Impact of high-dose vitamin D3 supplementation on hematological, hepatic and immune biomarkers, and viral load in patients infected with human immunodeficiency virus: A randomized, double-blind, multicenter controlled trial

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Abstract. Vitamin D plays an essential role in certain critical physiological processes, such as in the control of infections. The present study aimed to evaluate the impact of vitamin D supplementation on hematological, immunological and hepatic biomarkers, as well as viral load in patients infected with human immunodeficiency virus (HIV). A multicenter, double-blind, randomized, placebo-controlled clinical trial (1:1) was conducted according the Consolidated Standards of Reporting Trials recommendations. Following screening, 95 patients diagnosed with HIV and who were on antiretroviral therapy were randomly assigned to two groups as follows: The experimental group (n=48) supplemented with 100,000 IU vitamin D3, and the placebo group (n=47) treated with 1 ml olive oil, for 24 weeks. The results revealed a significant improvement (P<0.001) in the circulating vitamin D concentration, the total count of CD4 cells, leukocytes, lymphocytes, platelets, neutrophil percentage, hemoglobin concentration and hematocrit values in the vitamin D3-supplemented group compared to the placebo group. However, no significant differences (P>0.05) were observed between both groups (the vitamin D3-supplemented group and the placebo group) regarding the levels of alkaline phosphatase, serum bilirubin, serum glutamic-oxaloacetic and glutamic-pyruvic transaminases, as well as the HIV viral load. Althogether, these findings indicated that nutritional supplementation with 100,000 IU vitamin D3 enhanced immune cell counts (CD4) and selected hematological parameters in HIV-infected individuals under antiretroviral treatment. These results support the potential role of targeted vitamin D supplementation as an adjunctive strategy to optimize immune-related hematological indices in this population.

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Introduction

Since the epidemic of human immunodeficiency virus (HIV), >84 million individuals have been infected with the virus worldwide, with >40 million related deaths (1). Patients who have HIV not only experience the deterioration of their immune system, but also the worsening of the gastrointestinal system, with deficient nutrient absorption, as well as nutrient metabolism (2). This critical condition affects their overall quality of life (QoL) and intensifies the progression of the disease. One of the metabolic disorders associated with the prognosis of HIV infection is the malabsorption of vitamin D (Vit-D), resulting in Vit-D deficiency (VDD). Previous

studies have demonstrated that the majority of HIV-infected individuals have deficient levels (<20 ng/ml) or insufficient levels (<30 ng/ml) of Vit-D (3,4). Although the development of antiretroviral therapy (ART), such as efavirenz has been a revolutionary innovation for HIV-infected individuals, no improvements have been documented regarding Vit-D absorption and metabolism (5).

Among HIV-positive individuals, VDD is linked to high levels of inflammation caused by the upregulation of inflammatory markers, including stimulated monocyte phenotypes ($CCR2^+$ and CX3CR1), tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 (6). This condition further leads to the development of comorbidities, tissue dysfunction, the progression of autoimmune deficiency syndrome (AIDS), and ultimately, in premature death in HIV-infected individuals (7).

Vit-D is a pro-hormone that exerts anti-inflammatory effects (8). Optimal levels of Vit-D are not only required for the proper absorption of phosphorous and calcium (9), but also they play a critical role in other critical physiological, muscle, aging, skeletal and non-skeletal processes (10), including the prevention of tumor growth, and the control of infections, allergies and autoimmune diseases, the production of antibodies, the improvement of flexibility and strength in muscle, and the normal regulation of blood clotting and thyroid function (11-15). It is well-established that cells displaying the cluster of differentiation 4 (CD4) are greatly influenced by HIV infection, presenting as a high viral load (VL), count and other blood profile parameters. Previous studies have demonstrated an association between VDD and suboptimal levels of CD4 cell counts, impaired T-cell function, and increased levels of inflammatory markers among HIV-infected individuals undergoing ART (16,17).

Even with ART, HIV-infected individuals present suboptimal levels of Vit-D. This condition has been shown to be associated with the progression of comorbidities, including tuberculosis (TB), diabetes mellitus, cardiovascular diseases and osteoporosis (14,18). HIV infection itself is a risk factor for the premature development of numerous diseases, whereas VDD can be a catalyst for these pathological processes. Given the role of Vit-D in maintaining the adaptive, as well as innate immune response, the present study aimed to examine the hypothesis that Vit-D supplementation plays a crucial role in improving the CD4-cell count, blood count (CBC) and levels of liver markers among HIV-infected individuals undergoing ART.

