.7
70–79	33	55.0
>80	8	13.3
Gender		
Male	26	43.3
Female	34	56.7
Nutrition Status (BMI)		
Underweight	2	3.3
Normal Weight	21	35.0
Overweight		
High Risk	16	26.7
Obese I	15	25.0
Obese II	6	10.0
Nutrient Intake		
Energy		
<90% TER*	31	51.7
90–110% TER	14	23.3
>110% TER	15	25.0
Protein		
<10% TER	18	30.0
10–15% TER	24	40.0
>15% TER	18	30.0
Fat		
<20% TER	1	1.70
20–30% TER	27	45.0
>30% TER	32	53.3
Zinc		
< Indonesian RDA** 2013	54	90.0
Indonesian RDA 2013	3	5.0
> Indonesian RDA 2013	3	5.0

*TER = total energy requirement; **RDA = recommended daily allowance

Table 2. Characteristic of Subject's Serum Zinc level and Erythrocyte SOD Activity Based on Frailty Status

	Subject		
	Non-*Frail* (n=30)	*Frail* (n=30)	Total (n=60)
sZn Level			
Normal	3 (10%)	1 (3.3%)	4 (6.7%)
Deficiency	27 (90%)	29 (96.7%)	56 (93.3%)
eSOD Activity			
High	13 (43.3%)	14 (46.7%)	27 (45%)
Normal	10 (33.3%)	11 (36.7%)	21(35%)
Low	7 (23.3%)	5 (16.7%)	12 (20%)

Median energy intake was 86.97 (51.8–58.84)% total energy requirement (TER), low energy intake was found in 51.7% of the subjects. While the median intake of protein was 12.14 (3.39–50.66)% TER, there was no difference in energy and protein intake between the two groups. Despite 51.7% of the subjects had low energy intake, 53.3% of the subjects had fat intake >30% TER, with a mean value of fat intake of 32.5 ± 8.19% TER, with no differences

Table 3. Correlation between Serum Zinc Levels and Erythrocyte SOD Activity

Subject	r	p
Overall (n=60)	-0,04	0,076
Non-Frail	-0,099	0,604
Frail	0,01	0,957

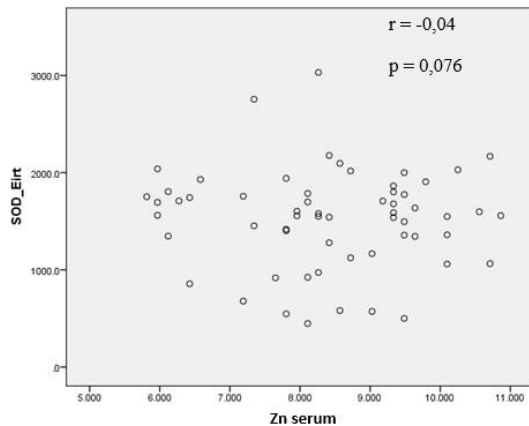neither between the frail nor non-frail group nor among BMI groups.

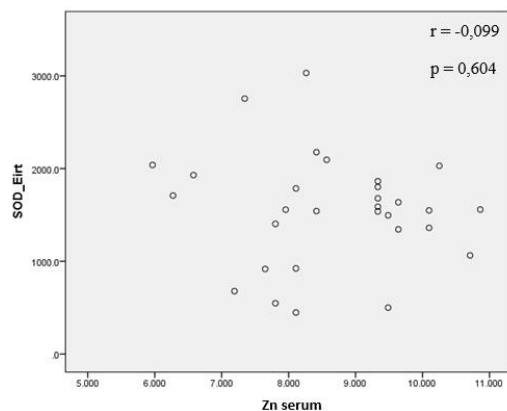Figure 1. Correlation between sZn level and eSOD Activity in Subjects

Figure 2. Correlation between sZn level and eSOD Activity in Non-frail Group

Median of zinc intake, determined by semiquantitative FFQ method, was 4.99 mg (2.50–15.76mg), when compared with the Indonesian *recommended daily allowance* (RDA) 2013, 90% of the subjects had zinc intake below the recommendation. The median intake of zinc in both groups was not significantly different, although the intake of the frail group (4.60 (2.50–11.0) mg) was lower than the non-frail (6.52 (2.79–15.8) mg). The characteristic of subjects based on energy, protein, fat, and zinc intake is shown in Table 1.
Mean sZn level was $8.41 \pm 1.36\ \mu$mol/l, which 93.3% of the subjects categorized as zinc deficient (45% in

non-frail group vs 48.3% in frail group). The mean of eSOD activity was 1526 ± 508 U/gHb, while the mean eSOD activity of both groups was 1551 ± 599 U/gHb and 1501 ± 405 U/gHb. There were no significant differences, neither in the mean of sZn level (p = 0.174) nor eSOD activity (p = 0.707) between both groups.

