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Research Article
Vitamin D Deficiency and Hematological Parameters in People Living with HIV/AIDS

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Abstract

Background and Objective: Patients with Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) infection experiences deficiency of vitamin D and abnormality of hematological parameters related to inflammatory and thrombotic activities. This study was designed to determine the relationship between changes in hematological parameters and vitamin D in HIV/AIDS infection. **Materials and Methods:** A cross-sectional study was conducted on 70 HIV/AIDS patients consuming Efavirenz (EFV)-based Antiretroviral Therapy (ART) for less than 6 months. Parameters including 25-hydroxy-vitamin D [25(OH)D] level and complete blood count. All parameters were measured in the Special Treatment Center (Pusat Pelayanan Khusus, Pusyansus) of the Voluntary Counseling and Testing (VCT) Clinic at Rumah Sakit Umum Pusat (RSUP) Haji Adam Malik, Medan, Indonesia. **Results:** There was a significant difference in terms of platelet count [mean±standard deviation: 329531.25 (79175.99) μL^{-1} vs 282710.53 (69895.25) μL^{-1}, p = 0.011], Mean Platelet Volume/Platelet count (MPV/PLT) [Median (Interquartile Range): 2.86×10^{-5} (1.70×10^{-5}-5.06×10^{-5}) vs 3.27×10^{-5} (1.94×10^{-5}-10.0×10^{-5}), p = 0.022] and plateletcrit (PCT) [mean±standard deviation: 0.29 (0.08)% vs 0.25 (0.06)%, p = 0.018] in the group with 25(OH)D level of <21 ng mL^{-1} compared to the group with 25(OH)D level of ≥21 ng mL^{-1}. There was a significant difference in terms of platelet count between the vitamin D sufficiency and insufficiency groups [mean±standard deviation: 300166.67 (71387.33) μL^{-1} vs 274653.85 (69095.84) μL^{-1}, p = 0.036]. A significant difference was found in terms of platelet distribution width (PDW) between the vitamin D deficiency and insufficiency groups [mean±standard deviation: 9.02 (1.14)% vs 9.48 (1.03)%, p = 0.020]. **Conclusion:** Low level of vitamin D significantly correlated with platelet index in HIV/AIDS patients consuming EFV-based ART.

Key words: 25-hydroxyvitamin D, complete blood count, deficiency, HIV/AIDS

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Decreased level of vitamin D in HIV infection may be due to a pre-existing deficiency of 25(OH)D which contributes to the incidence of HIV, or through chronic inflammation in HIV and induction of pro-inflammatory cytokine production may also play a role, leading to disruption in vitamin D metabolism. This decrease may also be caused by complications of infection, leading to less exposure to sunlight and inadequate nutritional intake[1,2]. Vitamin D deficiency may occur in both healthy individuals and individuals with illnesses. The incidence of vitamin D deficiency also increase in patients with tuberculosis (TB)[3-5]. Deficiency in healthy individuals is related to several risks, such as old age, living in areas with low ultraviolet radiation and a habit to avoid exposure to sunlight[6]. Medication may also play a role in decreasing vitamin D level. Medications such as EFV has been reported to contribute to decreased vitamin D level, due to it being a strong inducer of CYP2B6 and CYP3A enzymes, which were known to be associated with vitamin D metabolism. Induction of CYP3A causes catalysis of 4-hydroxylation 25(OH)D and contributes to vitamin D deficiency[7].

HIV/AIDS patients also experienced problems related to coagulation abnormalities. Age, 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 diseases, stroke, thromboembolism and even 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[8].

Anti-thrombogenic, anti-inflammatory and anticoagulant activities may be affected by vitamin D level. Low-level vitamin D is often associated with inflammation, endothelial dysfunction, higher risk of cardiovascular diseases, higher risk of infection, HIV progressivity and mortality. Persistent inflammation is often associated with low-level vitamin D and an increase in MPV[7,9-11]. Several studies have reported a correlation between vitamin D level and platelet index[6,9,11-14].

