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Vitamin D and Hemostasis Parameters in Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Patients with Pulmonary Tuberculosis Coinfections

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Abstract

BACKGROUND: Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) remains one of the most common causes of Vitamin D deficiency and homeostasis disorders due to its progressiveness and complications.

AIM: This study aims to determine the relationship of Vitamin D levels with hemostasis in HIV/AIDS patients with and without pulmonary tuberculosis (TB), who were consuming efavirenz (EFV)-based antiretroviral therapy (ART) for <6 months, with or without rifampicin-based antituberculosis treatment.

METHODS: 25(OH)D concentration, prothrombin time (PT), and platelet index were measured in HIV/AIDS patients with and without pulmonary TB, who were consuming EFV-based ART for <6 months, with or without rifampicin-based antituberculosis treatment. This study was conducted in the Special Treatment Centers (*Pusat Pelayanan Khusus*, *Pusyansus*) Voluntary Counseling and Testing clinic at Rumah Sakit Umum Pusat (RSUP) Haji Adam Malik, Medan, Indonesia, between August and October 2019.

RESULTS: We found no significant difference in terms of 25(OH)D concentration, PT, and platelet index between the two groups, except for platelet distribution width (PDW) differs significantly between HIV/AIDS-pulmonary TB group and HIV/AIDS only group ($p = 0.026$). We observed a significant difference in terms of mean platelet volume and PDW between baseline and after treatment for <6 months ($p \leq 0.05$) in the HIV/AIDS-pulmonary TB group and in the HIV/AIDS only group. A significant difference was also observed in terms of platelet count ($p = 0.021$) before and after EFV-based ART for <6 months $p \leq 0.05$) in the HIV/AIDS-pulmonary TB group.

CONCLUSION: There is no correlation between 25(OH)D concentration and PT or platelet index in HIV/AIDS patients with and without pulmonary TB who were consuming EFV-based ART- and rifampicin-based antituberculosis for <6 months.

Introduction

Human Immunodeficiency Virus (HIV)/Acquired immunodeficiency syndrome (AIDS) remains a global problem to date [1]. With the development of effective ARV therapy (ART), HIV/AIDS had evolved into a chronic illness [2]. The morbidity and mortality of HIV/AIDS patients are often associated with coinfections. One of the most common coinfections in HIV/AIDS patients is pulmonary tuberculosis (TB). Problems related to abnormal coagulation had also been reported in HIV/AIDS patients [1]. The acute inflammation process experienced by HIV patients may trigger abnormalities in the coagulation process due to stimulation of the extrinsic rather than the intrinsic pathway. Endothelial dysfunction in HIV/AIDS infection, leading to the activation and consumption of coagulation factor which results in coagulopathy [3]. Age, cluster of differentiation-4 (CD4) concentration, viral load, opportunistic infections, medications, and viral factors may play a role in the coagulation abnormalities experienced by HIV/AIDS patients. Complications due to these abnormalities may include cardiovascular disease, stroke, thromboembolism, among others, and may even cause an increase in HIV/AIDS mortality. Abnormalities in coagulation may be observed through changes in platelet counts, as well as changes in coagulation factors, both extrinsic and intrinsic [1].

Platelet plays an important role in hemostasis [3]. As an effector immune or inflammatory cell platelet releases the inflammatory mediators, interacting with external organisms, and increasing vascular permeability, and leads to hemostasis abnormality and thrombosis, as well as atherosclerosis. In chronic inflammation, an increase in platelet consumption causes an increase in mean platelet volume (MPV). MPV is a marker of platelet activation connecting the inflammation process to thrombosis. The higher the MPV, the more reactive and thrombogenic the hemostasis [4], [5]. Compared to

platelets with a smaller size, larger platelets have more granules, are more aggregative in nature, possess a higher concentration of thromboxane A2, and express more glycoprotein Ib and IIb/IIIa receptors [6]. In addition, platelet indices also consist of plateletcrit (PCT) which refers to a ratio between platelet volume and whole blood volume as well as platelet distribution width (PDW) which determines the distribution of particle size in platelets [7].

Previous studies have reported a deficiency of micronutrients in HIV/AIDS patients, including changes in Vitamin D concentration. Aside from maintaining calcium homeostasis in the human body, Vitamin D is also known to play a role in the natural and adaptive immune response [2]. Vitamin D concentration within the body is affected by general factors, including genetics, exposure to ultraviolet light, skin color, culture, seasons, as well as latitude [8]. Inflammation may interfere with renal hydroxylation, leading to a decrease in Vitamin D concentration [9].