Patients and methods

Study design and setting. A multicenter, double-masked, parallel-group, individually randomized placebo-controlled clinical trial for HIV-diagnosed cases treated with ART was conducted in Lahore (Pakistan), according to the 'Consolidated Standards of Reporting Trials' (CONSORT) (19) for parallel-group randomized trials. In the present study, HIV-positive patients prescribed with ART in the HIV treatment centers of three major public sector hospitals in Lahore, Pakistan, were recruited with a 1:1 allocation ratio. The study was conducted in those hospitals that host HIV/AIDS treatment centers operated by the Punjab AIDS Control Program. These hospitals include Mayo Hospital, Jinnah Hospital and Government-Said Mitha Teaching Hospital. Predefined inclusion and exclusion criteria were used to recruit the participants in the present study.

Inclusion and exclusion criteria. The selection of participants was based on the following inclusion criteria: i) HIV-positive patients undergoing ART who were ≥18 years of age; and ii) Vit-D levels <50 ng/ml. The following exclusion criteria were defined for the study: i) A history of Vit-D supplementation at a dose >100,000 IU over the past 3 months; and ii) pregnant and lactating women.

Sample size. Considering the role of olive oil (the placebo used in the present study) in improving the lipid profile of HIV-infected individuals (20), it was assumed that 60% of patients in the control group and 85% of patients in the treatment group (25% absolute increase) would attain >30% of their baseline Vit-D status at 6 months. This allowed the authors to determine a sample of 98 participants (49 per group) with a 5% significance level, 95% confidence interval (CI) and 80% power using the following formula:

$$n\ (per\ arm) = \{z_{\frac{1-\alpha}{2}}\sqrt{\underline{p} * \underline{q}(1+\tfrac{1}{k})} + z_{1-\beta} * \sqrt{p_1 * q_1 + (\tfrac{p_2 * q_2}{k})}\}^2/\Delta^2$$

$$n\ (per\ arm) = \{1.96\sqrt{(0.725)(0.275)(1+1)} +$$

$$0.84\sqrt{0.60(0.40) + (\tfrac{0.85*0.15}{1})}\}^2/(0.25)^2$$

$$n\ (per\ arm) = \{1.96\ (0.631) + 0.84\ (0.606)\}^2/(0.25)^2$$

$$n\ (per\ arm) = \{1.237 + 0.509\}^2/(0.25)^2$$

$$n\ (per\ arm) = \{3.049\}/(0.0625)$$

$$n\ (per\ arm) = 48.78$$

where, $z_{\frac{1-\alpha}{2}}$ is the critical value for α at 95% CI (1.96), $z_{1-\beta}$ is the critical value for β at 80% power of the study (0.84), k is the allocation ratio (1), p_1 is the incidence proportion of the control group (0.60), q_1 is 1-p_1 (0.40), p_2 is the incidence proportion of the treatment group (0.85), q_2 is 1-p_2 (0.15), $\underline{p}$ is $\frac{p_1+kp_2}{1+K}$ (0.725), $\underline{q}$ is 1-$\underline{p}$ (0.275) and Δ is the absolute difference between the proportions of both groups (0.25).

The calculated sample size of 98 participants (49 per group) was then inflated 16% to account for the loss to follow-up cases, making a total sample size of 114 participants (57 per group).

Baseline (pretest) assessment. A baseline assessment was conducted on the participants who fulfilled the inclusion criteria. A structured questionnaire was administered to collect the demographic details of the participants, including their occupation, sex, marital status, education, monthly family income, exposure to sunlight and HIV screening status. Recently registered patients with HIV were recruited in the study, and a 3 ml blood sample was obtained to assess their baseline values of CD4-cell count and CBC.

Intervention. All the participants recruited in the present study were enrolled for 24 weeks. Participants allocated to the control group were provided with a placebo (1 ml commercially available extra virgin olive oil). The placebo was administered orally in the form of oil. The participants in the experimental

group received a 100,000 IU vitamin D3 ampule administered orally by trained staff in different multicentric setups. Olive oil was used as a placebo as it has a similar appearance and texture as vitamin D3 ampules, and its consumption is harmless. The commercial product name of the study medication was ED-3, manufactured by GT Pharma (Pvt.) Ltd. (https://khasmart.pk/product/ed3-injection-200000-an-oral-i-m/?srsltid=AfmBOooc9CTGGTvIkF7UR8vr7NhezQELH_l13NNx-w3r66VTX-ezE4afx). Each ampule had 200,000 IU vitamin D3 with a thick and transparent liquid texture, whereas the product name of the placebo medication was olive oil procured commercially. Both products, the placebo and Vit-D supplement, were stored in a well-closed container at room temperature (25-30˚C).

