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Submission date: 10-Mar-2022 04:01PM (UTC+0800)
Submission ID: 1780938736
File name: burst_in_the_elderly_Pertiwi_Rosanti_Gatot_Soegiarto,_2019.pdf (219.44K)
Word count: 3892
Character count: 19628

THE NEW ARMENIAN MEDICAL JOURNAL
Vol.13 (2019), No 1, p. 79-84

EFFECT OF VITAMIN E SUPPLEMENTATION ON THE INCREASE OF NEUTROPHIL-MEDIATED OXIDATIVE BURST IN ELDERLY

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Received 15.12.2018; accepted for printing 10.01.2019

Abstract

***Background**: Aging process decreases function of neutrophils as effector cells in the first-line defense of the body, including their oxidative burst activity. The oxidative burst is related to the production of ROS in neutrophils through the NADPH oxidase enzyme system that generally occurs via arachidonic acid pathway. In this pathway, Vitamin E plays a role as a stimulator of phospholipase A2 activation to increase of superoxide production in cells. We evaluated the effect of vitamin E supplementation on the increase of neutrophil-mediated oxidative burst in elderly.*

***Methods**: The study design was a randomized controlled trial and single-blind with pre- and pos-test control group. 28 elderly who met the inclusion but not exclusion criteria were enrolled. Oxidative burst was measured before and after the vitamin E or a placebo treatment for 7 days. PMA (Post Menstrual Age) as the stimulant of oxidative burst used was used and its functions were measured using flowcytometry. The difference between neutrophils-oxidative burst between pre and post-treatment was analyzed using Wilcoxon Signed Rank.*

***Results**: Most of the subjects were female, mean age 73 years old with the number of leucocytes and neutrophils within normal limits. The mean oxidative burst before the vitamin E and the placebo treatment were 76.64 ± 3.98 % and 80.32 ± 12.08 % (N 98-100 %), respectively. Oxidative burst after vitamin E and placebo treatment were 87.79 ± 11.25 % and 81.92 ± 18.21 %, respectively. There was significant difference between neutrophils-mediated oxidative burst and vitamin E (p = 0.011), but not significant in placebo group (p = 0.594), with mean difference is higher in vitamin E (11.15 ± 1.49 %) compared to placebo (1.60 ± 1.94).*

***Conclusion** : There was an increase ofneutrophils-mediated oxidative burst in the elderly after vitamin E intake for seven days.*

Keywords: *neutrophils, oxidative burst, vitamin E*

Introduction

Aging is a physiological process that occurs in humans (1). Aging gives the effect of dysregulating to the body's immune system that known as immunosenescence (2). Neutrophils as part of the immune system also suffer from dysfunction due to the aging process (3, 4). Aging causes a decrease in microbicidal capacity of neutrophils due to decreased oxidative burst function (4-6). The function of the neutrophils-mediated oxidative burst is influenced by several things, including the intake of micronutrient vitamin E. There has been no research evaluating the effect of vitamin E on the increase of neutrophils-mediated oxidative burst in the elderly.

The incidence of highly infectious diseases in the elderly group becomes as the proportion of the elderly in the world increases. According to the demographic data of the world population, by 2050 there is an estimated increase in the elderly population more than 50% (7). Based on WHO data, mor-

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tality rates from infectious diseases in the elderly population (aged> 60 years) in Africa and some developing countries in Asia were about 20% (8).

The increase of mortality rates described above can be explained by a phenomenon called immunosenescence, a process that could occur in the innate and adaptive immune system. Associated with this, function of neutrophils as effector cells in the first-line defense of the body including their oxidative burst function that plays an important role in the intracellular killing process. , The oxidative burst function is related to the production of ROS in neutrophil cells through the NADPH oxidase enzyme system (9). In general, ROS formation might occur via NADPH oxidase, arachidonic acid pathway, xanthine-xanthine oxidase, cytochrome p-450 and mitochondrial electron transport chains (10). In this case, vitamin E plays a role in the arachidonic acid pathway as a stimulator of phospholipase A2 activation which further induces the synthesis of leukotriene B4 leading to increase of superoxide production in cells (11, 12).

