

## Effect of Vitamin D3 2000 IU Tablets on Serum Vitamin D 25-OH Levels, Hemoglobin Levels, Body Mass Index and CD4/CD8 Ratio in Patients Infected with Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome Who Have Been Treated with Antiretrovirals (ARVs)

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Article History	Abstract
<p>Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 16 Nov 2023</p>	<p><b>Background:</b> Human Immunodeficiency Virus (HIV) is a retrovirus subgroup of lentivirus family that has RNA genetic characteristics that attack the body's immune system, especially infecting Cluster Differential Four (CD4) lymphocytes and replicating according to the host's DNA. Vitamin D is important in stimulating innate immunity. Availability of vitamin D has an important role to improve the nutritional status of HIV/AIDS patients, improve the immune system, and improve prognosis. <b>Research Objectives:</b> To assess the effect of administration of vitamin D3 tablet therapy on serum vitamin D 25-OH levels, hemoglobin levels, body mass index (BMI), and CD4/CD8 ratio in HIV/AIDS infected patients who have been treated with antiretrovirals (ARV). <b>Methods:</b> This study was a cross-sectional study using 40 samples of HIV patients who were routinely controlled at the VCT polyclinic at Dr. Moewardi who received regular antiretrovirals. The inclusion and exclusion criteria are intended to obtain samples that are currently in HIV stages I and II so that it is expected to reduce other factors that cause bias levels of vitamin D apart from the chronic inflammatory process that occurs in HIV-infected patients. Patients who have come informed their consent to take blood samples during one of their visits to the VCT poly to check BMI, serum vitamin D (25-(OH)), and routine blood to determine the value of hemoglobin and the CD4/CD8 ratio. The statistical test uses an analysis of the difference in the average of the two groups. <b>Results:</b> The results of testing the mean difference between the two groups of serum levels of vitamin D 25-OH in the Vitamin D3 treatment and control groups showed a value of <math>Z = -2.277</math> (<math>p = 0.023</math>). The results of the different test mean that the two groups of hemoglobin in the Vitamin D3 treatment group and the control showed a value of <math>Z = -3.484</math> (<math>p = 0.001</math>). The results of different tests mean that the two BMI groups in the Vitamin D3 treatment group and the control group show a value of <math>t = -5.098</math> (<math>p = 0.001</math>). The results of the mean difference test of the two CD4/CD8 ratio groups in the Vitamin D3 treatment group and the control group showed a value of <math>t = -6.203</math> (<math>p = 0.001</math>). <b>Conclusion:</b> Administration of 2000 IU vitamin D3 tablets can increase serum vitamin D 25-OH levels, hemoglobin, BMI, and CD4/CD8 ratio in HIV/AIDS-infected patients treated with antiretroviral (ARV).</p>
<p>CC License CC-BY-NC-SA 4.0</p>	<p><b>Keywords:</b> HIV, AIDS, hemoglobin, BMI, CD4/CD8 ratio, vitamin D</p>

### 1. Introduction

Human Immunodeficiency Virus (HIV) is a retrovirus subgroup of lentivirus family that has RNA genetic characteristics that attack the body's immune system, especially infecting Cluster Differential Four (CD4) lymphocytes and replicating according to the host's DNA. Acquired Immuno Deficiency Syndrome (AIDS) is a collection of signs and symptoms that appear caused by a weakened immune system due to HIV infection, characterized by low CD4 T lymphocytes and the appearance of opportunistic infections or cancer.

Data in 2015 from WHO for the Southeast Asian region there were about 180,000 new HIV cases in the population and the death rate due to AIDS in the same year was around 130,000 people. 2 In Indonesia, HIV has spread in 386 districts/cities in all provinces in Indonesia. 1

One of the interventions used to date is the administration of ARV drugs. Indications that show someone with HIV in general are drastic weight loss, chronic diarrhea, wasting, and the person's white blood cell count (CD 4) decreases to below 200 cells/mm<sup>3</sup>.

In HIV patients the most common hematological complications found include anemia. Factors causing anemia in HIV patients can be caused by inadequate intake, opportunistic infections, malignancy, medication, the effect of the HIV virus on the formation of erythrocytes and on the CD4 immune system.