Platelets release inflammatory mediators, activate the complement factors and increase vascular permeability, contribute to blood coagulation, hemostasis and thrombosis, as well as atherosclerosis. Platelet consumption in chronic inflammation causes an increase in MPV, which is a marker of platelet activity, linking it to inflammatory and thrombotic processes. The higher the MPV, the more reactive and thrombogenic the hemostasis[11,15]. Compared to smaller platelets, larger platelets have more granules, are more aggregative in nature, possess higher concentration of thromboxane A2 and express more glycoprotein (Gp) Ib and IIb/IIIa receptors[16]. PCT and PDW are platelet parameters that provide thrombotic activities. PCT is a platelet concentration parameter and describe changes of platelet production and activity as well as thrombotic potentials[6].

In vitamin D deficiency, inflammatory response, pro-inflammatory cytokine (TNF-α and IL-6) levels, platelet reactivity and MPV are all increased[13,16]. Due to the fact and HIV infection is often accompanied by vitamin D deficiency and due to the fact that this deficiency is often correlated to abnormalities in hematological parameters, so, this study aimed to determine the relationship between changes in hematological parameters and vitamin D in HIV/AIDS infection.

MATERIALS AND METHODS

Study design: This is an analytical descriptive study using cross-sectional approach, conducted between August and October 2019 in the Special Treatment Center (*Pusat Pelayanan Khusus*, Pusyansus) of the Voluntary Counseling and Testing (VCT) Clinic in Rumah Sakit Umum Pusat (RSUP) Haji Adam Malik Medan, Sumatera Utara, Indonesia. The study involved HIV/AIDS patients with the following inclusion criteria: aged $\geq$20 years and being treated with EFV-based ART for less than 6 months. Exclusion criteria include history of vitamin D supplement consumption, history of anticoagulant consumption, history of thromboembolism, history of chronic kidney disease, a history of cirrhosis, increased in liver function markers for more than 5 times normal values, history of blood transfusion and pregnancy. Patients fulfilling the inclusion criteria will be provided with a written explanation and subsequently asked for consent to participate as subjects in the study. This study has been approved by the Health and Medical Research Ethics Committee of the Faculty of Medicine of Universitas Sumatera Utara/ RSUP Haji Adam Malik Medan with an ethical clearance certificate. [Reference Number: 625/TGL/KEPK FK USU-RSUP HAM/2019].

Data collection and procedure: Data were collected using anthropometric measurements for weight, height, to obtain body mass index (BMI) and using a questionnaire. Data related to currently-consumed ART and its duration were obtained from the medical records. Measurements of serum vitamin D 25(OH)D level were conducted using chemiluminescent microparticle immunoassay (CMIA) ARCHITECT 25-OH Vitamin D. Levels of 25(OH)D were classified based on US

Endocrine Society Classification, as vitamin D deficiency (≤20 ng mL^{-1}), vitamin D insufficiency (21-29 ng mL^{-1}) and vitamin D sufficiency (≥30 ng mL^{-1})[17].

Complete blood count was measured with Hematology Analyzer using flow cytometer principles. Results were considered normal if: hemoglobin concentration is ≥10 g dL^{-1}, White Blood Cells(WBC) 4.000-11.000 µL^{-1}, platelet count between 150,000-450,000 µL^{-1}, MPV 6.5-9.5 fL, PCT 0.10-0.50% and PDW between 10.0-18.0%.

Statistical analyses: 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 median and IQR for numerical variables with normal and non-normal distribution, respectively.

Statistical significance between study groups was determined using non-paired t-test and Mann-Whitney test for numerical variables with normal and non-normal distributions, respectively; as well as χ^2 and Fisher exact tests for categorical variables with normal and non-normal distribution, respectively. Comparative statistics analyses were conducted on more than 2 unpaired groups, with One-way ANOVA test used on data with normal distribution and Kruskal-Wallis test used on data with non-normal distribution followed by post-hoc analysis. An unpaired analysis was performed on categorical variables using crosstabs and Mann-Whitney test. Correlation tests using Pearson and Spearman correlations were conducted on numerical variables with normal and non-normal distributions, respectively. p-value of less than 0.05 was considered significant.

