ASSOCIATION BETWEEN ZINC SERUM LEVEL AND CD4+ COUNT IN HEALTHY ELDERLY WOMEN AT AISYIAH ELDERLY CLUB KOTA GEDE YOGYAKARTA

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ABSTRACT

Background. *Old ages are associated with physiologic deteriorations of all organs and tissues, such as involution of thymus and decreased function of gastrointestinal tract which lead to malnutrition. Involution of thymus will caused reduction of T cell subset for instance T cell CD4+. Prevalence of nutrient deficiency in old age was high, according to a survey that 35% of subjects with age above 50 years old showed deficiency for one or more vitamins and trace-elements. Zinc as one of trace-elements has vital role for immune system.*

Aim. *Aims of this study are to know the mean of zinc serum level, proportions of zinc deficiency in elderly and correlation between zinc and CD4+ count.*

Methods and Subjects. *Design of this study is cross sectional, with study subjects including healthy elderly women from Aisyiah elderly club at Kota Gede Yogyakarta. Elderly who were eligible with study criteria were measured height, weight, zinc serum level, and CD4+ count. Correlation test was performed to know correlation between ages, weight, body mass index (BMI), and zinc serum level with CD4+ count. Significant variables will be tested with multiple linier regressions. Level of significance is p < 0.05.*

Results. *A total healthy elderly women subjects were 60 persons with median of age 63.5 years old. Median zinc serum level was 5.47 umol/L with range between 3.25- 7.95 umol/L. Zinc deficiency (zinc serum level < 7.65 umol/L) occured in 95% of subjects. Mean of CD4+ count was 767.22±232.95 count/mm³. There were correlations between age, weight, and zinc serum level with CD4+ count (r= -0.296; 0.345; 0.324; 0.792, respectively). Results from linear regression model showed zinc serum level was the only variable that affect to CD4+ count (adjusted R² = 0.622).*

Conclusion. *Ninety five percent of healthy elderly women at elderly club Aisyiah Kota Gede Yogyakarta suffer from zinc deficiencies, and there is strong correlation between zinc serum level and CD4+ count.*

Keyword: zinc serum, CD4+ count, elderly

INTRODUCTION

Elderly population is increasing all over the world.[1] In Indonesia according to national survey in 1985 number of elderly increased 3.09%/years, and people who are 70 years of age or above increased higher than 5.65%/years.[2]

The immune system changes during the lifespan of man. Many described changes in the immune system of the elderly were dependent on illness or chronic diseases.[3] Increased of age will decreased all organ function and tissues physiologically,[4] like involution of thymus,[5] and changes of gastrointestinal function which increases risk of malnutrition.[6]

Thymus is vital for acquired cellular immune response, where the differentiations and proliferation of lymphocyte T (T cell) takes place. The involution of thymus will cause reduction of T cell subset for instance T cell CD4+ (T cell helper 1 [Th1] and T cell helper 2 [Th2]).[5]

Elderly tends to have a high prevalence of nutrient deficiencies. Based on surveys it has been estimated that as many as 35% of persons who are 50 years of age or above have a demonstrable deficiency of one or more vitamins and trace elements.[7] Zinc as one of trace elements have vital

role for immune system. Twenty five to thirty percent of elderly population suffered from zinc deficiency.[8] Innate or acquired immune system influenced by zinc deficiency even at mild grade. A disturbance in acquired cellular immune system will affect T cell functions, and production of cytokines.[9,10]

Both factors stated above have a great deal for decreasing immune system in elderly.[5] Decreased immune function has been implicated in the increased incidence of cancer and infections in elderly.[8]

Aims of this study are to know level of zinc serum, proportions of zinc deficiency in elderly and correlation between zinc and CD4+ count.

METHODS AND SUBJECTS

Design of this study is cross sectional. The subjects were healthy elderly (60 years of age or above). Subjects are from Aisyiah elderly club at Kota Gede Yogyakarta. All members are women. Inclusion criteria were (1) elderly (2) willing to participate. Exclusion criteria were (1) consumption of immunosuppressant drugs within one month (2) suffered from metabolic diseases or infection that causes impairment in immune system (diabetes mellitus (DM), tuberculosis, malignancy, renal failure, and chronic liver disease) (3) chronic gastrointestinal disease.

