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The effect of dietary antioxidant vitamins on malondialdehyde, white blood cells, and platelets

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ABSTRACT

Introduction: Vitamins have a main role in controlling metabolism in the body and the immune system. Vitamins A, C, and E are known as antioxidant nutrients and vitamin D is essential for innate and adaptive immune function. This study is aimed to analyze the effect of vitamin intake regularly on malondialdehyde (MDA) levels, white blood cell (WBCs) and platelet counts, and differences count of leukocyte.

Method: The study used 6 groups including the control group without intervention and the intervention group intake of one tablet of vitamin a day for 7 days which were divided into groups of vitamin A 2000 IU, vitamin C 1000 mg, vitamin D 400 IU, vitamin E 100 IU and multivitamins C 1000 mg and D 400 IU.

Results: Based on the study was explained that vitamins C, D, and E and multivitamins C and D were able to reduce MDA levels and leukocyte counts descriptively but not significantly. The differential leukocyte count has various levels depending on the type of vitamin.

Conclusion: Therefore, the consumption of vitamins A, C, D, and E and multivitamins C and D had no significant effect on MDA levels, WBCs count, platelets count, and differential count of leukocyte.

Keywords: *vitamin, antioxidant, malondialdehyde, leukocyte, platelet*.
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INTRODUCTION

The increasing number of metabolic diseases such as obesity and diabetes mellitus every year is still a major problem for public health. Those metabolic diseases can be induced by increased inflammation and oxidative.[1] Oxidative stress caused by reactive oxygen species (ROS) is one of the causes of increased oxidative activity in disease cells. Vitamins A, C, and E are noted among the non-enzymatic mechanisms against this phenomenon. Vitamin D deficiency is also caused by cardiovascular disease.[2] Vitamin D can affect insulin secretion, and insulin metabolism by altering insulin proinsulin and increasing insulin sensitivity. The active role of vitamin D is highly recommended for people with diabetes mellitus to lower blood glucose.[3] Vitamin D supplementation can be done by giving an injection or oral administration to patients.[4] Vitamin D along with vitamin C has been widely combined for immune enhancement.[5]

Vitamins A, C, and E are also known as compounds that are high in antioxidants.[6] Antioxidants are needed by the body to ward off free radicals, therefore they can overcome and prevent oxidative stress.[7] Free radicals that take electrons from DNA can cause changes in DNA structure so that mutant cells arise.[8] If this mutation occurs for a long time can become cancer. Free radicals also play a role in the aging process, where free radical initiation reactions in the mitochondria cause the production of reactive oxygen species (ROS).[7,8] Free radicals can be generated from the body's metabolism and external factors such as cigarette smoke, ultraviolet radiation, chemicals in food, and other pollutants.[9]

The use of dietary vitamins C, D, and E are expected to suppress oxidative stress in the body by reducing malondialdehyde (MDA) levels from blood serum. High MDA content in the blood means high levels of free radicals in the blood. This will indirectly relate to the profile of blood cells, both white blood cells (WBCs) and platelets. Therefore, this study was conducted to analyze the effect of dietary vitamins A, C, D, and E and multivitamins C and D in blood malondialdehyde (MDA) levels by spectrophotometry and blood cell profiles using the peripheral blood smear method. This study is aimed to analyze the effect of vitamin intake regularly on malondialdehyde (MDA) levels, white blood cell (WBCs) and platelet counts, and differences count of leukocyte

METHODS

Study Design

The study was experimental research with a cross-sectional approach. The respondents used were healthy males or females aged 19-35 years with a total of 24 respondents.

Exclusion respondents in this study were men and women aged < 19 years or > 35 years, men or women who were sick, and people who were taking drugs.

Data collection procedures

The study used 24 respondents who were divided into 6 groups with 4 samples each, namely control without intervention, sample with the intervention of Vitamins A 2000 IU, C 1000 mg, D 400 IU, E 100IU, multivitamin C 1000 mg and D 100 IU. Commercial vitamins A, C, D, and E tablets were given to respondents to be taken orally one tablet a day for 7 days. Meanwhile, commercial multivitamins C and D effervescent must be dissolved in water and then taken once a day for 7 days.