HIV/AIDS infection also decreases the body's immune response and the patients are prone to coinfections, with pulmonary TB being the most common. The incidence of Vitamin D deficiency increases not only in patients with TB but also in normal people [10], [11], [12] HIV-TB coinfection is also associated with Vitamin D deficiency [13]. The use of antituberculosis regiments such as isoniazid and rifampicin may interfere with the metabolism of Vitamin D, in addition to ARV. Efavirenz (EFV) and rifampicin are strong inducers of CYP2B6 and CYP3A enzymes, which are known to be associated with Vitamin D metabolism. Rifampicin is a more potent inducer compared to EFV in reducing 25(OH)D concentration. Induction of CYP3A causes catalysis of 4-hydroxylation 25(OH)D and contributes to Vitamin D deficiency. In administering cotherapy of ART and antituberculosis, one must pay attention to potential drug interaction and excessive toxicity [14].

Low Vitamin D level is associated with an increased risk for cardiovascular diseases, bone diseases, infections, HIV progressivity, and mortality [2], [14]. Vitamin D deficiency may result in calcium deposition in smooth muscle cells, affecting the renin-angiotensin system, disturbs glucose control, and increases pro-inflammatory cytokines. This indirectly impacts hypertension and cardiac risks [6].

Vitamin D also plays a role in hemostasis. $1,25(OH)_2D$ influences coagulation effects through upregulating the expression of anticoagulant glycoprotein (thrombomodulin) and through downregulating the expression of coagulation factors tissue factor on monocytes. In addition, $1,25(OH)_2D$ also inhibits the effect of tumor necrosing factor (TNF)-α which upregulates HIV RNA transcription in CD4 cells with latent infection [15], [16], [17].

In Vitamin D deficiency, inflammatory response, pro-inflammatory cytokine (TNF-α and interleukin-6) levels, platelet reactivity, and MPV are all increased [6], [18] Vitamin D is able to shift hemostasis toward fibrinolysis and decreases coagulation [19].

Due to varying results in previous studies on the relationship between Vitamin D level and hemostasis parameters, this study aims to compare Vitamin D level and coagulation abnormality in HIV/AIDS patients with and without pulmonary TB coinfection treated with EFV-based ART with and without rifampicin-based antituberculosis.

Materials and Methods

Study design

This is an analytic descriptive study with a cross-sectional approach conducted from August to October 2019 in Special Treatment Centers (*Pusyansus*) Voluntary Counseling and Testing (VCT) Clinic at Rumah Sakit Umum Pusat (RSUP) Haji Adam Malik, Medan, Indonesia. The study involved HIV/AIDS patients with and without pulmonary TB coinfection, aged ≥20 years, who were given EFV-based ART- and rifampicin-based antituberculosis for <6 months. Exclusion criteria include history of Vitamin D supplement consumption, history of anticoagulant consumption, history of thromboembolism, history of chronic kidney disease, and history of cirrhosis, increased in liver function markers for more than 5 times normal values, and pregnancy.

Diagnosis of pulmonary TB in study subjects was based on clinical signs and symptoms as well as diagnostic work-ups such as positive sputum smear in acid-fast bacilli staining or positive acid-fast bacilli culture. Other diagnostic work-ups include GeneXpert *Mycobacterium tuberculosis* – rifampicin (MTB-RIF) or polymerase chain reaction as well as chest X-ray examinations with features suggestive of pulmonary TB. Among the many problems in establishing the diagnosis of pulmonary TB are difficulties in producing sputum for bacteriological examination, especially the inability to produce sputum with good quality and in a sufficient amount.

Data collection and procedure

The sample was collected after obtaining Ethical Clearance for Health Research from the Medical and Health Research Ethics Committee of Faculty of Medicine, Universitas Sumatera Utara/RSUP Haji Adam Malik, Medan, Indonesia [Reference No: 625/TGL/KEPK FK USU-RSUP HAM/2019]. Anthropometry parameters were measured, including weight and height, and the subjects were subsequently asked to complete the questionnaire. Data related to currently consumed ART and antituberculosis as well as the duration of ART were obtained from the medical records.