Outcomes. The primary outcome was to determine whether the proposed oral high dose of vitamin D3 is sufficient for achieving the physiological concentration (30-50 ng/ml) in HIV-positive patients under ART after 24 weeks. The secondary outcome was to determine whether this intervention improves CD4-cell count, VL count, liver markers, such as serum glutamate pyruvate transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (AP) and total serum bilirubin (SB) in the study participants. The efficacy outcome and the tertiary outcome were to determine the effects of Vit-D supplementation on the CBC [i.e., total leukocyte count (TLC) and hematocrit (HCT)], total neutrophil count, monocyte count, platelet count, hemoglobin (Hb) count and lymphocyte count] in HIV-infected patients.

Safety endpoints. The following safety endpoints were included: Mortality, pregnancy, the incidence of hyperkalemia (high levels of potassium in blood), and any adverse effects due to study Vit-D supplementation (Vit-D toxicity signs and symptoms including persistent nausea, vomiting, increased thirst, dehydration and muscle weakness).

Randomization. Once written consent was obtained, eligible participants were assigned to a screening number by the recruitment hospital. The statistician, unrelated to the study, prepared and maintained the hospital randomization list prior to the start of recruitment. Of note, one hospital randomization list and three separate participant randomization lists (one for each hospital) were prepared. The subject identification code was then designated using the screening number during the study. Equal numbers were assigned to the participants of the supplemented and placebo groups. There were no restrictions (for example, stratification or block size). A screening number was given in the format of 'XXXS###' (for example, 101S001), which indicated the following: 'XXX': Site number, 'S': Screening; '###': Order of subjects to participate at each site.

Implementation. A random allocation sequence was then generated using an Excel sheet by a researcher unrelated to the study. Equal numbers were assigned to the supplemented and placebo groups. The study pharmacy used this sequence to label pairs of ampules that contained placebo and Vit-D supplement bearing the numbers assigned to both groups. The staff assigned consecutive ID numbers to the participants based on the order in which they were enrolled, and the hospital pharmacy then supplied ampules of placebo and Vit-D supplement bearing this ID number.

Blinding. All study participants, doctors and staff nurses who enrolled participants and performed study assessments were blinded to the allocation. The Vit-D supplement and placebo with a label of ID number were presented in identical 1 ml ampules and had the same appearance and texture.

Laboratory methods. The quantification of 25(OH)D in human serum and plasma was used to diagnose Vit-D deficiency. Serum 25(OH)D concentrations were measured using chemiluminescent microparticle immunoassay (CMIA) (sensitivity 99-100%; measurement range, 0.02 to 30.5 nmol/l) technology in Chughtai Laboratories in Lahore, Pakistan. CMIA is a delayed one-step immunoassay for quantitatively determining 25(OH)D in human serum and plasma. All other biochemical testing was performed in the same laboratory following standard procedures and techniques.

Follow-up assessment. Participants in the study were allocated an HIV enrollment card. Patients were advised to return to the treatment center after the initial dose of supplementation on the 5th, 12th and 24th weeks for follow-up visits. The doses were administered orally in the 1st, 5th and 12th weeks of the study, whereas a 3-ml blood sample for outcome measurements was obtained in the 24th week. The patients were monitored at follow-up visits at the centers and were also assessed for any medical or nutritional complications and adverse events. Local community health workers tracked down participants who did not attend follow-up visits. Those who missed three consecutive visits were withdrawn from the study.

Timeline of the study. Participant enrollment began on November 22, 2019. However, due to the COVID-19 pandemic, enrollment remained very low. Enrollment ended on July 31, 2021, while the study was concluded on January 31, 2022.

Ethical considerations. The study was approved by the Punjab AIDS Control Program (D. No. PACP/Admin/26285), as well as by the Institutional Review Board (IRB), University of Punjab (D. No. 292/IIM). Written informed consent was obtained from all the participants recruited in the study. In addition, the present study is registered at clinical trial.gov (NCT05306704).