Materials

Materials needed for blood smears included slides and cover slips 7101 Sail brand, Olympus CX-23 microscope, and Giesa Merck cat. 1.09204.0500 - Azur Eosin Methylene Blue Solution from Giemsa for staining. Meanwhile, the equipment needed for MDA testing used a Thermo Scientific Spectrophotometer UV-Vis, cuvette, micropipette, and materials included Phosphate Buffer Saline (PBS) solution, 0.25N Hydrochloric Acid (HCl), 15% Trichloracetic Acid (TCA), 0.37% Thiobarbituric Acid (TBA) Solution. Blood samples were taken using a Terumo 5 cc syringe.

Blood smear

A blood smear test was carried out by dripping one drop of blood on a slide, then covered with another slide and smearing it at an angle of 30-40° then drying it. Furthermore, the specimen was immersed in a fixative solution for 2-3 minutes, then Giemsa dye was dripped on the specimen. WBCs, platelets, and leukocyte profiles were observed using an Olympus CX-23 microscope with a magnification of 400x.[10]

Malondialdehyde Assay

The MDA assay was carried out by taking 500 ml of serum samples and adding 4.5 ml of cold PBS solution. Afterward, 4 ml of supernatant was taken, then 1 ml of 15% TCA was added, and given 1 ml of 0.37% TBA in 0.25 N HCl then added in an 80°C water bath for 15 minutes. Then allowed to stand at room temperature for 60 minutes and centrifuged at 3000 rpm for 15 minutes. The absorbance measurement of the MDA sample supernatant was calculated using a spectrophotometer UV-Vis at a wavelength of 532 nm based on the standard curve (standard) of the MDA solution.[11,12]

Data analysis

The data was collected and analyzed by using the Wilcoxon test to determine the effect of vitamins A, C, E, D, and multivitamins C and D on MDA levels, leukocyte and platelet counts, and differential leukocyte counts.

RESULTS

Malondialdehyde

The results of the MDA assay explained a decrease in MDA levels after consuming vitamins C, D, and E and a combination of vitamins C and D for 7 days although not significantly different. However, in contrast to the results of respondents who consumed vitamin A, which increased. The results of the MDA assay could be seen in table 1.

White Blood Cells and Platelets

Based on the results of the blood smear test in table 2, showed that the WBC count decreased in all groups but was not significantly related before and after treatment for 7 days. On the other hand, the platelet count increased descriptively in the control group, vitamins A, C, E, and the combination of D and E, but decreased in vitamin D.

Differential Count of Leukocyte

Based on the observation of the leukocyte profile in each group, it was explained that the basophils were still in normal condition both before and after treatment for 7 days. Eosinophils increased in the control group but decreased in all vitamin treatment groups. The band neutrophil decreased in all groups, except for the vitamin A group which experienced an increase in the percentage. The segmented neutrophil increased in the control group, vitamin D, C, and E, a combination of vitamins C and D groups, but decreased in the vitamin C group, and was stable in the vitamin A group. The percentage increase in lymphocytes was found in the vitamins A, and C, a combination of vitamins C and D groups, and decreased in the control group, vitamins D, and E. The percentage of monocytes increased in the control and vitamin A groups, decreased in the vitamin C, D, and E groups, and stabilized in the C and D groups. However, the increase and decrease in the leukocyte profile did not have a significant difference both before and after therapy. The results of the differential count of leukocytes could be seen in Tables 3a, 3b, and 3c.

DISCUSSION

This study found a not significant decrease in MDA levels after consuming vitamins C, D, and E and a combination of vitamins C and D for 7 days. Meanwhile, this study found an increasing level of MDA after consuming vitamin A. Several studies reported that vitamin A indirectly has an antioxidant effect, thus it couldn't affect the MDA levels directly.[13] This study also found that WBC count was decreased in all groups but was not significantly related before and after treatment for 7 days. But, the platelet count was decreased after vitamin D administration. Vitamin D could act as a direct antioxidant by protecting the cell membrane from lipid peroxidation, it could decrease platelets level in this study.[14]

Based on the results of the leukocyte profile, it showed zero blood disorders, and myeloproliferative disease due to basophils was 0%. Meanwhile, eosinophils decreased on the 7th day, indicating that the respondents did have not allergies or parasitic infections due to taking vitamins. However, neutrophil levels had fluctuating counts for each group. These neutrophils are responsible for fighting bacterial infections and inflammatory disorders that show various conditions after taking vitamins. Lymphocytes play a role in fighting viral and bacterial infections and monocytes play a role in fighting chronic infection.[15,16]