The purpose of this study was to determine the effect of vitamin E supplementation on the neutrophils-mediated oxidative burst in the elderly group. We hypothesized that the vitamin E might improve oxidative burst function of neutrophils in the elderly.

METHODS

Study Design and Participants: This study used randomized clinical trial, single-blind, pre and post-test control group with the treatment of vitamin E (400 IU/d) and placebo for 7 days in 28 elderly people. Inclusion criteria were as follows: age of at least 60; no chronic diseases such as Diabetes mellitus, Hypertension, AIDS, another infection disease and blood disorder; no consumption of supplement, steroid and statin in past 6 months; no habit of smoking; no consumption of alcohol, no traumatic experience in the last 3 months; and no obesity.

Sampling was done by simple random sampling in 28 elderly that divide into 2 groups at random, i.e., the vitamin E supplement group and the placebo group. All study participants were blinded to treatment that given during the trial.

Interventions: This study evaluated the administration of vitamin E (RRR-α-tocopheryl acetate) (400 IU/d) against placebo. The vitamin E and placebo pill formulations were manufactured (Darya Varia, Indonesia) to be indistinguishable by size, color, weight, taste, or dissolution in water. There were no adverse events or changes in any physiological parameters that could be attributed to vitamin E or have unmasked blinding.

Study Objective and Main Outcome Measures: The main objective of this trial was to evaluate whether supplementation with vitamin E increased the oxidative burst function of neutrophil cells. The oxidative burst function was measured by laboratory flow-cytometry to measure the quantitative determination of oxidative burst leukocytes in the heparin-supplemented whole blood. The reagent kit used in this study was PhagoburstTM produced by Glycotope Biotechnology Germany. PhagoburstTM consists of anatomized E. coli bacteria, 12-myristate 13-acetate phosphate (PMA), N-formyl-methionyl-leucine-phenylalanine and dihydrorhodamine (DHR) peptides 123. The production of ROS could be calculated from the addition and observed of the DHR 123 oxidation increase in fluorency intensity and the result was expressed as a percentage of the number of oxidized cells (13).

Statistical Analysis: Research analysis begins with normality test using Saphiro Wilk test. Then, the difference of neutrophils-mediated oxidative burst between pre and post-treatment was analyzed using Wilcoxon Signed Rank. All analysis were performed by SPSS version 17.0.

RESULTS

Patient Characteristics and Adherence; The basic characteristics of vitamin E and placebo groups are shown in Table 1. The number of leukocytes and neutrophils was an important parameter in this study. Based on the Saphiro Wilk test results, both had a normal distribution and there was no significant difference in vitamin E or placebo group ($p > 0.05$).

Effects of Vitamin E on Oxydative Burst Function

Determination of oxidative burst function of neutrophil cells was performed using flow-cytometry. The results of the flow cytometric examination resulted in the pattern of blood cell distribution based on the results of light exposure that reflect the shape and size of cells also the presence or absence of granules in the cytoplasm. The percentage of the oxidative burst was measured by the percentage of neutrophil cells containing the R 123

TABLE 1.

Basic Characteristics of All Subjects

Characteristic		Received Vitamin E (n = 14)		Received Placebo (n = 14)		p value
Gender						
Male		10		11		0.633
Female		4		3		
Sports activity						
Routine		5		4		0.680
Non-routine		9		10		
	Normal value	mean	SD	mean	SD	
Age, (years)		73.14	4.64	73.28	4.57	0.937
Body mass index,†	-	22.3	2.9	22.11	1.88	0.830
Haemoglobin, g/dL	12.9-14.2	13.10	1.83	13.4	0.86	0.510
Glucose, g/dL	70 -105 mg/dL	134.14	22.0	121.5	19.9	0.750
Creatinine, mg/dL	0.6-1.3	0.80	0.28	0.81	0.21	0.881
Leucocytes, cell/µL	3.7-10.1x10^3/µL	7664.3	1468.4	6622.1	1278.9	0.061
Neutrophil, cell/µL	1.63-6.96x10^3/µL	4564.1	1332.4	3587.9	1160.1	0.055