Statistical analysis. Data were analyzed using SPSS version 25 software (IBM Corp.). Frequencies and percentages were calculated for the descriptive statistics of qualitative variables, while the mean and standard deviation were calculated for quantitative variables. As all the pairs of variables were skewed distributed (the results of the normality test are presented in Table SI), to evaluate the difference in outcome for the treatment group before and after the intervention, the Wilcoxon signed-rank test was used, while assessing the difference in outcome between the treatment and placebo groups, analysis of covariance (ANCOVA) was applied. Both tests were applied at a 95% CI. A value of $P<0.05$ was considered to indicate a statistically significant difference.

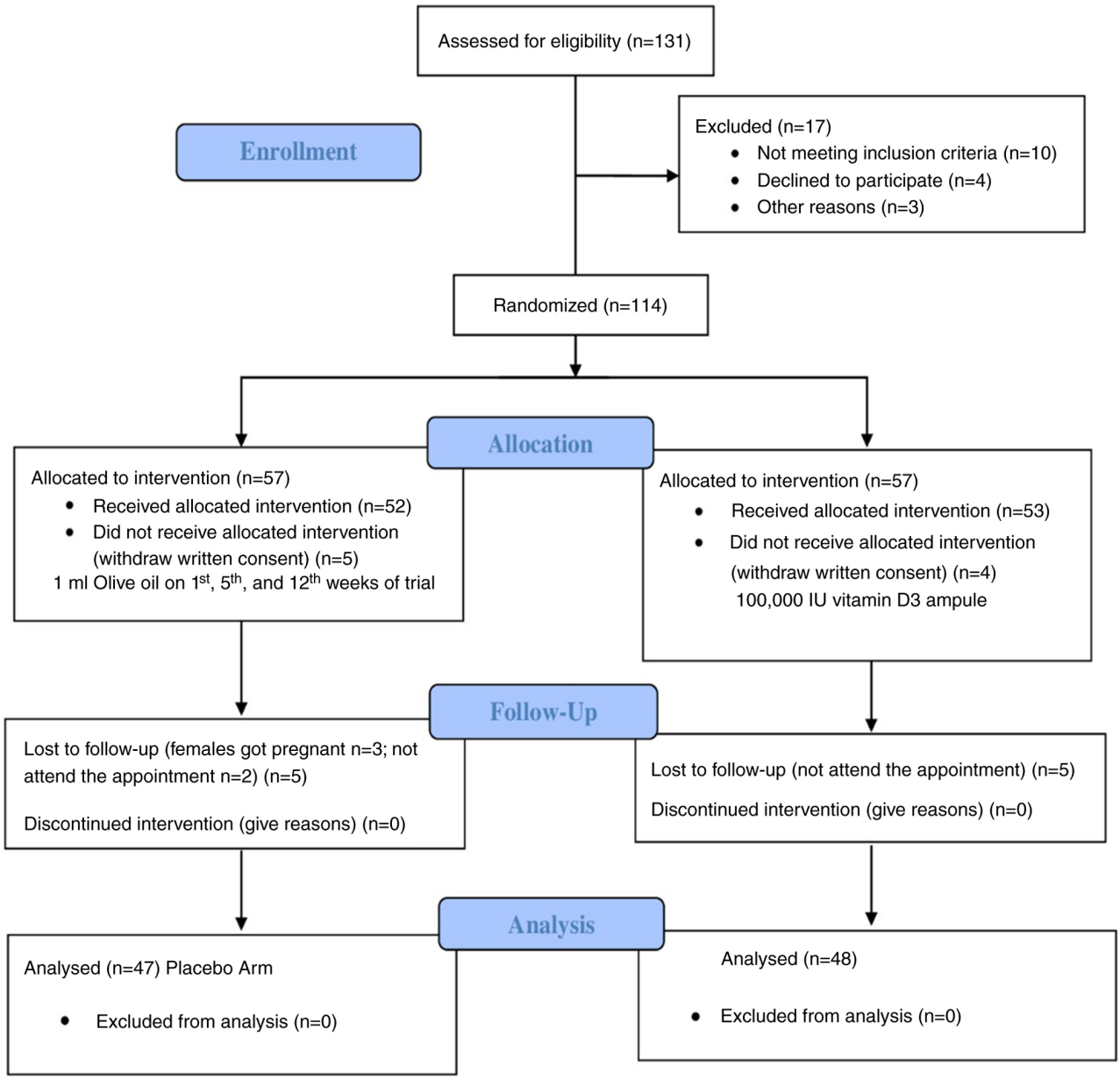

Figure 1. Flow diagram demonstrating the selection of participants according to the CONSORT criteria (19).