Zinc serum level and CD4+ count were measured at biochemistry laboratory in Faculty of medicine Gadjah Mada University. Normal zinc serum level is 7.65 - 22.95 umol/L. Elderly is person who are 60 years old or above according to birth of date. Immunosuppressant drugs are corticosteroid. Healthy criteria were based on anamnesis and physical examination, to exclude. DM there should be no sign of polyphagia, polydypsia, polyuria as well as history of diabetes medications. For excluding tuberculosis, there should be clinically no chronic cough and medication of tuberculosis. Exclusion of malignancy was based on no history of operation, radiation or chemotherapy. Renal impairment was

confirmed by no history of renal disease. Chronic liver disease is confirmed from anamnesis of no history of chronic jaundice. Chronic gastrointestinal disease is excluded when there is no sign of diarrhea more than 15 days.

Elderly who are eligible with study criteria were examined by doctors and stated healthy clinically were subsequently measured for height, weight, zinc serum level, and CD4+ count. Two CC blood specimen for examination of zinc serum level was stored in sterile eppendorf vacutainer free mineral. The sample was centrifuged until serum formed and added with HNO3 solution 1:5. An examination by atomic absorption spectrophotometry (AAS) method was subsequently done. Examination of CD4+ count used enzyme linked immuno assay (ELISA) method.

STATISTICAL ANALYSIS

Normality test was performed to know distribution of data. Correlation between zinc serum level, age, weight, body mass index and CD4+ count was analyzed with Pearson correlation test. Significance variables will be tested with multiple linier regressions. Data analyses used computer software with level of significance of $p < 0.05$.

RESULTS

Total member of Aisyah elderly club were 108 female. Sixty persons fulfilled research criteria. Baseline characteristics of study subject are shown in table 1. Lowest age of the study subjects was 60 years old and the highest 71 years old with median 63.5 years. Median of weight was 52.5 kg with lowest weight 33.5 kg and highest weight was 102 kg. Median of height was 151 cm with lowest height was 142 cm and highest height 160 cm. Median of body mass index (BMI) is 22.66 kg/m2 with lowest 15.5 kg/m2 and highest 45.94 kg/m2. Median of zinc serum level was 5.47 umol/L with lowest zinc serum level was 3.25 umol/L and highest zinc serum level was 7.95 umol/L. Mean of CD4+ count was 767.22 with standard deviation 232.95 count/mm3.

Table 1. Baseline Characteristics Elderly Subjects at Aisyiah Elderly Club Kota Gede

Variable	Median	Minimum-Maximum
Age (years)	63.5	60-71
Weight (kg)	52.5	33.5-102
Height (cm)	151	142-160
Body mass index (kg/m^2)	22.66	15.5-45.94
Zinc serum level (umol/L)	5.47	3.25-7.95
CD4+ (count/mm^3)	767.22±232.95¶	707.04-827.39§

Note : ¶ mean ± standard deviation. § 95% confidence interval (CI)

Table 2 showed the result of Zinc deficiency (<7.65umol/L). Fifty seven elderly (95%) of study subjects were suffered zinc deficiency.

Table 2. Zinc Serum Level of Elderly at Aisyiah Elderly Club Kota Gede

Variable	n	Percentage
Zinc serum level < 7.65 umol/L	57	95
Zinc serum level > 7.65 umol/L	3	5

Note : n number of patients

Correlation between zinc serum level and CD4+ count showed strong and positive correlation ($r = 0.792$; $p < 0.05$). Correlation between age and CD4+ count showed weak correlation ($r = -0.296$; $p < 0.05$). Sign "-"showed that an increasing of age is accompanied by the decreasing CD4+ count. Table 3 showed a weak correlation between weight and CD4+ count ($r = 0.345$; $p < 0.05$), and also between BMI and CD4+ count ($r = 0.324$; $p < 0.05$). All of the correlations can be seen in table 3.

Table 3. Correlations between Age, Weight, Body Mass Index, and Zinc Serum Level with CD4+ Count

Variable	r	p	95% CI
Age	-0.296	0.033	0.000-0.079
Weight	0.345	0.000	0.000-0.049
BMI	0.324	0.033	0.000-0.049
Zinc serum level	0.792	0.000	0.000-0.049

Note : r correlation coefficient . CI confidence interval. tested with Pearson correlation

Significant variables like age, weight, BMI, and zinc serum level were tested again with multiple linier regressions. Result from multiple linier regressions (table 4) showed constance value -363.724, and from four significances variables zinc serum level was the only variable that affect to CD4+ count (Beta = 146.334. p = 0.000. 95% CI = 109.538-183.129). Regression equation can be defined as stated below:

$$CD4+ \text{ count} = -363.724 + 146.334 \text{ (zinc serum level)}$$

Constance value -363.724 means if there was no zinc serum level, CD4+ count will show a value of -363.724 count/mm3. Regression coefficient value 146.334 showed that every addition of 1 umol/L zinc serum level increased CD4+ count 146.334 count/mm3. Adjusted R square from multiple linier regressions is 0.622, meaning that 62.2% CD4+ count can be explained by zinc serum level and the rest (100%-62.2% = 37.8%) are caused by other factors.