Vitamin D is important in stimulating innate immunity. Vitamin D stimulates CD4 in T cells so that it can increase the CD4/CD8 ratio. 4 In addition to playing a role in the immune system, vitamin D has been shown to suppress hepcidin transcription so that it has the potential to increase iron levels in the circulation which in turn can trigger erythropoiesis. 5 Vitamin D also stimulates CD4 on T cells so as to increase the ratio of CD4/CD8.4

Based on these facts it can be concluded that the presence of vitamin D has an important role in improving the nutritional status of HIV/AIDS patients, improving the immune system, and improving prognosis. However, how big is the role of Vitamin D in predicting the prognosis of HIV/AIDS patients, there is still much to be done in further research. Therefore, this study will assess the effect of vitamin D therapy on HIV/AIDS patients who have been treated with antiretroviral (ARV).

## 2. Materials And Methods

### Types of research

This study used experimental research using the Randomized Controlled Trial (RCT) method with a pretest-posttest approach with a control group design. This research was conducted at the Department of Internal Medicine, VCT (Vouuntary, Counseling and Testing) polyclinic at RSUD Dr. Moewardi Surakarta from June 2020 – December 2020. Researchers have obtained ethical clearance from the ethical commission for basic/clinical research at RSUD Dr. Moewardi, Surakarta / Faculty of Medicine, Universitas Sebelas Maret Surakarta.

### Population and Sample

The population in this study were all HIV patients who were taking outpatient treatment at the VCT Internal Medicine Polyclinic at RSUD Dr. Moewardi. The sample selection was carried out by random sampling. the minimum sample in this study is 40 respondents who will be divided into 2 groups; the intervention group (20 respondents) and the control group (20 respondents). To avoid dropping out, the number of samples used in this study was 44 respondents (22 respondents in the intervention group and 22 respondents in the control group).

### Statistical analysis

To prove the hypothesis, an analysis of the mean difference between the two groups was performed. For the mean difference between the two groups in the control and treatment groups, both pre and post-treatment used the unpaired t-test if the data distribution is normal. If the data distribution is not normal, then use the Mann-Whitney test. To prove the mean difference between the two groups in the pre and post-treatment, in the control and treatment groups, a paired t test is used if the data distribution is normal. If the data distribution is not normal, the Wilcoxon test is used. To test how much influence the independent variables have on each dependent variable, a linear regression test is used. The test uses the Statistical Product and Service Solution (SPSS) for Windows version 23.0.

## 3. Results and Discussion

### Basic characteristics

**Table 1** Description and Comparison of Basic Characteristics of Research Subjects

Variable	Group Intervention	Group Control	p
Age (th)	40.05 ± 8.85	36.70 ± 11.16	0,299

Gender				
Man	13 (65,0)	17 (85,0)		
Woman	7 (35,0)	3 (15,0)		0,144
D 25-OH (ng/ml) <sup>3</sup>	28,10 (13,87 – 43,1)	22,08 (9,6 – 70,0)		0,588
Hemoglobin (g/dl)	14,15 (7,1 – 17,0)	14,5 (10,7 – 15,7)		0,735
BMI (kg/m <sup>2</sup> )	21,7 (18,0 – 32,9)	21,15 (13,1 – 27,0)		0,330
CD4/CD8	0,40 (0,08 – 1,29)	0,40 (0,07 – 0,86)		0,892

There were no significant differences in age ( $p = 0.299 > 0.05$ ) and sex ( $p = 0.144 > 0.05$ ) between the two experimental groups. The results of this statistical test confirm the homogeneity of the two characteristics so it can be said that the effect of the role of the two was controlled in this experiment.