RESULTS

Table 1 presents a comparison between 32 subjects with 25(OH)D level of <21 ng mL^{-1} and 38 subjects with 25(OH)D level of ≥21 ng mL^{-1}. Sociodemographic and clinical characteristics were also presented in Table 1. There was no statistically significant difference in terms of gender (p = 0.785) and age (p = 0.300) between the two study groups. We found no significant difference in terms of WHO HIV stage between the group with 25(OH)D level of <21 ng mL^{-1} [stage I/II (12.5%) and stage III/IV (87.5%)] and the group with 25(OH)D level of ≥21 ng mL^{-1} [stage I/II (23.7%)] and III/IV [(76.3%); p = 0.373]. There was no significant difference in terms of BMI between the two groups (p = 0.262). No significant difference was observed in terms of sunlight exposure duration between the two groups (p = 0.144). The frequency of sunlight exposure was similar between the two groups (p = 0.364).

We found a statistically significant difference in terms of platelet count, MPV/PLT and PCT (p = 0.011; p = 0.022 and p = 0.018, respectively). Platelet and PCT were higher in the group with 25(OH)D level of <21 ng mL^{-1} while MPV/PLT was higher in the group with 25(OH)D level of ≥21 ng mL^{-1}. There was no statistically significant difference in terms of hemoglobin concentration, hematocrit, WBC count, platelet distribution width (PDW), neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) between the two study groups (Table 2).

We found a statistically significant difference in terms of age (p = 0.034), platelet count (p=0.024), MPV/PLT (p = 0.027) and PDW (p = 0.034) when comparing between vitamin D deficiency, insufficiency and sufficiency (Table 3).

There was no statistically significant difference between vitamin D status and hemoglobin concentration, Total Lymphocyte Count (TLC), platelet count, MPV and PCT (p>0.05); however, we found a statistically significant difference between vitamin D status and PDW (p = 0.024) (Table 4). A total of subjects experienced anemia, one in the vitamin D deficiency group and 1 in the vitamin D sufficiency group. Twelve subjects had low TLC across the three vitamin D status groups. Higher platelet count was observed in 2 subjects belonging to the vitamin D deficiency group. High MPV was also observed in 13 subjects belonging to the vitamin D deficiency and insufficiency groups. Low PDW was observed in 54 subjects across the three vitamin D status groups. Low PCT was observed in 1 subject from the vitamin D insufficiency group.

We also found no relationship between BMI, age, hemoglobin concentration, hematocrit, Neutrophil to Lymphocyte Ratio (NLR) and Platelet to Lymphocyte Ratio (PLR) with platelet count. We found a positive correlation between BMI and MPV and negative correlation between NLR-PLR and MPV (p<0.05). We found negative correlation between age and PCT and positive correlation between WBC count as well as PLR and PCT (p<0.05). BMI, hemoglobin concentration and hematocrit were positively correlated with PDW (p<0.05) (Table 5).

Across the study groups, we found statistically significant difference in terms of vitamin D level with duration of sunlight exposure (p = 0.007) with mean 25(OH)D level of 19.97 ng ng mL^{-1} in the group with <30 min sunlight exposure/day and 25.39 ng mL^{-1} in the group with ≥30 min sunlight exposure/day. In relation to frequency of sunlight exposure, we found no statistically significant difference in terms of vitamin D level across the three sunlight exposure frequency groups (p = 0.280). Mean 25(OH)D levels were 21.45, 21.15, 21.15 and 24.63 ng mL^{-1} in the groups with rare

Table 1: Sociodemographic and clinical characteristics of HIV/AIDS subjects with 25(OH)D levels of <21 and ≥21 ng mL^{-1}

	25(OH)D <21 ng mL^{-1} (n = 32)		25(OH)D ≥21 ng mL^{-1} (n = 38)			
Parameters	No.	Percentage	No.	Percentage	p-value	OR
Sex						
Male	27	84.4	30	78.9	0.785	1.440
Female	5	15.6	8	21.1		
Age						
>40 years old	5	15.6	11	28.9	0.300	0.455
≤40 years old	27	84.4	27	71.1		
Education						
Higher	29	90.6	30	78.9	0.314	2.578
Lower	3	9.4	8	21.1		
Occupation						
None	9	28.1	10	26.3	1.000	1.096
Working	23	71.9	28	73.7		
Mode of transmission						
Homosexual	5	15.6	3	7.9	-	-
Heterosexual	17	53.2	21	60.6		
IDU or tattoo	1	3.1	1	2.6		
Blood transfusion	1	3.1	1	2.6		
Unknown	8	25.0	10	26.3		
Marital status						
Married	21	65.6	11	28.9	0.005	4.686
Unmarried	11	34.4	27	71.1		
WHO stage						
I/II	4	12.5	9	23.7	0.373	0.460
III/IV	28	87.5	29	76.3		
Body mass index (BMI)						
BMI ≥25 kg m^{-2}	5	15.6	3	7.9	0.262	2.160
BMI <25 kg m^{-2}	27	84.4	35	92.1		
Duration of ART						
>90 days	21	65.6	26	68.4	1.000	0.881
≤90 days	11	34.4	12	31.6		
Duration of exposure to sunlight						
<30 minutes day^{-1}	20	62.5	16	42.1	0.144	2.292
≥30 minutes day^{-1}	12	37.5	22	57.9		
Frequency of exposure to sunlight						
1-3 times week^{-1} and 4-5 times week^{-1}	22	68.8	21	55.3	0.364	1.761
6-7 times week^{-1}	10	31.3	17	44.7		