Results

Study participants. To enroll 114 participants in the present study, a total of 131 patients were screened. All the enrolled participants were then randomized, and 57 participants were recruited in each of the two groups, i.e., the placebo and Vit-D-supplemented groups. Nevertheless, a total of 9 participants withdrew written consent, 8 of whom were lost during the follow-up period, while 2 female participants became pregnant during the trial. Consequently, a sample of 47 participants in the placebo group and 48 participants in the Vit-D supplementation group were included in the final data analysis (Fig. 1).

Socio-demographic characteristics of the participants. The majority of the participants was male (84.2%), literate (60.0%), process/factory workers (37.9%), and married (65.3%), with only a small number of participants being exposed to regular sunlight (12.6%), with a mean age of 33.68±7.78 years (Table I). Other characteristics of the participants are also presented in Table I.

Pre and post-intervention comparisons of 25(OH)-D levels, CD4 cell count, viral load, hepatic markers and CBC for the treatment group. The analysis revealed a significant (P<0.05) difference for all parameters before and after the 25(OH)-D supplementation (Table II). Significant increases (P<0.05) were observed in the concentration of 25(OH)-D levels, CD4 cell count, SGPT, SGOT, SB, AP, Hb, TLC, HCT, % neutrophils, % lymphocytes, % monocytes, platelets and eosinophils. However, a significant (P<0.05) decrease was observed in the VL count (Table II and Fig. 2).

Differences in circulating parameters comparing the Vit-D-supplemented group to the placebo group. In a following step, the values of some of the circulating parameters analyzed

Table I. Socio-demographic characteristics of the study participants.

Socio-demographic characteristics	Placebo (n=47)			25(OH)-D (n=48)			Total		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Age (years)	19	53	33.1±7.4	19	55	34.1±8.1	19	55	33.6±7.7
Basal circulating 25(OH)-D (nmol/l)	21.0	42.9	25.0±4.8	21.0	44.4	27.8±5.3	20.0	44.4	26.9±5.1
	No. of participants		%	No. of participants		%	No. of participants		%
Sex									
Male	41		87.2	39		81.3	80		84.2
Female	3		6.4	6		12.5	9		9.5
Transgender	3		6.4	3		6.3	6		6.3
Literacy									
Literate	25		53.2	32		66.7	57		60.0
Illiterate	22		46.8	16		33.3	38		40.0
Occupation									
Skilled trade worker	12		25.5	12		25.0	24		25.3
Process/factory worker	16		34.1	20		41.7	36		37.9
Elementary worker	15		31.9	11		22.9	26		27.4
Sex worker	4		8.5	5		10.4	09		9.5
Marital status									
Married	30		63.8	32		66.7	62		65.3
Unmarried	17		36.2	16		33.3	33		34.7
Sunlight exposure									
Yes	7		14.9	5		10.4	12		12.6
No	40		85.1	43		89.6	83		87.4

Max, maximum; Min, minimum; SD, standard deviation.

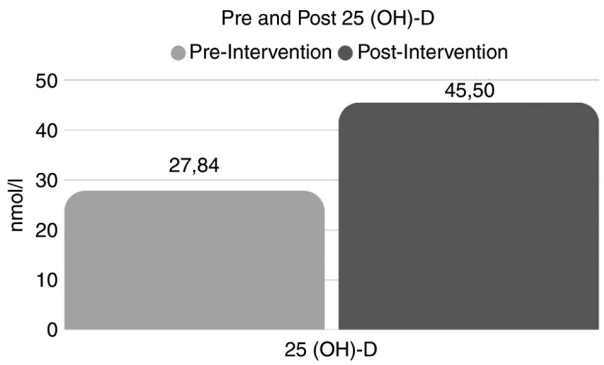

Figure 2. Mean Pre and Post 25 (OH)-D levels in treatment group.

in Table II, were compared between the Vit-D-treated group and the placebo group (Table III). The analysis revealed significantly (P<0.05) higher values in the treatment group for the 25(OH)-D concentration, CD4 cell count, Hb, TLC, HCT, % neutrophils, % lymphocytes and platelets. The comparison was performed in the patients treated with the placebo compared to the patients supplemented with Vit-D, when their mean values were adjusted for their pre-intervention mean values. However, no significant differences (P>0.05) were observed for the VL count, SGPT, SGOT, SB, AP, % monocytes and % eosinophils between the Vit-D-supplemented patients and the patients administered the placebo post-intervention. These results highlight that there was a significant (P<0.05) difference when patients undergoing ART are supplemented with Vit-D, suggesting that Vit-D supplementation is more beneficial than ART alone.