NOTES: *Data are presented as No. unless otherwise specified.
†Body mass index was calculated as weight in kilograms divided by the square of height in meters

metabolite product (the result of DHR 123 oxidation by superoxide produced from oxidative burst). Interpretation of oxidative burst function before and after the treatment tended to increase in both study groups (Table 2).

Normality test was performed in the data that results in abnormal data distribution, thus Wilcoxon Signed Rank test was used to determine the difference of neutrophils-mediated oxidative burst before and after the treatment . The results showed that the increase of neutrophils-mediated oxidative burst was statistically significant in the vitamin E group (p = 0.011), while in the placebo group there was also a tendency to increase the oxidative burst function value, but it was not statistically significant (p = 0.594) (Table 3). In this study, the mean difference of oxidative burst increase in vitamin E group (11.15 ± 1.49%) was greater than the placebo group (1.60 ± 1.94%).

DISCUSSION

The elderly population as subjects in this study had an average age of 73.21 ± 4.52 years old. Wenisch et al study (England), using a research sample with a mean of 71 ± 7.5 years old to determine the decrease in oxidative burst function in elderly (14). In another study about decreased neutrophil function in age by

TABLE 2.

Neutrophils-mediated oxydative burst before and after treatment

Oxydative Burst Function (%)					
Vitamin E			Placebo		
No. Subject	Pre	Post	No. Subject	Pre	Post
1.	87.39	91.02	1.	87.30	61.18
2.	87.34	91.56	2.	87.92	99.88
3.	94.23	98.66	3.	89.37	90.33
4.	70.86	86.80	4.	83.18	57.83
5.	62.76	88.76	5.	62.35	99.90
6.	64.28	84.71	6.	78.75	73.47
7.	69.12	87.86	7.	91.05	81.43
8.	78.80	85.02	8.	85.52	99.07
9.	51.85	97.44	9.	87.37	99.97
10.	92.82	96.12	10.	8-5.20	99.70
11.	92.72	73.54	11.	78.26	99.30
12.	78.25	93.31	12.	65.26	66.76
13.	84.88	97.92	13.	91.14	60.59
14.	57.69	56.40	14.	51.83	57.57

TABLE 3.

Wilcoxon Signed Rank Test on Oxidative Burst Functions before and after the vitamin E and placebo treatment

Group	Oxydative Burst Function (%), Median (Range)						p value
	Pre treatment			Post treatment			
	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.	
Vitamin E	78.52	51.85	94.23	89,89	56.40	98.60	0.011
Placebo	85.36	51.83	91.14	85.88	57.57	99.97	0.594

Butcher et al was using samples with mean age 68.2 years old (15). This due to the increasing life expectancy of people in Indonesia. Based on the World Population Prospect report, the life expectancy of Indonesians has increased from 67.8 years (from 2000 to 2005) to 70.6 years (in 2015) (16).

This study involved the subject of healthy elderly research. A healthy elderly criterion that often used in studies field of immunogerontology is the SENIEUR protocol (SENior EURopean). The SENIEUR Protocol eliminates the presence of various immunologic and severe disease disorders to obtain a healthy elderly population with fairly strict criteria(17, 18). In this study did not fully apply the SENIEUR protocol in subject selection, due to resource constraints. However, through detailed anamnesis, physical examination and some investigations such as hemoglobin, leukocyte count, absolute neutrophil count, serum creatinine and blood glucose were expected to exclude diseases such as diabetes, hypertension, blood disorders, kidney and heart disease, and obtained a healthy elderly population that approaching SENIEUR criteria.