25(OH)D: 25-hydroxycholecalciferol/Calcidiol, IDU: Injection drug users, WHO: World health organization, BMI: Body mass index, ART: Antiretroviral therapy, EFV: Efavirenz, RIF: Rifampicin, p≤0.05 is considered statistically significant

Table 2: Laboratory examination characteristics of HIV/AIDS subjects with 25(OH)D levels of <21 and ≥21 ng mL^{-1}

Parameters	25(OH)D <21 ng mL^{-1} (n = 33)	25(OH)D ≥21 ng mL^{-1} (n = 37)	p-value
Hemoglobin (g dL^{-1}), mean (SD)	13.12 (1.99)	13.58 (1.86)	0.320
Hematocrit (%), mean (SD)	38.97 (4.99)	39.61 (4.82)	0.590
WBC (μL^{-1}), median (IQR)	6750 (3050-11240)	6620 (3230-16250)	0.883
Neutrophil (%), mean (SD)	51.65 (10.46)	56.29 (12.27)	0.097
PLT (μL^{-1}), mean (SD)	329531.25 (79175.99)	282710.53 (69895.25)	0.011
Lymphocytes (%), mean (SD)	32.71 (9.80)	28.07 (9.92)	0.054
TLC, mean (SD)	2206.28 (922.75)	1861.17 (755.05)	0.090
MPV (fL), mean (SD)	8.96 (0.72)	9.04 (0.66)	0.642
MPV/PLT, median (IQR)	2.86×10^{-5} (1.70×10^{-5}-5.06×10^{-5})	3.27×10^{-5} (1.94×10^{-5} -10.0×10^{-5})	0.022
PCT (%), mean (SD)	0.29 (0.08)	0.25 (0.06)	0.018
PDW (%), mean (SD)	9.02 (1.14)	9.18 (1.05)	0.534
NLR, median (IQR)	1.66 (0.52-4.46)	1.92 (0.84-89.44)	0.191
PLR, median (IQR)	8970.16 (5405.41-21497.01)	8844.92 (4594.38-439325.84)	0.962

IQR: Interquartile Range, SD: Standard deviation, WBC: White blood cell, 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, NLR: Neutrophil to lymphocyte ratio, PLR Platelet to lymphocyte ratio, p≤0.05 is considered statistically significant

Table 3: Demographic, clinical and laboratory examination characteristics in vitamin D deficiency, insufficiency and sufficiency groups