Adverse events. No adverse events were reported during the duration of the trial.

Discussion

Similar to previous studies (21-23), the present study also confirmed insufficient levels of Vit-D among HIV-infected individuals undergoing ART (28.08±5.40 nmol/l). The primary source of Vit-D is exposure to sunlight. Although Pakistan is a country with high levels of sunlight exposure (Lahore has >70% high-intensity sunny daylight hours per year). However, the majority of the participants recruited in the present study reported no sunlight exposure (85.1%). This reduced exposure could be due to weak health conditions and concomitant infections caused by HIV (24).

Table II. Comparison of 25(OH)-D circulating levels, CD4-positive cells, viral load, liver markers, and complete blood count for the Vit-D-treated group.

Pair nos.	Pairs	V1[a] Mean ± SD	V4[b] Mean ± SD	Z Score	P-value
Pair 1	Pre 25 (OH)-D - post 25 (OH)-D (nmol/l)	27.84±5.32	45.50±8.74	-6.208	<0.001
Pair 2	Pre CD4 - post CD4 (cells/mm^3)	436.96±140.75	642.85±264.76	-5.694	<0.001
Pair 3	Pre VL - post VL (viral particles/ml)	10942.85±17646.20	740.13±945.60	-4.038	<0.001
Pair 4	Pre SGPT - post SGPT (U/l)	34.10±17.87	37.84±14.12	-3.112	0.002
Pair 5	Pre SGOT - post SGOT (U/l)	35.67±19.11	41.72±22.31	-4.108	<0.001
Pair 6	Pre serum bilirubin - post serum bilirubin (mg/dl)	0.60±0.22	1.02±1.07	-4.147	<0.001
Pair 7	Pre-AP - post-AP (U/l)	205.18±115.46	329.44±252.92	-4.657	<0.001
Pair 8	Pre Hb - post Hb (g/dl)	13.14±1.87	14.96±1.59	-4.978	<0.001
Pair 9	Pre TLC - post TLC (cells/l)	5.87±2.20	8.57±2.57	-5.498	<0.001
Pair 10	Pre HCT - post HCT (%)	37.22±9.72	46.08±5.54	-5.126	<0.001
Pair 11	Pre neutrophil - post neutrophil (%)	45.54±12.67	57.45±10.61	-5.549	<0.001
Pair 12	Pre lymphocytes - post lymphocytes (%)	30.83±10.41	38.24±7.36	-4.298	<0.001
Pair 13	Pre monocytes - post monocytes (%)	5.42±3.25	7.46±3.03	-4.657	<0.001
Pair 14	Pre platelet - post platelet (platelets/nl)	230.10±94.03	321.81±98.32	-4.684	<0.001
Pair 15	Pre eosinophil - post eosinophil (%)	2.17±1.49	3.91±1.73	-4.764	<0.001

[a]Baseline values for the treatment group; [b]endline values for the treatment group. A P-value <0.05 was considered to indicate a statistically significant difference. AP, alkaline phosphatase; Hb, hemoglobin; HCT, hematocrit; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; TLC, total leukocyte count; VL, viral load.

Previous research has also exhibited the stable persistence of CD4-positive cells as a significant obstacle to the treatment of HIV with ART (25). This condition occurs together with suboptimal levels of other circulating parameters. Taken together, this is a challenging issue among patients with HIV undergoing ART. The present study also revealed suboptimal levels of CD4 cell count, VL count, SGOT, SGPT, SB, AP, Hb, TLC, HCT, neutrophils, lymphocytes, monocytes and platelet counts in HIV-infected individuals on ART. However, after administering the corresponding dose of Vit-D, there was a significant (P<0.05) improvement in circulating Vit-D levels, CD4 cell count, Hb, TLC, HCT, neutrophils, lymphocytes and platelet counts.

