

Prevalence of vitamin D deficiency in PLHIV and its relation to CD4 count and ART: A cross sectional study

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Abstract: *Introduction:* HIV (Human Immunodeficiency Virus) continues to be a major global public health issue with no cure. Vitamin D is a fat-soluble hormone that is majorly involved in the classical function of calcium and phosphorus hemostasis and bone mineralization as well as non-classical functions of immune modulation in various viral and autoimmune diseases. A combination of both traditional risk factors, HIV-specific and antiretroviral therapy (ART)-specific contributors leave HIV-infected persons (PLHIV) at a greater risk for low 25-OH-Vitamin D levels and frank vitamin D deficiency. *Aims and Setting:* The current study was conducted to assess and characterize the prevalence of Vitamin D deficiency in PLHIV-on-ART attending a tertiary care hospital and assess the factors that may be affecting it. *Methods:* 95 PLHIV registered at an ART center were selected over a period of 6 months based on Inclusion and Exclusion criteria. Flow cytometry estimation of CD4 count and ELISA based quantitative assessment of serum 25-OH Vitamin D3 were done along with detailed clinical examination. $P < 0.05$ was considered to be statistically significant. *Results:* About half of the PLHIV assessed were deficient in vitamin D. Severe vitamin D deficiency was noted in one-fourth of subjects. Serum vitamin D levels were significantly less in subjects on ZLN regime compared to TLE regime. No significant difference was found between vitamin D deficiency and duration of treatment, different treatment regimens or differing CD4 counts. No significant association of serum levels of Vitamin D with duration of treatment or varying CD4 count was found. *Conclusion:* There is greater prevalence of subnormal levels of Vitamin D in PLHIV on ART. ZLN regime appears to have a negative impact on Vitamin D levels in comparison to TLE regimen. More research needs to be done to further evaluate the physiology of Vitamin D in PLHIV on ART.

Keywords: HIV, Internal Medicine, Infectious Disease Medicine, Medicine, Nutritional Sciences, PLHIV, PLHA, Vitamin D.

Introduction

HIV continues to be a major global public health issue, having claimed 36.3 million (27.2 - 47.8 million) lives so far. There is no cure for HIV infection. However, with increasing access to effective HIV prevention, diagnosis, treatment and care, including for opportunistic infections, HIV infection has become a manageable chronic health condition, enabling people-living-with-HIV (PLHIV) to lead long and healthy lives. There were an estimated 37.7 million (30.2–45.1 million) PLHIV at the end of 2020 [1].

National adult (15-49 years) HIV prevalence was estimated at 0.22% (0.17%–0.29%) in 2020; 0.23% (0.18%–0.31%) among males, and 0.20% (0.15%–0.26%) among females. The total number

of people living with PLHIV in India was estimated at 23.19 lakh (18.33 lakh–29.78 lakh) in 2020. There were approximately 51,000 (34,800–77,200) deaths among PLHIV in 2020, with almost 63% deaths being AIDS-related. Even with low prevalence, India had the second highest HIV burden globally with an estimated 23.19 lakh PLHIV in 2020. In view of this burden, HIV/AIDS continues to be a public health challenge in India [2].

Vitamin D is a fat-soluble hormone that is majorly involved in the classical function of calcium and phosphorus hemostasis and bone mineralization as well as non-classical functions of immune modulation in various viral and autoimmune diseases. Both innate and adaptive immunity is aided by vitamin D.

Deficiency of vitamin D is not only linked with bone and muscle disorders, but it has a critical role in many infectious and noninfectious diseases. Vitamin D deficiency, assessed by low levels of 25-hydroxy vitamin D (25(OH)D), has been associated with diverse conditions, many of which have become a cause of concern in PLHIV such as infections, cardiovascular disease, insulin resistance and diabetes, dyslipidemia, cancer, neurocognitive impairment, frailty, renal function alteration, osteopenia/ osteoporosis, and autoimmune diseases [3-8].