	25(OH)D (ng mL^{-1})			
	Deficiency (≤20) (n = 32)	Insufficiency (21-29) (n = 26)	Sufficiency (≥30) (n = 12)	p-value
Age (years), median (IQR)	28.00 (22.00-52.00)	34.00 (24.00-68.00)	36.50 (22.00-66.00)	0.034[a]
BMI (kg m^{-2}), mean (SD)	21.65 (3.25)	22.09 (2.87)	21.73 (1.95)	0.848
Duration of ART treatment (days), mean (SD)	111.06 (57.05)	108.23 (55.79)	126.08 (56.00)	0.651
Hemoglobin (g dL^{-1}), mean (SD)	13.12 (1.99)	13.86 (1.78)	12.97 (1.95)	0.253
Hematocrit (%)	38.97 (4.99)	40.38 (4.29)	37.92 (5.65)	0.305
WBC (µL^{-1}), median (IQR)	6750 (3050-11240)	6800 (4180-16250)	6035 (3230-9580)	0.312
Neutrophil (%), mean (SD)	51.65 (10.46)	56.47 (12.26)	55.89 (12.83)	0.252
PLT (µL^{-1}), mean (SD)	329531.25 (79175.99)	274653.85 (69095.84)	300166.67 (71387.33)	0.024[b]
Lymphocytes (%), mean (SD)	32.71 (9.80)	28.67 (10.19)	26.78 (9.60)	0.137
TLC, mean (SD)	2206.28 (922.75)	1993.99 (803.06)	1573.39 (565.57)	0.085
MPV (fL), mean (SD)	8.96 (0.72)	9.19 (0.68)	8.72 (0.53)	0.134
MPV/PLT, median (IQR)	2.86×10^{-5} (1.70×10^{-5}-$5.06\times10\times^{5}$)	3.50×10^{-5} (1.94×10^{-5}-10.0×10^{-5})	2.80×10^{-5} (2.15×10^{-5}-4.78×10^{-5})	0.027[c]
PCT (%), mean (SD)	0.29 (0.08)	0.25 (0.06)	0.26 (0.06)	0.058
PDW (%), mean (SD)	9.02 (1.14)	9.48 (1.03)	8.53 (0.80)	0.034[d]
25(OH)D (ng mL^{-1}), mean (SD)	15.53 (4.09)	25.10 (2.30)	36.05 (5.04)	<0.001
NLR, median (IQR)	1.66 (0.52-4.46)	1.71 (0.84-89.44)	2.16 (0.84-8.44)	0.390
PLR, median (IQR)	8970.16 (5405.41-21497.01)	8576.82 (4595.38-439325.84)	11888.20 (4826.18-18541.67)	0.326

[a]Kruskal-Wallis Test. Mann-Whitney Analysis: Deficiency vs. Insufficiency p = 0.073, Deficiency vs. Sufficiency p = 0.020, Insufficiency vs. Sufficiency p = 0.256. [b]One-way Anova Test. Bonferroni *post-hoc* analysis: Deficiency vs. Insufficiency p = 0.299, Deficiency vs. Sufficiency p = 0.534, Insufficiency vs. Sufficiency p = 0.036. [c]Kruskal-Wallis Test. Mann-Whitney Analysis: Deficiency vs. Insufficiency p = 0.009, Deficiency vs. Sufficiency p = 0.474, Insufficiency vs. Sufficiency p = 0.137. [d]One-way anova test. Bonferroni *post-hoc* analysis: Deficiency vs. Insufficiency p = 0,020, Deficiency vs. Sufficiency p = 0.741, Insufficiency vs. Sufficiency p = 0.986. IQR: Interquartile Range, SD: Standard deviation, BMI: Body mass index, WBC: White blood cell, 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, NLR: Neutrophil to lymphocyte ratio, PLR Platelet to lymphocyte ratio, p≤0.05 is considered statistically significant

Table 4: Hematological parameter categories based on vitamin D status across study groups

		25(OH)D (ng mL^{-1})						
		≤20		21-29		≥30		
		No.	Percentage	No.	Percentage	No.	Percentage	p-value
Hemoglobin (g dL^{-1})	Anemia (<10)	1	3.1	0	0.0	1	8.3	0.702
	No anemia (≥10)	31	96.9	26	100.0	11	91.7	
TLC	Low (<1200)	4	12.5	4	15.4	3	25.0	0.372
	Normal (≥1200)	28	87.5	22	84.6	9	75.0	
Platelet (µL^{-1})	Low (<150,000)	0	0.0	1	3.8	0	0.0	0.381
	Normal (150,000-450,000)	30	93.8	25	96.2	12	100.0	
	High (>450,000)	2	6.3	0	0.0	0	0.0	
MPV (fL)	Low (<6.5)	0	0.0	0	0.0	0	0.0	0.111
	Normal (6.5-9.5)	26	81.3	19	73.1	12	100.0	
	High (>9.5)	6	18.8	7	26.9	0	0.0	
PDW (%)	Low (<10)	26	81.3	16	61.5	12	100.0	0.024
	Normal (10-18)	6	18.8	10	38.5	0	0.0	
	High (>18)	0	0.0	0	0.0	0	0.0	
PCT (%)	Low (<0,1)	0	0.0	1	3.8	0	0.0	1.000
	Normal (0,1-0,5)	32	100.0	25	96.2	12	100.0	
	High (>0,5)	0	0.0	0	0.0	0	0.0	