There was no significant correlation found among age with sZn level and eSOD activity of the subjects, both overall as well as per group. There was no significant correlation between sZn level and eSOD activity, neither overall nor per group (Table 3).
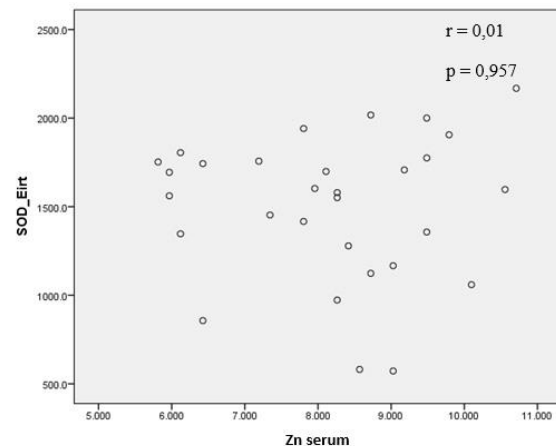

Figure 3. Correlation between sZn level and eSOD Activity in Frail Group

DISCUSSION

In this study, 61.7% of the subjects were classified as overweight, even 10% of subjects were obese II. The elderly rarely perform regular minimum recommended physical activity; moderate intensity for 5 x 30 minutes at least per week; only 22% of the elderly who have continuous sports activities.[15] Sedentary lifestyle often occur in the elderly, due to physiological changes, such as the decreased organ capacity, and reduced muscle strength.[15,16]

The difference in mean BMI between the non-frail vs frail group is 2.79, which was higher in the frail group. Other physiological changes in the elderly, like body composition changes, increased fat

and visceral fat mass, decrease in muscle mass and body metabolic rate, were found.[15] Excessive fat mass and loss of muscle mass, a condition called sarcopenic obesity,[15] together with excess body weight causes a decrease in muscle strength, functional capacity in daily activities.[4,15] Overweight and sedentary lifestyle accelerate the occurence of sarcopenia, and ultimately increase the incidence of frailty syndrome.[15]

Food record concluded 51,7% of the subjects had low energy intake, it should be considered that elderly intake of food is reduced up to 25% compared to adults, caused by the physiological changes in the elderly.[15,17,18] In this study, 30% of the subjects had low protein intake, and we also should be concerned in terms of the quality of protein which is consumed by the subjects. Essential amino acids in animal protein is more complete than vegetable protein, also vegetable protein is bound to carbohydrates therefore it is more difficult to digest due to the limitations of the human digestive enzymes.[19] Although 51.7% of the subjects had low energy intake, on the other hand 53.3% of the subjects had fat intake >30% TER and only 1 subject had a low fat intake (<20% TER). Overweight in the elderly was not caused by a high intake of fat, but it is referring to the lack of the subject's physical activity (in this study we did not explore it further). The main therapy in obesity is weight loss without causing a decrease in muscle mass and increased risk of frailty, with physical exercise and adequacy of dietary protein, vitamins, and minerals, that without causing any increase in the amount of calories.[20]

In this study, 93,3% of the subjects had zinc deficiency, which is in line with the most of the subjects (90%) had a lower zinc intake compared with Indonesian RDA 2013 (13 mg/day for males and 10 mg/day for females). The result is consistent with several studies in other countries which were done in elderly subjects. A cross sectional study by Mariani et al[21] in 981 healthy elderly subjects showed 69% of the subjects had zinc deficiency (plasma zinc level <10 µM). A study by Sfar et al[7] showed a prevalence of zinc deficiency in elderly (age >75 years old) of 65%.[7] The study also showed that the mean plasma zinc level in healthy adults (age 30–40 years old) was 11.75 ± 1.65 µmol/L.