A growing body of literature suggests prevalence of vitamin D deficiency in PLHIV. Vitamin D deficiency is frequent even in ambulatory HIV-positive patients [9-12]. A combination of both traditional risk factors, HIV-specific and antiretroviral therapy (ART)-specific contributors may leave HIV-infected persons to be at a greater risk for low 25(OH) D levels and frank vitamin D deficiency. The current study was conducted to assess and characterize the prevalence of Vitamin D deficiency among PLHIV attending a tertiary care hospital, its relation to CD4 counts and to assess the effect of NACO (National AIDS Control Organization, India)-based ART regimens on it.

Material and Methods

95 HIV positive patients registered at ART center, Dr B. R. Ambedkar Hospital, Raipur, Chhattisgarh, India were selected randomly from among the patients attending the center over a period of 6 months based on Inclusion and Exclusion criteria. Detailed history and clinical examination of each patient was done using a pre-designed proforma along with assessment of laboratory and other parameters as required. Blood was collected from anterior cubital vein of patients using standardized protocol for phlebotomy and used for flow cytometry estimation of CD4 count. ELISA based quantitative assessment of serum 25-OH Vitamin D3 was used to determine Vitamin D levels.

Inclusion criteria:

1. Patients above 18 years of age of both sexes.
2. Patients taking ART for more than 6 months.

Exclusion criteria:

1. Pregnant women.

2. Patients on Vitamin D or Calcium supplementation.
3. Known cases of Chronic Kidney Disease, Parathyroid disorders, deranged Renal function tests, malignancy.
4. Known or cases of Rickets, Osteoporosis

Operational Definitions:

1. Vitamin D levels were categorized using the following classification:[13]

Interpretation	Values
Normal	>32ng/mL
Insufficient	≥20ng/mL and ≤32 ng/mL
Deficient	<20ng/mL

2. ART regimens:
 - a. ZLN - Zidovudine (300 mg) + Lamivudine (150 mg) + Nevirapine (200 mg)
 - b. TLE - Tenofovir (TDF 300 mg) + Lamivudine (3TC 300 mg) + Efavirenz (EFV 600 mg) as Fixed Dose Combination (FDC) in a single pill once a day.

Statistical Analysis: Data has been expressed as percentage, mean and standard deviation. Kolmogorove Smirnov analysis used to assess the linearity of the data. Student’s t test used to assess the significance of difference between two parameters. ANOVA followed by post hoc Benferroni’s test used to assess the significance of difference between more than two parameters in case of parametric data and Kruskal Wallis test followed by Dunn test used in case of non-parametric data. Frequency distribution analyzed using Chi-square test or Fischer’s exact test. P<0.05 considered to be statistically significant. SPSS™ (IBM Corp. Pvt. Ltd.) and Microsoft Excel™ (Microsoft Corp.) used to analyze the data.

Results

Our study population comprised of total 95 subjects. The demographic profile of the patients in the study was as per Table 1. Levels of Vitamin D were as per Table 2. The duration of ART treatment was as per Table 3. The ART regime used under National AIDS Control Programme of India was as per Table

4. CD4 count $<200 /\text{mm}^3$ was found in 12 (13.65%) subjects. Forty-four (46.42%) subjects reported CD4 counts of $200\text{-}500/\text{mm}^3$. CD4 count

$>500/\text{mm}^3$ was present in thirty-eight subjects (40%). Pulmonary Tuberculosis was present in 19 (20%) of subjects.

Age group	Male	%	Female	%	Total
<30	5	5.26	17	17.89	22
31-40	21	22.01	23	24.21	44
41-50	19	20	4	4.21	23
>50	5	5.26	1	1.05	6
Total	50	54.73	45	47.36	95

Vitamin D levels (ng/ml)	Category / Status	No. of subjects	%
<20	Deficiency	23	24.21
20-32	Insufficiency	24	25.26
>32	Normal	48	50.52
Total		95	100

Duration of treatment (Months)	No. of subjects	Percentage
6-12	24	25.26
12-36	28	29.47
>36	43	45.26
Total	95	100