Table 4.
Multiple linier regressions of Age. Weight. Body Mass Index and Zinc Serum Level to CD4+ Count

Variables	Unstandardized Coefficient		Standardized Coefficient	t	p	95% CI	
	Beta	SE	Beta			Lower level	Higher Level
Constance	-363.724	518.691		-0.701	0.486	-1403.205	675.757
Age	2.641	7.487	0.034	0.353	0.726	-12.363	17.645
Weight	8.145	5.974	0.383	1.363	0.178	-3.828	20.117
BMI	-13.464	13.835	-0.270	-0.973	0.335	-41.189	14.261
Zinc	146.334	18.361	0.780	7.970	0.000	109.538	183.129

Notes : SE *standard error*. p significances. CI confidence interval

DISCUSSION

Old ages are risk factors for malnutrition/nutrient deficiencies. Nutrient deficiencies consist of one or more vitamins and trace elements.[7] Results from this study showed 95% elderly suffered zinc deficiency. This is different from other reports that stated zinc deficiency between 25-30%.[8] Increasing of age will cause changes in sensation and perception for food and often precipitate complex psychosocial disorder.[11] Increasing of age also make changes on teeth structure and oral tissues that caused complaints.[12] Interaction between those factors plus low income will magnitude inadequate intake in elderly.[11] Malnutrition can also lower the balmy taste even pain suffering that magnitude inadequate intake.[13,14] Zinc deficiency in elderly can be caused by decrease intake of zinc lower than recommended daily allowance, and impairment of absorption.[15] Zinc deficiency is foung to be more prevalent in Indonesia due to less consumption of animal source like meat, milk, liver, and egg.[16]

Increasing of age is in opposite with CD4+ count. These phenomena showed decreasing of immune function in elderly. Changes of immune system were strongly correlated with involution of thymus. Effects from involution of thymus are disorders of T cell that consist of decreasing quantity and quality of T cell; decreasing of T cell memory differentiation; decreasing of T cell protection and changes of homeostasis T cell.[17,18] Reports from study on elderly in Germany showed there are decreasing of total T cell CD3, decreasing

of T cell CD8 that is higher than CD4, decreasing of T cell naïf (CD45RA), and increasing of T cell memory (CD45RO).[3]

Nutrition had effect on immune system. Most of immunodeficiency causes are due to energy protein malnutrition.[19] Judgment on nutrition status with anthropometrics measure like weight and BMI showed correlations (although weak) with CD4+ count. Result from a study published in AIDS Research and Therapy showed that BMI of HIV (human immunodeficiency virus) patients are different according to CD4+ count.[20]

Positive correlations between zinc and CD4+ count in elderly women consistent with previous study.[21] Lukito et al.[21] reported there were positive correlations between zinc serum and absolute count CD3 (total T cell). CD4 (T helper cell) and CD19 (total B cell) in women elderly but not in men (r = 0.32; 0.28; 0.50 respectively). Differences between coefficient correlation of zinc serum level and CD4+ count in this study (r = 0.792) may be due to differences in zinc serum level and CD4+ count, while this study median zinc serum level is 5.47 umol/L and CD4+ count is 767.22±232.95 count/mm^3. From previous study zinc serum level was 11.8±0.2 umol/L and CD4+ count 773±314 count/mm^3.

Zinc has several functions on cellular specific immune system (T cell). Thymus is a vital organ for development of lymphocyte T, requires functioning zinc. Studies on animal proved that zinc deficiency will decrease size and thymus cellularity, as well as apoptosis from thymocyte (T cell in

thymus). Decreasing activity of Zn-dependent thymulin also happened in man that suffered zinc deficiency. Thymulin is 9-peptida hormone which is secreted by thymus epithelial cell. Thymulin induced several marker of T cell, and precipitate T cell function include allogenic cytotoxicity, suppressor function, and production of IL-2.[19,22]

This study used cross sectional design that owned limitation to prove caused-effects, therefore further research with cohort design or clinical trial will be needed. Further studies that involved elderly men also need to be done. This study only draw sample from one elderly club and cannot be generalized for elderly population.

Results from this study showed 95% elderly women in elderly club Aisyiah Kota Gede Yogyakarta suffered zinc deficiencies, and there is positive correlation between zinc serum level and CD4+ count.

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