Measurement of serum Vitamin D 25(OH) D levels was conducted using chemiluminescent microparticle immunoassay ARCHITECT 25-OH Vitamin D. Based on US Endocrine Society Classification, levels of 25(OH)D were classified as Vitamin D deficiency (≤20 ng/mL), Vitamin D insufficiency (21-29 ng/mL), and Vitamin D sufficiency (≥30 ng/mL).

Complete blood count was measured with a hematology analyzer using flow cytometer principles. Results were considered normal if: Platelet count is between 150,000 and 450,000/μL, MPV 6.5–9.5 fL, PCT 0.10–0.50%, and PDW between 10.0 and 18.0%.

Prothrombin time (PT) was measured using semi-automatic Coatron, which recorded clotting time. PT was normal between 11 and 14 s and was considered prolonged if exceeding normal PT time by 2 s.

One subject with clinical HIV/AIDS-pulmonary TB in the current study was found to have thrombocytopenia, but the measuring device was unable to measure MPV, PDW, and PCT; thus, the subject was excluded from this study.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 25.0. Variables were described using proportion for categorical variables and using mean and standard deviation as well as the median and interquartile range (IQR) for numerical variables with normal and non-normal distribution, respectively.

Statistical significance between study groups was determined using χ^2 and Fisher tests for categorical variables with normal and non-normal distribution, respectively, as well as non-paired t-test and Mann–Whitney test for numerical variables with normal and non-normal distributions, respectively. Statistical significance of paired numerical variables was tested using paired t-test and Wilcoxon test for data with normal and non-normal distribution, respectively. $p < 0.05$ was considered significance.

Results

A total of 70 subjects fulfilled the inclusion criteria, with 33 subjects having HIV/AIDS-pulmonary TB treated with EFV-based ART- and rifampicin-based antituberculosis as well as 37 subjects with HIV/AIDS only, treated with EFV-based ART. There was no statistically significant difference in terms of mean age and gender between the two groups. We also observed no statistically significant difference in terms of the duration of EFV-based ART between the two groups (Table 1).

Platelet index, PT, and Vitamin D levels were evaluated by categorizing these parameters based on pulmonary TB coinfection. We found a statistically significant difference in terms of PDW level between the two groups ($p \leq 0.05$), while MPV ($p = 0.099$) and PCT ($p = 0.625$) showed no statistically significant difference. The level of 25(OH)D ($p = 0.855$) and PT ($p = 0.206$) also showed no statistically significant difference between the two study groups (Table 2).

Clinical characteristics, Vitamin D levels, and laboratory examinations categorized based on TLC levels on ART can be seen in Table 3. We found a statistically significant difference in terms of BMI ($p = 0.032$) and PDW ($p = 0.050$). There was no statistically significant difference in terms of 25(OH)D levels ($p = 0.88$) and PT ($p = 0.858$) between the two study groups.

Table 4 showed that most of the subjects treated with EFV-based ART- and rifampicin-based antituberculosis had deficiency Vitamin D (51.5%) and subjects treated with EFV-based ART only had insufficiency Vitamin D (48.6%). Most of the subjects in the two study groups had normal PT (11–15 s). We found that 5 of 50 subjects had prolonged PT, and none with shortened PT, but these were not statistically significant. Most of the subjects in the group treated with EFV-based ART- and rifampicin-based antituberculosis had normal platelet counts. Two subjects had high platelet counts, and 1 had low platelets. All subjects in

Table 1: Demographic and clinical characteristics in the study groups

Parameters	EFV-based ART + RIF-based antituberculosis (n=33)	EFV-based ART (n=37)	p-value
Sex, n (%)			
Male	28 (84.8)	29 (78.4)	0.699
Age, median (IQR)	33 (22–52)	34 (23–68)	0.416
WHO Stage, n (%)			
I/II	0 (0)	13 (35.1)	0.001
III/IV	33 (100)	24 (64.9)	
BMI (kg/m^2)	20.98 (2.72)	22.58 (2.87)	0.020
Duration of ART treatment (days), median (IQR)	112 (13–209)	116 (29–203)	0.702

IQR: Interquartile range, WHO: World Health Organization, BMI: Body mass index, ART: Antiretroviral therapy, EFV: Efavirenz, RIF: Rifampicin, p≤0.05 is considered statistically significant.