The data on the number of leucocytes and neutrophils were important parameters in this study and the mean number of both groups was normal. The study of Bovill et al and Nilsson et al also obtained the average number of normal leukocytes at the elderly has no significant differences in both men and women (19, 20). This contributed to Shaw et al's conclusion that showed no significant change in the number leukocyte in elderly (1). Another study conducted by Schroder et al also obtained results that in accordance with this study. He stated that in elderly there was a non-significant change in a number of neutrophils in the peripheral circulation(21). However, in the elderly, there was a decrease in the response of neutrophil precursor cells to G-CSF which was an essential material to initiate the production of neutrophils (22, 23).

In this study, the elderly were given the treatment of vitamin E and placebo. Vitamin E was given in the form of the d-α-tocopherol compound, which is one of the isomers of vitamin E that capable of modulating the function of neutrophil cell oxidative burst through the performance of the phospholipase A2 enzyme in the arachidonic acid pathway (12). Subsequently, the formation of arachidonic acid induces the synthesis of leukotriene B4 (LTB4) which has the effect of inducing the occurrence of neutrophil cell oxidative burst (11). Vitamin E was administered for 7 days at a dose of 400 IU / day, on the basis of Robson et al's study suggesting that elevated levels of vitamin E in plasma after 7 days of administration (24).

In this study, the function of oxidative burst of neutrophil cells statistically increased significantly in the vitamin E group. This was in accordance with the results of a study by Robson et al, which obtained an increase in the function of neutrophil cell oxidative burst in groups receiving vitamin E, vitamin C and beta-carotene also physical exercise compared to the group that receiving placebo and physical exercise ($p < 0.05$) (24). There was no comparison of oxidative burst values from other studies that could be aligned with this study because other studies had different subjects and or had different interventions from this study .

The function of neutral oxidative burst cells was a function of neutrophils that influenced by various factors such as hyperglycemia, hypertension, trauma, depression, alcohol, and cigarettes that have become the exclusion criteria in this study. Other factorial influences that unknown to researchers might still influence the results of the oxidative burst function in this study such as different degrees of inflammation between subjects. The influence of sample handling during laboratory examination, the pattern of nutrients that have not been homogeneous among the subjects, as well as differences in the mechanism of vitamin E me-

tabolism in the body. Improvement of supplementation method, duration of administration, handling of blood samples and comparison between vitamin E content and oxidative burst function was needed to know the effect of supplementation on oxidative burst function of the neutrophil cell.

Administration of vitamin E did not give any side effects in this study. In one study mentioned that the provision of vitamin E (α-tocopherol) might be at risk of forming a radical of tocopheryl. If it not reduced to co-antioxidants, the tocopheryl radicals could react with lipids to form lipid radicals. Therefore, sometimes vitamin E supplementation should be accompanied by vitamin C (25). In general, vitamin E administration has a good level of safety as long as it was given in the 200-800 IU / day range and did not cause significant clinical side effects (26-28). From this study, we suggest that vitamin E supplementation of 400 IU per day for 7 days had an effect on the function of neutrophil cell oxidative burst in the elderly group. There was a significant increase in the function of oxidative burst of neutrophil cells in stasis compared to placebo.

Several limitations including a minimal sample size of the population, thus it less to reflect a diverse population of elderly people. This study used a single-blind, so the possibility of subjective bias still exists that might affect the results of the study. Confounding variables in this research was the form of diet pattern research subject that still could not be controlled. This was because the study population was the general public and it was difficult to equate the diet among the research subjects. In an effort to minimize the bias of equating diet could be done by education and monitoring diet. The organ function parameters that associated with vitamin E metabolism and plasma vitamin E levels were not calculated in this study, thus there was no comparison between plasma vitamin E levels and their activity when inducing an increase in oxidative burst neutrophil.

CONCLUSIONS

There was an increase of oxidative burst in the elderly after vitamin E intake for seven days.

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