25(OH)D: 25-hydroxycholecalciferol/Calcidiol, TLC: Total lymphocyte count, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, p≤0,05 is considered statistically significant

exposure (1-3 times week^{-1}), moderate exposure (4-5 times week^{-1}) and frequent exposure (6-7 times week^{-1}), respectively. We also found no statistically significant difference in terms of vitamin D levels with BMI of ≥25 kg m^{-2} (p = 0.190) with mean 25(OH)D level of 18.93 ng mL^{-1} in the group with BMI of ≥25 kg m^{-2} and 23.08 ng mL^{-1} in the group with BMI of <25 kg m^{-2}. No statistically significant difference was found in terms of vitamin D levels with duration of ART treatment (p = 0.342) with mean 25(OH)D level of 23.27 ng mL^{-1} in the group with duration of ART treatment >90 days and 21.23 ng mL^{-1} in the group with duration of ART treatment ≤90 days.

Table 5: Correlation between platelet index and independent variables

	PLT		MPV		PCT		PDW	
	r	p-value	r	p-value	r	p-value	r	p-value
BMI	-0.155	0.201	0.337	0.004	-0.053	0.662	0.445	<0.001
Age	-0.210	0.081	0.010	0.936	-0.268	0.025	0.039	0.750
Duration of ART treatment (days)	-0.058	0.631	0.143	0.239	-0.020	0.869	0.198	0.100
Hemoglobin	-0.210	0.081	0.035	0.772	-0.205	0.088	0.344	0.004
Hematocrit	-0.145	0.231	0.031	0.799	-0.107	0.380	0.343	0.004
WBC	0.201	0.096	-0.098	0.418	0.247	0.040	0.104	0.393
Neutrophil	-0.117	0.334	0.011	0.925	-0.108	0.372	-0.101	0.405
Lymphocytes	0.016	0.897	0.057	0.637	0.029	0.813	0.221	0.066
TLC	0.147	0.225	0.016	0.895	0.151	0.212	0.282	0.018
NLR	0.113	0.350	-0.244	0.042	0.030	0.803	-0.196	0.104
PLR	0.185	0.125	-0.252	0.036	0.584	<0.001	-0.202	0.093

r: Clinical significance, PLT: Platelet, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, WBC: White blood cell, TLC: Total lymphocyte count, ART: Antiretroviral therapy, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, $p \leq 0.05$ is considered statistically significant

DISCUSSION

Vitamin D has anti-inflammatory properties due to a direct connection between vitamin D and antimicrobial peptide hepcidin which regulates systemic iron concentration. In addition, pro-inflammatory cytokines may disrupt erythropoiesis through inhibition of erythropoietin production as well as through the disruption of erythroid progenitor cell differentiation and proliferation[18]. The current study did not find significant difference in terms of hemoglobin concentration across the study groups. This is similar to a study by Ernst *et al.*[19] which reported no statistically significant difference in terms of hemoglobin concentration before and after supplementation of 2800 IU cholecalciferol for 8 weeks in patients with hypertension and vitamin D deficiency. However, a study by Kim *et al.*[20] on patients with End-Stage Renal Disease reported a significant positive correlation ($\beta = 0.292$; $p = <0.001$) between hemoglobin concentration and serum level of 25(OH)D. They also found that the risk of anemia was higher in patients with 25(OH)D level of <10 ng dL^{-1} compared to patients with 25(OH)D level of ≥10 ng dL^{-1}.

A decrease in platelet count in HIV infection may be due to autoimmune destruction as a result of direct infection to megakaryocytes, as well as coagulopathy consumption in AIDS[8]. Vitamin D deficiency may also trigger platelet reactivity[13,16]. A study by Park *et al.*[11] on general population found that platelet counts were higher in the group with vitamin D deficiency compared to groups with vitamin D sufficiency and insufficiency. A similar result was found in the current study, where platelet count was significantly higher in the group with 25(OH)D level of <21 ng mL^{-1} compared to the group with 25(OH)D level of ≥21 ng mL^{-1}. In post-hoc analysis, no statistically significant difference was found in terms of platelet count between the group with vitamin D deficiency and vitamin D insufficiency.