Table 2: Vitamin D and laboratory measurements in the study groups

Parameters	EFV-based ART + RIF-based antituberculosis (n=33)	EFV-based ART (n=37)	p-value
TLC, median (IQR)	1669.92 (126.65–3582.26)	2080.80 (1088.51–4019.80)	0.017
Platelet (/μL), mean (SD)	313121.21 (91443.21)	296081.08 (62478.61)	0.372
MPV (fL), mean (SD)	8.86 (0.68)	9.13 (0.68)	0.099
MPV/PLT, median (IQR)	2.77×10^{-5} (1.70×10^{-5}–10.00×10^{-5})	3.09×10^{-5} (2.05×10^{-5}–5.60×10^{-5})	0.202
PCT (%), mean (SD)	0.28 (0.09)	0.27 (0.05)	0.625
PDW (%), mean (SD)	8.80 (1.04)	9.38 (1.07)	0.026
25(OH)D (ng/mL), mean (SD)	22.406 (9.47)	22.78 (9.44)	0.855
PT (s), median (IQR)	12.00 (11.00–21.00)	12.20 (11.0–16.4)	0.206

IQR: Interquartile range, SD: Standard deviation, TLC: Total lymphocyte count, MPV: Mean platelet volume, MPV/PLT: Mean platelet volume/platelet count, PCT: Plateletcrit, PDW: Platelet distribution width, 25(OH)D: 25-hydroxycholecalciferol/Calcidiol, PT: Prothrombin Time, p≤0.05 is considered statistically significant.

the other group had normal platelet counts (p = 0.177). In terms of MPV, 90.9% subjects treated with EFV-based ART- and rifampicin-based antituberculosis had normal platelet size, with the rest having enlarged platelet. In the other group, 73% subjects had normal platelet size and the rest 27% had enlarged platelets. There was no statistically significant difference in terms of MPV between the two groups. Most of the subjects in the two study groups had low PDW and we found no statistically significant difference. We also observed no statistically significant difference in terms of PCT between the two study groups (p > 0.05).

There was no statistically significant difference in terms of mean 25(OH)D levels in the two groups (even when broken down into Vitamin D deficiency, insufficiency, and sufficiency) (Table 5).

The comparison of platelet count, MPV, PCT, and PDW before and after treatment with EFV-based ART is presented in Table 6. Fifty-five subjects had complete records of MPV, PCT, and PDW, with 29 subjects treated with EFV-based ART- and rifampicin-based antituberculosis and 26 treated with EFV-based ART only. We observed statistically significant differences in terms of MPV and PDW (p ≤ 0.05) before and after treatment with ART. The difference of platelet count before and after treatment with EFV-based ART was statistically significant in the EFV-based ART only group (p = 0.021), but not in the other group. There was no statistically significant difference in terms of PCT in all study groups before and after EFV-based ART (p > 0.05).

PDW was found to be higher in subjects with 25(OH)D level of ≥21 ng/mL in both study groups, but this was not statistically significant. In contrast, higher platelet count was found in subjects with 25(OH)D level of <21 ng/mL in both study groups. This was also not statistically significant (Table 7).

Discussion

Various things may affect Vitamin D levels in HIV/AIDS patients with and without pulmonary TB

Table 3: Clinical characteristics, Vitamin D, and laboratory measurements in the study groups with TLC on ART <1200 and TLC on ART ≥1200

Parameters	TLC on ART <1200 (n=11)	TLC on ART ≥1200 (n=59)	p-value
IMT (kg/m^2), mean (SD)	20.12 (2.75)	22.14 (2.83)	0.032
Platelet (/µL), mean (SD)	295818.18 (111053.88)	305661.02 (70581.97)	0.700
MPV (fL), mean (SD)	8.95 (0.84)	9.01 (0.66)	0.770
MPV/PLT, median (IQR)	2.99x10^{-5} (1.94 x10^{-5}-10.00x10$^{-5)}$	3.09x10^{-5} (0.83 x10^{-5}-1.70x10^{-5})	0.916
PCT (%), mean (SD)	0.26 (0.11)	0.27 (0.06)	0.780
PDW (%), mean (SD)	8.52 (1.12)	9.21 (1.05)	0.050
25(OH)D (ng/mL), mean (SD)	22.96 (8.81)	22.54 (8.4)	0.880
PT (s), median (IQR)	11.4 (11–21)	12.2 (11–16.2)	0.858

IQR: Interquartile range, SD: Standard deviation, TLC: Total lymphocyte count, MPV: Mean platelet volume, MPV/PLT: Mean platelet volume/Platelet count, PCT: Plateletcrit, PDW: Platelet distribution width, 25(OH)D: 25-hydroxycholecalciferol/Calcidiol, PT: Prothrombin time, p≤0.05 is considered statistically significant.