### Effect of Vitamin D3 Administration on D 25-OH, Hemoglobin, BMI, and CD4/CD8

**Table 2** Comparison of Initial and Final Values of D 25-OH, Hemoglobin, BMI, and CD4/CD8 in the Intervention Group

Variable	Initial Value ( <i>Pre</i> )	Final Value ( <i>Post</i> )	p
D 25-OH (ng/ml)	28,10 (13,87 – 43,1)	33,85 (15,3 – 41,6)	0,023
Hemoglobin (g/dl) <sup>3</sup>	14,15 (7,1 – 17,0)	15,3 (10,8 – 17,9)	0,000
BMI (kg/m <sup>2</sup> )	21,7 (18,0 – 32,9)	22,36 (20,2 – 32,8)	0,000
CD4/CD8	0,40 (0,08 – 1,29)	0,48 (0,22 – 1,40)	0,000

**Table 3** Comparison of Final Values of D 25-OH, Hemoglobin, BMI, and CD4/CD8 between the Intervention Group and the Control Group

Variable	Group Intervention	Group Control	p
D 25-OH (ng/ml)	33,85 (15,3 – 41,6)	26,65 (7,1 – 38,5)	0,002
Hemoglobin (g/dl)	15,3 (10,8 – 17,9)	14,35 (10,5 – 15,5)	0,020
BMI (kg/m <sup>2</sup> )	22,36 (20,2 – 32,8)	21,2 (13,0 – 26,8)	0,042
CD4/CD8	0,48 (0,22 – 1,40)	0,40 (0,13 – 0,64)	0,044

Based on table 2, the increase in D 25-OH in the intervention group was stated to be significant ( $p = 0.023 < 0.05$ ). Based on table 3 the final value of D 25-OH in the intervention group was higher than in the control group. Statistically, the difference was stated to be significant ( $p = 0.002 < 0.05$ ). Thus, it is proven that the administration of vitamin D3 has an effect on D 25-OH.

Based on table 2, the increase in hemoglobin in the intervention group was stated to be significant ( $p = 0.000 < 0.05$ ). Based on table 3, the final hemoglobin value in the intervention group was higher than that in the control group. Statistically, the difference was stated to be significant ( $p = 0.020 < 0.05$ ). Thus, it is proven that the administration of vitamin D3 has an effect on hemoglobin.

Based on table 2, the increase in BMI in the intervention group was stated to be significant ( $p = 0.000 < 0.05$ ). Based on table 3, the final value of BMI in the intervention group was higher than that in the control group. Statistically, the difference was stated to be significant ( $p = 0.042 < 0.05$ ). Thus, it is proven that the provision of vitamin D3 has an effect on BMI.

Based on table 2, the increase in CD4/CD8 in the intervention group was stated to be significant ( $p = 0.000 < 0.05$ ). Based on table 3, the final CD4/CD8 score in the intervention group was higher than that in the control group. Statistically, the difference was stated to be significant ( $p = 0.044 < 0.05$ ). Thus, it is proven that the administration of vitamin D3 has an effect on CD4/CD8.

### Contribution of Vitamin D3 to D 25-OH, Hemoglobin, BMI, and CD4/CD8

The magnitude of the contribution of the effect of giving vitamin D3 to the four response variables in this experiment can be known based on the value of the square coefficient of beta ( $\beta^2$ ) which can be obtained from linear regression analysis.

**Table 4** Linear Regression Analysis of the Effect of Vitamin D3 Administration on D 25-OH, Hemoglobin, BMI, and CD4/CD8

Dependent Variables	B	$\beta$	$\beta^2$	p
D 25-OH (ng/ml)	9,748	0,499	0,249	0,001
Hemoglobin (g/dl)	1,288	0,384	0,147	0,000
BMI (kg/m <sup>2</sup> )	1,048	0,154	0,024	0,000
CD4/CD8	0,118	0,237	0,056	0,000

The effect of vitamin D3 on D 25-OH remained significant ( $p = 0.001 < 0.05$ ) by controlling for the effect of age, sex and baseline value. The regression coefficient (B) of 9.748 indicates that the value of D 25-OH in patients who were given vitamin D3 for 3 months was 9.748 ng/ml higher than in patients who were not given vitamin D3. The magnitude of the effect contribution ( $\beta^2$ ) of vitamin D3 on D 25-OH was 0.249 or 24.9%.

The effect of vitamin D3 on hemoglobin was still significant ( $p = 0.000 < 0.05$ ) controlling for the effect of age, sex and baseline value. The regression coefficient (B) of 1.288 indicates that the hemoglobin value in patients who were given vitamin D3 for 3 months was 1.288 g/dl higher than in patients who were not given vitamin D3. The magnitude of the effect contribution ( $\beta^2$ ) of vitamin D3 on hemoglobin was 0.147 or 14.7%.