Vitamin C tends to increase lymphocytes because it has been proven to increase interferon levels and immune cell activity thus increasing the activity of lymphocytes and macrophages as well as increasing leukocyte migration and mobility.[17] Based on the results of previous

Table 1. The results of malondialdehyde levels of pre and post-vitamin supplementation

Group	N	Malondialdehyde Levels					P-value Wilcoxon Test
		Pre-Treatment		Post-Treatment			
		Mean	STD	Mean	STD		
Control	4	10.116	4.326	8.102	0.863	↓	0.465
Vitamin A	4	5.274	1.839	7.671	0.743	↑	0.068
Vitamin C	4	11.842	5.965	9.349	4.975	↓	0.465
Vitamin D	4	17.595	12.826	14.958	2.613	↓	0.715
Vitamin E	4	40.512	21.478	18.314	4.613	↓	0.144
Vitamin C&D	4	25.697	13.360	16.492	7.596	↓	0.465

Table 2. The results of WBCs count in each group

Group	N	WBC (x10³ uL⁻¹)					P-value Wilcoxon Test
		Pre		Post			
		Mean	STD	Mean	STD		
Control	4	146.000	77.357	124.750	57.314	↓	0.465
Vitamin A	4	180.625	97.309	131.125	38.473	↓	0.465
Vitamin C	4	981.25	57.716	89.875	18.589	↓	0.715
Vitamin D	4	96.500	45.190	92.000	29.941	↓	0.715
Vitamin E	4	103.500	25.341	94.750	21.152	↓	0.273
Vitamin C&D	4	91.750	30909	27.750	19.589	↓	0.715

Table 3. The results of platelets count in each group

Group	N	Platelets (x10³ uL⁻¹)					P-value Wilcoxon Test
		Pre		Post			
		Mean	STD	Mean	STD		
Control	4	113.600	56.933	146.800	75.597	↑	0.715
Vitamin A	4	109.400	75.664	124.600	91.357	↑	0.715
Vitamin C	4	131.000	75.297	134.400	95.319	↑	0.715
Vitamin D	4	145.800	45.921	86.400	34.334	↓	0.066
Vitamin E	4	83.600	35.887	145.000	41.091	↑	0.465
Vitamin C&D	4	114.800	30.375	133.600	82.019	↑	0.068

studies, vitamin C has been shown to suppress pro-inflammatory processes through a pleiotropic mechanism while increasing anti-inflammatory and pro-resolution effects on macrophages.[18] Supplementation of vitamin C 1-5 grams per day and if consumed in a few weeks will increase the proliferation of T lymphocytes, therefore it can improve the body's immune system such as antimicrobial activity and natural killer cells (NK cells), lymphocyte proliferation, chemotaxis, and delayed-type hypersensitivity reactions.[19] Vitamin C plays a role in maintaining cell integrity and protecting cells against ROS (reactive oxygen) during the inflammatory response.[18,20]

The study used a dose of vitamin D 400 IU quietly lower to reduce neutrophil levels. The use of vitamin D3 is recommended 1000-2000 IU per day to optimize the positive role of vitamin D in increasing immunity, and maintaining the hard and soft tissues in the body.[21] Meanwhile, based on the results of previous studies, it was shown that vitamin D was an anti-lymphoproliferative agent that inhibits the differentiation of monocytes into macrophages thereby limiting the production of various inflammatory cytokines, including TNF-α and IL-6.[22] Deficiency of vitamin D could affect the balance of pro-inflammatory cytokines and cause impaired platelet function.[23] The results of this research on monocyte count were following the results of previous studies which showed that vitamin D caused a decrease in monocytes. However, the effect was different in multivitamins C and D.

The enhancement of neutrophil counts and function during the supplementation of vitamin E, particularly in patients with type Ib glycogen storage disease.[24] Vitamin E improves neutropenia by reducing reactive oxygen species (ROS) and undergoing apoptosis. Vitamin E supplementation enhances lymphoproliferative capacity, and neutrophil-mediated functions including the phagocytic function of PMNs and production of monocyte chemoattractant protein-1.[24] Meanwhile, the relationship between supplementation of vitamin A and leukocytes profile is unclear yet.