Table 4: Category of Vitamin D concentration, PT, and platelet index throughout the period of ART consumption

Parameters	EFV-based ART + RIF-based Antituberculosis (n=33)		EFV-based ART (n=37)		p-value
	n	%	n	%	
25(OH)D (ng/mL)					
Deficiency (≤20)	17	51.5	15	40.5	0.078
Insufficiency (21–29.9)	8	24.2	18	48.6	
Sufficiency (≥30)	8	24.2	4	10.8	
PT (s)					
Shortened (<11)	0	0	0	0	0.555
Normal (11–15)	31	93.9	34	91.9	
Prolonged (>15)	2	6.1	3	8.1	
Platelet (/µL)					
Low (<150,000)	1	3	0	0	0.177
Normal (150,000–450,000)	30	90.9	37	100	
High (>450,000)	2	6.1	0	0	
MPV (fL)					
Low (<6.5)	0	0	0	0	0.106
Normal (6.5–9.5)	30	90.9	27	73	
High (>9.5)	3	9.1	10	27	
PDW (%)					
Low (<10)	28	84.8	26	70.3	0.244
Normal (10–18)	5	15.2	11	29.7	
High (>18)	0	0	0	0	
PCT (%)					
Low (<0.1)	1	3	0	0	0.471
Normal (0.1–0.5)	32	97	37	100	
High (>0.5)	0	0	0	0	

BMI: Body mass index, ART: Antiretroviral therapy, EFV: Efavirenz, RIF: Rifampicin. 25(OH)D: 25-hydroxycholecalciferol/Calcidiol, PT: Prothrombin time, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, p≤0.05 is considered statistically significant.

Table 5: 25(OH)D concentration and Vitamin D status

Parameters	Total (n=70)	EFV-based ART + RIF-based Antituberculosis (n=33)	EFV-based ART (n=37)	p-value
25(OH)D (ng/mL), mean (SD)	22.60 (8.40)	22.96 (8.81)	22.54 (8.4)	0.880
Vitamin D deficiency	15.53 (4.09)	15.18 (4.32)	15.92 (3.91)	0.618
Vitamin D insufficiency	25.10 (2.30)	24.01 (2.18)	25.60 (2.24)	0.108
Vitamin D sufficiency	36.05 (5.04)	36.15 (4.08)	35.85 (7.36)	0.928

ART: Antiretroviral therapy, EFV: Efavirenz, RIF: Rifampicin, 25(OH)D: 25-hydroxycholecalciferol/Calcidiol, Vitamin D deficiency: 25(OH)D ≤20 ng/mL, Vitamin D insufficiency: 25(OH)D 21-29.9 ng/mL, Vitamin D sufficiency: 25(OH)D ≥30 ng/mL, p≤0.05 is considered statistically significant.

Table 6: Comparison of platelet counts, MPV, PCT, and PDW before and after ART administration in the study groups

Parameters	Total (n=55)			EFV-based ART + RIF-based Antituberculosis (n=29)			EFV-based ART (n=26)		
	Baseline	On ART	P value	Baseline	On ART	P value	Baseline	On ART	P value
Platelet (/µL), mean (SD)	297200.00 (114324.98)	305345.45 (81363.66)	0.534	319344.83 (139077.39)	312965.52 (91261.59)	0.783	272500.00 (73243.84)	296846.15 (69488.82)	0.021
MPV (fL), mean (SD)	9.63 (1.10)	9.04 (0.70)	<0.001	9.68 (1.26)	8.86 (0.71)	0.001	9.57 (0.93)	9.23 (0.64)	0.036
PCT (%)	0.28 (0.10)	0.28 (0.08)	0.580	0.30 (0.12)	0.28 (0.09)	0.217	0.26 (0.06)	0.27 (0.06)	0.11
PDW (%), median (IQR)	10.20 (7.00-18.00)	9.20 (7.00-11.30)	<0.001	10.00 (7.00-18.00)	8.80 (7.00-11.20)	<0.001	10.20 (8.00-16.00)	9.65 (7.60-11.30)	0.004

IQR: Interquartile range, SD: Standard deviation, ART: Antiretroviral therapy, EFV: Efavirenz, RIF: Rifampicin. PT: Prothrombin time, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, p≤0.05 is considered statistically significant.