The effect of vitamin D3 on BMI remained significant ( $p = 0.000 < 0.05$ ) by controlling for the effect of age, sex and baseline value. The regression coefficient (B) of 1.048 indicates that the BMI value of patients who were given vitamin D3 for 3 months was 1.048 kg/m<sup>2</sup> higher than patients who were not given vitamin D3. The magnitude of the effect contribution ( $\beta^2$ ) of vitamin D3 on BMI was 0.024 or 2.4%.

The effect of vitamin D3 on CD4/CD8 was still significant ( $p = 0.000 < 0.05$ ) controlling for the effect of age, sex and baseline values. The regression coefficient (B) of 0.118 indicates that the CD4/CD8 value in patients who were given vitamin D3 for 3 months was 0.118 higher than in patients who were not given vitamin D3. The magnitude of the effect contribution ( $\beta^2$ ) of vitamin D3 on CD4/CD8 was 0.056 or 5.6%.

This experimental study with RCT design was conducted to identify the role of vitamin D3 on serum vitamin D 25-OH levels, hemoglobin levels, body mass index (BMI), and CD4/CD8 ratio in HIV/AIDS infected patients who were already being treated with antiretrovirals (ARV).

Administration of vitamin D3 has been shown to significantly increase serum levels of vitamin D 25-OH. This is because serum D 25-OH or 25 hydroxyvitamin D is the main form of circulating vitamin D in metabolic processes. This serum is a product of the first phase of hydroxylation in the liver by vitamin D-25-hydroxylase (25-OHase). This 25-hydroxyvitamin D must then be further hydroxylated in the kidney by 25(OH)D-1-OHase (CYP27B1) to form the biologically active vitamin 1,25(OH)2D. This active form of the vitamin enhances the antimicrobial function of monocytes and macrophages, reduces adaptive immunity, and reduces the differentiation, proliferation and production of B cell immunoglobulin and also triggers apoptosis.

Data from several cohort studies indicate that HIV-infected patients overall have a body mass index (BMI) that is equal to or lower than the age-matched general population. 7 In the study Mehta et al (2011), they observed that vitamin D status was low. is associated with an increased risk of HIV-related complications including wasting (BMI < 18 kg/m<sup>2</sup>), mouth ulcers, and acute upper respiratory infections. Wasting is a feature of HIV disease and is associated with adverse HIV-related health and survival outcomes

In this study, the average initial BMI in the control group was 21.03 and in the treatment group was 22.52. After being given treatment, the average BMI in the treatment group was significantly higher than the control group, which was 23.41, while the control group that received a placebo was 21.03. In this study, administration of vitamin D3 was shown to have a significant effect on BMI which indicated an increase in body weight. These results indicate that improving immunity boosted by administration of vitamin D can prevent decreased appetite and decreased muscle mass.

Anemia is the most common complication found in HIV patients. This can occur due to inadequate intake, opportunistic infections, malignancy, medication, the effect of the HIV virus on the formation

of erythrocytes and on the CD4 immune system. Anemia in HIV-infected patients can also be caused or exacerbated by antiretroviral drug (ARV) side effects. 3 Research by Mehta et al (2010) proved that low vitamin D levels were significantly associated with an increased risk of developing HIV disease, severe anemia, and microcytosis. hypochromic in HIV-infected persons. The association between low vitamin D levels and lower hemoglobin levels or iron deficiency has also been observed in previous studies in individuals with diseases other than HIV.8

Low CD4 T lymphocytes and the appearance of opportunistic infections or cancer are signs of HIV infection. 1 In this study, administration of vitamin D3 was shown to have a significant effect on increasing the CD4/CD8 ratio, which mathematically means encouraging an increase in CD4 which increases the survival rate of HIV patients.

#### 4. Conclusion

Based on the results of the study, giving vitamin D3 2000 IU tablets can increase serum levels of vitamin D 25-OH, hemoglobin, BMI, and CD4/CD8 ratio in HIV/AIDS infected patients who have been treated with antiretrovirals (ARVs).

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