Continuous inflammation in vitamin D deficiency triggers the release of pro-inflammatory cytokines (TNF-α and IL-6), leading to oxidative stress and megakaryopoiesis, which results in the production of larger sized platelets[9,11]. The current study found no statistically significant difference in terms of MPV between the groups with 25(OH)D levels of <21 and ≥21 ng mL^{-1} and between the groups with vitamin D deficiency, sufficiency and insufficiency. A contrasting result was reported in a study by Erkus *et al.*[9] where a statistically significant difference was found in terms of MPV between the group with vitamin D deficiency and the group with normal vitamin D level ($p<0.001$). The study stated that MPV value (>6.22 fL) may be used to predict vitamin D deficiency (AUC 0.77). A study by Korzonek-Szlacheta *et al.*[13] on patients with stable coronary artery disease found that MPV value was highest in the group with 25(OH)D <10 ng mL^{-1} and lowest in the group with 25(OH)D 20-30 ng mL^{-1}. It was stated that MPV (>10.5 fL) had moderate ability (AUC 0.70) to predict vitamin D deficiency and the level of 25(OH)D (≤15.5 ng mL^{-1}) had low ability to (AUC 0.65) predict an increase in MPV. A study by Park *et al.*[11] on general population reported that MPV was significantly higher in the group with vitamin D deficiency compared to the groups with vitamin D sufficiency and insufficiency. Another study by Cure *et al.*[16] found that MPV was significantly lower in the groups with vitamin D deficiency and insufficiency and MPV was negatively correlated with vitamin D level.

There was a significant difference in terms of PCT between the groups with 25(OH)D level of <21 ng mL^{-1} and 25(OH)D ≥21 ng mL^{-1} in the current study. Esen *et al.*[6] conducted a regression analysis and reported that PCT was

most significantly correlated with vitamin D level in patients with End-Stage Renal Disease (ESRD) receiving Renal Replacement Therapy (RRT). Al-Nimer and Salih[12] reported that vitamin D3 supplementation in women with iron deficiency anemia may significantly reduce platelet count, plateletcrit and MPV by 7.4, 18.1 and 11.5%, respectively.

The current study found a significant difference in terms of PDW between the groups with vitamin D insufficiency and sufficiency, after post-hoc analysis. MPV/PLT was significantly different between the study groups with 25(OH)D level of <21 and ≥21 ng mL^{-1}. A similar result was observed when comparing the groups with vitamin D insufficiency and deficiency during *post-hoc* analysis. In a study by Korzonek-Szlacheta *et al.*[13] on patients with stable coronary artery disease, PDW was found to be highest in the groups with vitamin D deficiency and moderate vitamin D deficiency. MPV/PLT ratio was highest in the groups with vitamin D deficiency and moderate vitamin D deficiency. In contrast, Coşkun and Şahin[14]. in their study on healthy children aged 0-18 years reported that there was no correlation between 25(OH)D level and platelet index (platelet, PCT, PDW and MPV).

The current study found no significant difference in terms of NLR and PLR between the groups with 25(OH)D level of <21 ng mL^{-1} and 25(OH)D ≥21 ng mL^{-1}. However, Akbas *et al.*[21] in their study reported a significant difference in terms of PLR and NLR between the group with vitamin D level of <20 and ≥20 ng mL^{-1}. According to a study by Erkus *et al.*[9] NLR (>1.69) was found to be able to predict vitamin D deficiency (AUC 0.72). In addition, vitamin D3 supplementation may significantly lower PLR in the group of women with iron deficiency anemia receiving ferrous sulfate and vitamin D3 supplementation compared to the group receiving ferrous sulfate only[12].

The current study has several limitations. First, this is a cross-sectional study and therefore unable to determine the cause of lack of vitamin D. Second, the study disregards coinfections in the study groups. Third, the sample size was not adequate to illustrate correlation. Fourth, most of the subjects were below 40 years of age.

CONCLUSION

This study provided an overview regarding the role of vitamin D level on hematological parameters in HIV/AIDS patients consuming EFV-based ART due to association with inflammatory and thrombotic activities. From the results of this study we can conclude that low level of vitamin D significantly correlated with platelet index in HIV/AIDS patients consuming EFV-based ART. Further studies with a larger sample size are needed to generate a more precise conclusion.

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