Table 7: Relationship between Vitamin D concentration with PT and platelet index between study groups

Parameters	EFV-based ART + RIF-based Antituberculosis (n=33)			EFV-based ART (n=37)		
	25 (OH)D (ng/mL)		p-value	25(OH)D (ng/mL)		p-value
	<21 (n=17)	≥21 (n=16)		<21 (n=15)	≥21 (n=22)	
Hemoglobin (g/dL), mean (SD)	12.48 (1.84)	13.23 (2.20)	0.295	13.84 (1.96)	13.83 (1.57)	0.989
PT (seconds), median (IQR)	12.20 (11.0-21.0)	11.85 (11.00-13.80)	0.561	12.00 (11.0-15.00)	12.60 (11.00-16.40)	0.088
Platelet (/µL), mean (SD)	338647.06 (90680.03)	2860000.00 (86859.27)	0.099	319200.00 (16868.46)	280318.18 (56606.74)	0.062
MPV (fL), mean (SD)	8.89 (0.69)	8.83 (0.69)	0.834	9.05 (0.77)	9.19 (0.62)	0.544
PCT (%), mean (SD)	0.30 (0.09)	0.25 (0.08)	0.112	0.29 (0.61)	0.26 (0.47)	0.086
PDW (%), mean (SD)	8.69 (0.98)	8.92 (1.11)	0.531	9.39 (1.22)	9.37 (0.98)	0.960

BMI: Body mass index, ART: Antiretroviral therapy, EFV: Efavirenz, RIF: Rifampicin. 25(OH)D: 25-hydroxycholecalciferol/Calcidiol, PT: Prothrombin time, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, p≤0.05 is considered statistically significant.

coinfections. A decrease in Vitamin D levels in HIV infection may be attributed to pre-existing 25(OH)D deficiency, which contributed to the incidence of HIV. MTB and HIV-1 gp120 may stimulate toll-like receptors 1/2 and induce the expression of CYP27B1 on infected macrophages. In addition, chronic inflammation in HIV infection and induction of pro-inflammatory cytokines such as TNF-α may cause inhibition to renal 1α-hydroxylase, which in turn causes a decrease in parathyroid hormone's (PTH) ability to convert 25(OH) D into 1,25$(OH)_2$D, leading to accumulation of 25(OH) D. In addition, the complication may also play a role in the intensity of sunlight exposure, malnutritional, and decreased oral intake [20], [21]

ART also plays a role in decreased Vitamin D levels. EFV poses a risk for Vitamin D deficiency by increasing the catabolism of 25(OH)D and production of inactive metabolites through interaction with CYP450 enzyme in the form of CYP24A1 induction, which converts 25(OH)D into its inactive form, 24,25$(OH)_2$D, as well as reduction of CYP2R1 transcription which inhibits the hydroxylation of Vitamin D3 and Vitamin D2 [20], [21], [22].

Administration of rifampicin in HIV/AIDS-TB coinfections, which is a strong inducer for CYP3A4, affects the activation of 24,25-hydroxylase Vitamin D which leads to the inactivation of 25(OH)D. After 2 months of treatment with antituberculosis, 25(OH)D level may increase due to improvements in the patient's diet within the 1st week of antituberculosis treatment, or due to the decrease of inflammatory stimuli in the initial phase of antituberculosis treatment, leading to a decrease in 25(OH)D hydroxylation [23].

Chronic inflammation process in HIV infection, which may be aggravated by pulmonary TB infection, and the mechanism of action of EFV-based ART, which may be aggravated by treatment with rifampicin-based antituberculosis, may work together against CYP450, leading to disruption in Vitamin D metabolism. Based on this, Vitamin D level should be lower in the group treated with EFV-based ART- and rifampicin-based antituberculosis when compared to the group treated with EFV-based ART only. The actual result of this study, however, is different. We found no significant difference in terms of 25(OH)D level between the group treated with EFV-based ART- and rifampicin-based antituberculosis and the group treated without rifampicin, both with ART duration of <6 months. Studies by Mariana and Rusli [24] found no difference in plasma level of EFV in HIV/AIDS-TB patients treated with ART for 3–6 months and rifampicin when compared to HIV/AIDS without TB. They also found that the proportion of patients with viral load ≥40 copy/mL was higher in the group treated with ART and rifampicin compared to the group without rifampicin, although this was not statistically significant. We concluded that this might contribute to the result we observed in terms of 25(OH)D levels between our two study groups.