Previous similar studies (22,26,27) have also demonstrated the critical role of Vit-D supplementation in improving physiological concentrations among HIV-infected individuals. The study conducted by Piloya *et al* (22) found that daily Vit-D supplementation for 16 weeks was well-tolerated and effectively improved Vit-D levels among HIV-infected individuals. However, no significant (P>0.05) changes were observed in the CD4 cell count. By contrast, some studies claim the role of Vit-D supplementation in reducing the CD4-cell count among HIV-infected individuals under ART (26,27). However, the present study found a significant (P<0.05) role of Vit-D supplementation in achieving optimal levels for CD4-positive cells. Moreover, the present study suggests an association between Vit-D supplementation and improved enzyme/immune levels, including Hb, TLC, HCT, neutrophil percentage, lymphocyte percentage and platelets.

Further, the Vit-D-treated arm exhibited a significant within-group reduction in VL; however, the between-group comparison did not yield significant results. This pattern is consistent with prior HIV randomized controlled trials where Vit-D supplementation corrected deficiency and modestly improved immune indices without producing a clear between-group VL benefit once baseline values and ART effects were accounted for. For example, a previous randomized trial found vitamin D3 did not lower the risk of unsuppressed VL after 6 months of ART (RR 1.10; 95% CI, 0.87-1.41), despite biochemical repletion, suggesting any observed within-group VL decline (28). This likely reflects ART optimization, regression-to-the-mean, or adherence effects rather than a direct antiviral action of Vit-D alone (26,28,29).

In the present study, mild elevations in SGPT and SGOT were observed in the Vit-D-supplemented group. Such changes in the levels of transaminases are commonly reported among individuals living with HIV undergoing ART and are frequently attributed to drug-induced hepatocellular stress or underlying infection, rather than overt liver injury. For instance, studies in African settings have demonstrated a low incidence of clinically significant SGPT and SGOT elevations during ART, suggesting that mild enzyme elevations may often reflect subclinical effects or systemic inflammation rather than true hepatic damage (30,31). Nevertheless, given that liver enzyme elevations were observed, even if mild, this finding highlights the need for the ongoing monitoring of hepatic function when high-dose Vit-D is administered in ART-treated populations, to ensure early identification and management of potential hepatotoxicity.

Similarly, the post-intervention AP level exceeded the conventional adult reference range. AP is a composite enzyme with hepatic and bone isoforms; increased bone-specific AP is common during vitamin D repletion due to enhanced osteoblastic activity and bone remodeling (24). Efavirenz and

Table III. Comparison of outcomes for treatment and control group post-intervention.

Pairs	Mean		Mean difference	F-value	P-value
	Treatment group	Control group			
25 (OH)-D (nmol/l)	41.41	36.24	5.16	12.18	<0.001
CD4-cells (cells/mm^3)	789.32	635.0	154.32	16.32	<0.001
VL (viral particles/ml)	742.10	668.48	73.62	0.118	0.732
SGPT (U/l)	37.89	34.42	3.47	1.29	0.259
SGOT (U/l)	38.28	33.94	4.33	2.03	0.158
SB	1.02	0.70	0.32	3.13	0.08
AP (U/l)	321.58	255.53	66.05	2.01	0.160
Hb (g/dl)	14.87	13.73	1.14	12.85	<0.001
TLC (cells/l)	8.68	6.68	2.00	24.54	<0.001
HCT (%)	46.16	40.84	5.32	16.75	<0.001
Neutrophils (%)	57.49	50.04	7.45	11.26	<0.001
Lymphocytes (%)	37.80	32.49	5.31	7.85	<0.01
Monocytes (%)	7.380	6.457	0.922	0.95	0.089
Platelets (platelets/nl)	314.84	255.79	59.05	9.981	<0.01
Eosinophils (%)	3.93	3.41	0.513	1.55	0.217

F-value, refers to the f statistics of the ANCOVA test applied; A P-value <0.05 was considered to indicate a statistically significant difference. AP, alkaline phosphatase; Hb, hemoglobin; HCT, hematocrit; SB, Serum bilirubin; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; TLC, total leukocyte count; VL, viral load.

tenofovir exposure has also been associated with elevated AP levels in HIV-positive individuals (5), possibly through effects on bone metabolism. While no adverse events were reported herein, future studies should consider AP isoenzyme fractionation and concurrent bone turnover markers to differentiate hepatic from skeletal sources.

