STUDY PROTOCOL

Official Title: *The Effect of Vitamin D3 Supplementation on Interleukin-6 Levels and CD4 Counts in Patients with Human Immunodeficiency Virus Receiving Antiretroviral Therapy*
NCT Number: *NCTXXXXXXXX (To be updated after registration)*
Document Date: *March 24, 2025*

This study was designed as a randomized controlled trial (RCT) with a pretest–posttest control group design. The study was conducted at Dr. Moewardi General Hospital, Surakarta, Indonesia, where patient recruitment and blood sample collection were performed. Laboratory analysis of IL-6 and CD4+ T-cell counts was carried out at the Biomedical Laboratory, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. Ethical clearance for this study was obtained from the Health Research Ethics Committee of Dr. Moewardi General Hospital.

The study population consisted of all patients diagnosed with HIV and receiving ART at Dr. Moewardi General Hospital. Study subjects were selected from this population according to eligibility criteria. Inclusion criteria were: (1) patients diagnosed with HIV stage 1 or 2; (2) receiving ART for at least six months with a tenofovir/lamivudine/dolutegravir (TLD) regimen; (3) aged >18 to <60 years; (4) willingness to participate and provide written informed consent; and (5) not currently consuming other vitamin supplements. Exclusion criteria included: (1) patients who discontinued therapy (dropout) or died during the study period; (2) presence of opportunistic infections or infections other than HIV; and (3) documented allergic reaction to vitamin D3 supplementation.

Research instruments included informed consent forms, patient identity forms, vitamin D3 supplementation (5000 IU tablets), and laboratory examination tools for IL-6 and CD4+ T-cell measurements. Eligible subjects were randomly assigned to either the intervention or control group. Written informed consent was obtained prior to participation. Eligible participants were recruited based on predefined inclusion and exclusion criteria and randomly allocated into intervention and control groups. Baseline blood samples were collected for IL-6 and CD4+ T-cell assessment. Serum IL-6 levels were measured from 3 mL of venous blood collected from the cubital vein before and after intervention, analyzed using the enzyme-linked immunosorbent assay (ELISA) method, and reported in ng/L (ratio scale). CD4+ T-cell counts were measured from 3 mL venous blood samples using the latex immunoturbidimetry method on the ADVIA 1800 chemistry system and reported in cells/μL (ratio scale). The intervention group received oral vitamin D3 supplementation at a dose of 5000 IU daily for three months,

while the control group received standard antiretroviral therapy (ART) without additional vitamin D3 supplementation. Follow-up measurements of IL-6 and CD4+ T-cell counts were conducted after completion of the three-month intervention period. The study design assumed relative homogeneity of baseline population characteristics, and outcome variables were measured before and after treatment exposure to assess changes attributable to the intervention.

STATISTICAL ANALYSIS PLAN

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Data analysis began with assessment of normality using the Shapiro–Wilk test due to the sample size being fewer than 50 subjects. A p-value >0.05 indicated normal distribution, whereas $p<0.05$ indicated non-normal distribution. If data were normally distributed, parametric tests were applied, including the independent t-test to compare differences between control and intervention groups, and the paired t-test to assess within-group pretest–posttest changes. If data were not normally distributed, non-parametric alternatives were used: the Mann–Whitney U test replaced the independent t-test, and the Wilcoxon signed-rank test replaced the paired t-test. Statistical significance was determined at $p<0.05$.

To evaluate the magnitude of the intervention effect, effect size was calculated using Cohen's d, providing clinical interpretation beyond statistical significance. Cohen's d values were categorized as follows: 0.00–0.20 (very small effect), 0.21–0.50 (small effect), 0.51–0.80 (moderate effect), 0.81–1.30 (large effect), and >1.30 (very large effect). Additionally, analysis of covariance (ANCOVA) was performed to statistically control for potential confounding variables, particularly baseline differences between groups ($p<0.05$), thereby enhancing the validity of comparisons and isolating the true effect of vitamin D3 supplementation on IL-6 levels and CD4+ T-cell counts.

INFORMED CONSENT FORM

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GOVERNMENT OF CENTRAL JAVA PROVINCE

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CONSENT TO PARTICIPATE IN RESEARCH / INFORMED CONSENT

PATIENT LABEL

Patient Name: _____
Date of Birth / Sex: _____
Medical Record Number: _____
Address: _____

Room: _____
Date: _____
Time: _____

(Please fill in or attach patient label if available)

PATIENT STATEMENT

I, the undersigned (patient information):

Name: _____
Date of Birth / Sex: _____
Medical Record Number: _____
Address: _____

If the patient is under 21 years old / unable to receive information or provide consent due to other reasons:

Therefore, the hospital may obtain consent from a parent, spouse, closest family member, or legal guardian.

LEGAL REPRESENTATIVE (IF APPLICABLE)

I, the undersigned:

Name: _____
Date of Birth / Sex: _____
Address: _____

Relationship with patient:
☐ Wife ☐ Husband ☐ Child ☐ Father ☐ Mother ☐ Other: _____

INFORMATION STATEMENT

After receiving both verbal and written information regarding the research and fully understanding the benefits, procedures, advantages, and potential discomforts of the study conducted by:

Name: _____
Institution: _____
Title of Study: _____

For the purpose of:
☐ Scientific Paper ☐ Undergraduate Thesis ☐ Master Thesis ☐ Dissertation ☐ Other: _____

CONSENT STATEMENT

I hereby voluntarily agree to participate in this research.

I understand that:

- I may withdraw from the study at any time without penalty
- My decision will not affect the quality of healthcare services I receive
- I have received sufficient explanation regarding this study

This statement is made consciously and without any coercion from any party.

Surakarta, _____ Time: _____

Researcher
(Signature & Printed Name)

Participant / Representative
(Signature & Printed Name)