The subjects are not used to consume animal foods, either meat or seafood which are food sources of zinc. Degenerative disease suffered by the subjects (dyslipidemia, hypertension, diabetes mellitus, and hyperuricemia) is also an obstacle for the subjects to consume these foods. Zinc is found in all organs, tissues, and body fluids, as well as having various biologic functions. Homeostasis of zinc keeps plasma zinc level in a small range, therefore a decrease in plasma/serum zinc concentration serum showed zinc deficiency in individuals, especially in relation to long term low zinc intake.[22] In case of low zinc intake, enzymes containing zinc in plasma and metallothionin will be releasing zinc into the pool of zinc, and zinc will subsequently be distributed to other parts that are more in need of zinc.[23]

There are only few studies concerning SOD activity in elderly patients with frailty syndrome. A study in mice with knocked out SOD1 gen (SOD1KO) found that they had increased inflammation and sarcopenia, and also showed characteristics similar to frailty syndrome in humans which includes: weight loss, weakness, low physical activity, and exhaustion. The SOD1KO mice showed alterations in pathways that have been proposed to play roles in the patophysiology of frailty syndrome, which are oxidative stress, mitochondrial dysfunction, and cell senescence.[24] In the present study, there was no differences in erythrocyte SOD activity between the non-frail and frail groups. Meanwhile, 45% of subjects had high erythrocyte SOD activity, and 20% had low erythrocyte SOD activity. High activity of erythrocyte SOD is a response to high pro oxidant/ROS stimulation in the cells, as well as vice versa.[25] High pro oxidant can be due to various conditions, such as aging process, inflammation, anemia, free radicals, and obesity.[25–29] In this study, 33.3% subjects had altered kidney function, which may be caused by anemia in chronic disease and thus contribute to increased pro inflammation. Another factor that may increase ROS level in cells is obesity.[29] In this study, mean BMI was higher in the frail compared to the non-frail group (p=0.02). In fact, 20% subjects in the frail group had class II obesity (BMI $\geq$30 kg/m^2).

This study did not show any significant correlation between serum zinc level and erythrocyte SOD activity. An in vitro experiment study in tissues, which were made to be zinc deficient, showed altered antioxidant defense mechanism in terms of decreased SOD activity.[30] The result of the in vitro study was different from our study, which may be

due to compensation mechanism from free zinc pool and bound zinc which maintain zinc-related enzyme function. Studies in animal or human subjects still have not shown consistent results in the correlation between zinc status and SOD activity.[7,31–34] The inconsistent result may be due to insufficient number of subjects.[21] A study by Mariani et al[21] with a greater number of subjects (n = 981) showed weak yet significant negative correlation between zinc level and SOD activity (r = -0.1; p <0.01). However, other studies involving other trace element status (e.g Cuprum) showed correlation with SOD activity.[32]

Activity of SOD is influenced by many factors, such as free radicals, inflammation due to degenerative disease, and obesity. These confounding factors of SOD activity were not controlled in this study. Otherwise zinc as part of the structure of the CuZnSOD enzyme, in which the role of zinc is a stabilizer of the structure, can be replaced by other metals when the body is deficient. The role of zinc could be replaced by cuprum (Cu) or cobalt (Co),[35] it was probably the reason why the SOD activity can be increased even in the case of zinc deficiency, as it did in the subjects of this study.

In conclusion, in this study serum zinc level did not significantly correlate with erythrocyte SOD activity both overall and per group.

Study Limitation
This study had several limitations. Because it was a cross sectional design, the direction of the reported associations cannot be established. A cross sectional study does not have controls, and is susceptible to bias and confounding factors.

Dietary assessment is challenging in elderly, their abilities to record and remember their diet is limited. Under reporting of dietary analysis, which was performed by semi quantitative FFQ and 3 x 24 hours estimated food record, may be another limitations in this study. Using serum zinc level may not reflect total body zinc level, due to serum zinc level only shows less than 0.1% of body zinc.

The measurement of a single endogen antioxidant activity, which is erythrocyte SOD, was performed in this study, while other major endogenous enzymes were not examined. Fluctuations in the activity of endogen antioxidant enzymes are responsive to pro oxidant stimulation in the body, so that the level of other endogen antioxidants and oxidative stress markers need to be assessed. Further research with larger number of subjects and also considering other endogen antioxidants, oxidative stress marker, and trace elements may be needed to determine the correlation between zinc and SOD activity in elderly patients, especially in frailty syndrome.

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