Nutritional deficiencies cause the onset and progression of various diseases, including AIDS, by dysregulating the immune system. Similarly, Vit-D has a marked effect on the regulation of adaptive, as well as innate immune responses. Although ART is considered to be a revolutionary treatment for patients with HIV as it prolongs their life and improves their QoL, it cannot be neglected that the initiation of some ARTs has been associated with VDD in previous studies (24). Therefore, the present study strongly suggests that the setup of certain ARTs would be implemented with Vit-D supplementation, particularly in Vit-D-deficient patients. Optimal levels of Vit-D have antimicrobial, anti-inflammatory and immunomodulatory properties that reduce the risk of acquiring a number of infections (16,17).

In summary, in the present study, high-dose vitamin D3 supplementation in Vit-D-deficient adults living with HIV and undergoing ART resulted in significant improvements in CD4 cell counts and hematological indices, without significant between-group differences in VL or hepatic markers. The absence of calcium measurements is a limitation however, as high-dose Vit-D may, in rare cases, cause hypercalcemia; future studies are thus required to incorporate calcium and renal function monitoring. The selected 100,000 IU bolus dose was based on previous randomized controlled trials and aligns with the Endocrine Society's recommendations for deficiency correction, remaining within the safe upper intake level equivalent (<10,000 IU/day) (24,28,29). The findings of the present study therefore suggest a supportive, although not curative, role of Vit-D in enhancing selected immune and hematologic outcomes in this context. Optimal levels of Vit-D in the human body control the production of Th17 cells, Th-1-associated cytokines and T-lymphocytes, and encourage the proliferation of Th-2-associated cytokines. Notably, Vit-D supports antiviral and antibacterial immunity. It also attenuates disease progression among HIV-infected individuals and should, therefore, be made a part of the regular treatment of HIV.

The present study has some limitations which should be mentioned. First, the present study was not a dose-response study. Therefore, it is unclear whether a lower dose than the one used would help improve the studied parameters. Second, although no adverse events were encountered during the study, biochemical safety monitoring and calcium status were not performed with the high-dose of Vit-D supplementation. The increase observed in the levels of transaminases was likely due to the ART, increasing with Vit-D supplementation. Strategies to preserve liver function need to be implemented (further research is required in this context). Furthermore, the present study experienced an attrition rate of 17%, the initial sample size was prospectively increased by ~16% to account for anticipated loss to follow-up, based on previous HIV trial experiences in similar settings. Attrition was balanced between intervention and control groups, and baseline characteristics did not differ significantly between completers and non-completers. Therefore, the risk of attrition bias is considered minimal, and a per-protocol analysis was retained to reflect the physiological effect of high-dose Vit-D in participants who completed the regimen. While

this approach may limit generalizability to all individuals initiating supplementation, it provides a robust estimate of efficacy under ideal adherence conditions. Finally, the present study did not examine the long-term effects of high-dose Vit-D supplementation and the sustainability of improved parameters, as the study period was relatively short. Therefore, further trials need to be conducted with long follow-ups and different dosages in diverse settings.

In conclusion, Vit-D supplementation in deficient HIV-infected individuals undergoing ART significantly improved CD4 cell counts and selected hematological parameters, without producing significant changes in VL or hepatic markers. Nutritional deficiencies can cause dysregulation in the immune system, while nutritional supplementation can help protect individuals from infections by regulating the immune system. In this context, Vit-D supplements play an instrumental role.

While these results support the potential of Vit-D as a safe, low-cost adjunct to optimize immune-related hematologic outcomes, further research with longer follow-up periods, calcium monitoring and varied dosing regimens is warranted to confirm and extend these findings.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

FM, JS, RS, HWA, MY and JF carried out the investigation and methodology. ER performed data curation and was involved in the writing of the manuscript. AMCSM was involved in the formal analysis and data validation. DFL was involved in study supervision, in the conceptualization of the study, in project administration and in the writing of the original draft of the manuscript. Authors FM and JS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Punjab AIDS Control Program (D. No. PACP/Admin/26285) as well as by the Institutional Review Board (IRB), University of Punjab (D. No. 292/IIM). Written informed consent was obtained from all the participants recruited in the study, and they were informed that they could withdraw their participation from the study at any point in time. The present study is also registered at clinicaltrial.gov. The NCT number of this study on clinical trial.gov is 05306704. All subjects provided written informed consent in accordance with the Declaration of Helsinki and the 2013 Fortaleza revision..

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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