The various results of this study while taking vitamins A, C, D, and E and multivitamins C and D orally showed that there was no significant difference in the effect of taking antioxidant vitamins on reducing MDA levels, leukocyte counts, differential leukocyte counts, and increasing platelet counts. Among these vitamins that have received sufficient attention are vitamins C, D, and E which showed a decrease in descriptive

Table 3. a. The results of the leukocyte profile in the group of control and vitamin A

Types of Leukocytes	N	Control						Vitamin A					
		Pre (%)		Post (%)			P-value Wilcoxon Test	Pre (%)		Post (%)			P-value Wilcoxon Test
		Mean	STD	Mean	STD			Mean	STD	Mean	STD		
Basophils	4	0	0	0	0	-	-	0	0	0	0	-	-
Eosinophils	4	4.50	3.10	7.50	1.00	↑	0.068	4.75	3.86	4.00	3.83	↓	0.593
Band neutrophils	4	20.25	14.5	10.75	3.69	↓	0.197	10.75	2.21	9.50	3.11	↑	0.655
Segmented neutrophils	4	39.25	10.94	56.25	3.30	↑	0.068	53.50	7.85	53.50	5.32	-	1.00
Lymphocytes	4	25.50	1.91	18.00	5.22	↓	0.068	25.50	1.91	30.50	12.71	↑	0.465
Monocytes	4	7.25	5.50	7.50	4.20	↑	0.715	5.50	1.91	6.00	4.08	↑	0.854

Table 3. b. The results of the leukocyte profile in the group of Vitamin C and vitamin D

Types of Leukocytes	N	Vitamin C						Vitamin D					
		Pre (%)		Post (%)			P-value Wilcoxon Test	Pre (%)		Post (%)			P-value Wilcoxon Test
		Mean	STD	Mean	STD			Mean	STD	Mean	STD		
Basophils	4	0	0	0	0	-	-	0	0	0	0	-	-
Eosinophils	4	8.00	4.55	5.25	3.59	↓	0.269	4.50	2.38	1.50	1.29	↓	0.141
Band neutrophils	4	9.25	7.80	7.50	6.66	↓	1.00	8.00	4.163	7.00	5.35	↓	0.854
Segmented neutrophils	4	53.75	4.04	52.75	10.50	↓	0.715	42.50	15.50	52.50	7.33	↑	0.465
Lymphocytes	4	21.75	3.86	30.25	13.48	↑	0.273	38.00	15.98	32.75	4.27	↓	0.715
Monocytes	4	7.25	0.50	4.25	4.57	↓	0.141	7.00	5.22	6.25	0.96	↓	1.00

Table 3. c. The results of leukocyte profile in the group of Vitamin E and vitamin C-D

Types of Leukocytes	N	Vitamin E						Vitamin C and D					
		Pre (%)		Post (%)			P-value Wilcoxon Test	Pre (%)		Post (%)			P-value Wilcoxon Test
		Mean	STD	Mean	STD			Mean	STD	Mean	STD		
Basophils	4	0	0	0	0	-	-	0	0	0	0	-	-
Eosinophils	4	8.25	3.40	2.25	0.96	↓	0.066	5.75	5.91	3.25	3.59	↓	0.461
Band neutrophils	4	11.00	5.89	7.50	2.00	↓	0.73	8.00	4.16	4.00	3.27	↓	0.273
Segmented neutrophils	4	39.75	8.14	53.00	5.32	↑	0.144	52.00	6.27	56.50	10.66	↑	0.465
Lymphocytes	4	33.75	11.295	32.75	13.35	↓	0.854	29.25	10.37	31.25	17.02	↑	0.715
Monocytes	4	7.25	4.27	4.50	0.58	↓	0.273	5.00	3.37	5.00	2.94	-	1.00

MDA, leukocyte count, and differential leukocyte count such as neutrophil band and monocyte which were quite consistent with the results of previous studies. The limitation of this research is the small number of respondents, therefore further research can be developed by increasing the number of respondents and the length of treatment to get an accurate and significant value.

CONCLUSION

Consumption of vitamins A, C, D, and E and multivitamins C and D had no significant effect on MDA levels, WBC count, platelet count, and differential count of leukocytes. However, vitamins C, D, and E and multivitamins C and D were able to reduce MDA levels and WBCs count descriptively.

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CONFLICT OF INTEREST

There is no conflict of interest.

ETHICAL STATEMENT

This research has been declared to have received an ethical certificate with No.118/EC/KEPK/UNUSA/2022.

AUTHOR CONTRIBUTION

All authors contributed equally to this study.

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