Nylen *et al*. [14] reported that there was no significant difference in the mean plasma level of 25(OH) D in the 4th and 16th weeks of treatment with EFV-based ART between HIV and HIV with TB coinfection groups. They also found that the effect of EFV in decreasing 25(OH)D level was more prominent in the EFV-based ART only group when compared to EFV-based ART with rifampicin. In their study, Havers *et al*. [8] also stated that the largest proportion of decreased Vitamin D level was observed in the group treated with ART for 0–24 weeks compared to those treated for 24–48 weeks, and this level was significantly decreased in the group treated with EFV-based ART compared to the group treated with non-EFV-based ART. Musarurwa *et al*. [20], in their study on African subjects in Zimbabwe, reported that the median serum level of 25(OH)D was significantly higher in HIV/pulmonary TB group compared with HIV without pulmonary TB group.

The current study was not able to demonstrate changes in 25(OH)D levels before and after treatment with EFV-based ART, both with and without rifampicin-based antituberculosis, due to the unavailability of baseline data on pre-ART 25(OH)D levels. Gayatri *et al*. [13], in their

study in Bali, Indonesia, also reported that serum 25(OH) D concentration was significantly lower in the HIV-TB group compared to ARV-naive HIV group. They found that hypovitaminosis D was independently associated with the development of active TB in HIV patients (OR 26.154 [90% CI: 4.371–156.541]; p < 0.001).

In the current study, there was no evidence of a statistically significant difference in terms of PT and platelet count between the two study groups. Janssen *et al*. [25] in their study stated that PT, activated partial thromboplastin time (APTT), and INR values were increased in patients with HIV-TB.

In their study, Nasir *et al*. [3] reported a significant difference in platelet count between HIV/AIDS patients treated with ART and ART-naive group (p = 0.001). They also found no significant difference in terms of PT (p = 0.358). In contrast, Ephraim *et al*. [26] reported that PT was significantly lower in subjects treated with ART compared to ART-naive (p < 0.0001) but platelet count was higher in the ART group (p > 0.05). The decrease in platelet count in HIV infection may be due to autoimmune destruction and thrombopoiesis disruption as a result of direct infection to megakaryocytes, as well as coagulopathy consumption in AIDS [1], [27].

When the platelet count in subjects with TLC <1200 was compared to those with TLC ≥1200, higher platelet count was observed in subjects with TLC ≥1200; however, this was not statistically significant. (p = 0.70). Low CD4+ level and treatment with ART are risk factors for thrombocytopenia. ART causes bone marrow suppression, while HIV infection causes disruptions in thrombopoiesis [3] In their study, Raman *et al*. [1] reported no statistically significant difference in platelet count and PT between the groups with CD4 <200 cell/mm^3 and with CD4>200 cells/mm^3 (p > 0.05). However, they found a statistically significant difference in terms of APTT (p = 0.0006).

Table 7 showed that there was no statistically significant difference in terms of PT and platelet index between subjects with 25(OH)D levels of <21 ng/mL and ≥21 ng/mL. Coşkun and Şahin [7], in their study on children aged 0–18 years, reported that there was no correlation between 25(OH)D level and platelet index. Elbers *et al*. [28] stated that there were no changes in PT after 2 months of supplementation with Vitamin D in patients with Vitamin D deficiency and secondary hyperparathyroidism. Korzonek-Szlacheta *et al*. [18], in their study on patients with stable coronary artery disease, found that MPV value was highest in the group with Vitamin D deficiency (25(OH)D <10 ng/mL) and lowest in the Vitamin D insufficiency group (25(OH)D 20–30 ng/mL) (p < 0.001). PDW was highest in the Vitamin D deficiency and moderate Vitamin D deficiency groups.

The current study has several limitations. We were not able to determine whether the patients' Vitamin D deficiency was chronic due to unavailability of baseline data, lack of baseline CD4 data, and due to the fact that we did not assess factors which are commonly associated with Vitamin D levels such as skin color, food sources, and lifestyle. In addition, most of our subjects were below 40 years old. Further studies with a larger sample size were required to determine the difference of Vitamin D level and hemostasis parameters in the two study groups.

Conclusion

We found no relationship between 25(OH) D concentration and PT or platelet index in HIV/AIDS patients with and without pulmonary TB treated with EFV-based ART- and rifampicin-based antituberculosis for <6 months.

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