Treatment Regimen	No. of subjects	Percentage
TLE	45	49.5
ZLN	50	50.5
Total	95	100

Status of serum vitamin D was compared in subjects with variable duration of treatment. It was found that all three groups were Age ($p=0.1$) and Sex (0.6) matched. No significant difference was found in frequency distribution in subjects with various duration of treatment ($p=0.7$). Association of vitamin D levels with duration of treatment was assessed. No significant

association was found between Vitamin D levels and duration of treatment ($p=0.419$). (paragraph change)

Status of serum vitamin D was assessed in subjects with variable treatment regimes. It was found that all three groups were Age ($p=0.1$) and Sex (0.6) matched. No significant difference was found in frequency distribution in subjects with various duration of treatment ($p=0.7$). Serum vitamin D levels were compared between subjects who received different regimes for HIV treatment. Serum vitamin D levels were found to be higher in subjects who were on TLE regime (mean= 43.45 ± 10.1) compared to subjects with ZLN regime (mean= 32.55 ± 9.8). The difference was found to be significant on assessment by students-t test ($p=0.01$).

Status of serum vitamin D in subjects with differing CD4 counts was assessed. It was found that the three groups were matched for age ($p=0.4$) and sex ($p=0.2$) but the difference in frequency distribution of status of vitamin D was not statistically significant ($p=0.6$). Serum vitamin D levels were compared between subjects with varying CD4 count. It was found that serum vitamin D levels were higher in subjects with CD4 count >500 (mean= 42.1 ± 29.1) compared to those with CD4 count $200\text{-}500$ (mean= 34.6 ± 18.6) and CD4 count <200 (mean= 35.1 ± 21.7). The difference failed to achieve statistical significance ($p=0.7$).

Frequency of Pulmonary Tuberculosis in subjects on different ART regimes was also assessed. No significant difference in

frequency distribution was found ($p=0.3$). (paragraph change). CD4 count was found to be higher in subjects on ZLN regime compared to those on TLE regime. But the difference failed to reach statistical significance ($p=0.1$).

Discussion

In our study maximum number of subjects, 44(46.3%), were found to be in the age group of 31-40 years followed by age group 41-50 years. Less than 30 years age group consisted of 22 (23.1%) subjects and only 6 subjects (6.31%) were present in >50 years age group. Mean age among study subjects was found to be 37.3 years. Study population comprised of 50 male (54.73%) and 45 female (47.36%). In our study, total 24 (25.26%) subjects were found to have insufficient levels of vitamin D. Forty-eight (50.52%) subjects were found to have > 30 ng/ml of serum vitamin D and were labelled as normal. Serum levels <20 ng/ml were considered as Vitamin D deficiency, and Vitamin D levels 20-32 ng/ml were considered as vitamin D insufficiency. Hospital-based studies from India have showed a prevalence of Vitamin D deficiency ranging from 37% to 99% in non-disease specific conditions [14-17].

Rattan et al [18] have reported an incidence of Vitamin D deficiency of 11.3% in non-diseased individuals of the age group 30-45 years from coastal Odisha which is the closest available reference comparable to our study in terms of geographical and demographical profile. The above comparison suggests a greater deficiency of Vitamin D among HIV patients. In contrast, Zhang L et al [19] concluded that Vitamin D deficiency was prevalent regardless of HIV sero-status in well-treated HIV-infected men. However, considering the resource limited setting and taking into account the socio-demographic profile of patients, the possibility of malnutrition acting as a confounding factor cannot be ruled out. García et al found Vitamin D deficiency to be present in 86% of HIV infected subjects [21]. In another study authors found prevalence of vitamin D deficiency to be 29%. The criteria for deficiency was <35 nmol/liter from April to September and <25 nmol/liter from October to March [22].

Rodríguez M et al in 2009 found moderate and severe vitamin D deficiency to be present in

36.8% and 10.5% HIV positive subjects respectively. Authors have considered Vitamin D levels < or = 20 and >10 ng/ml to be moderate deficiency and < or =10 ng/ml to be severe deficiency. The use of inconsistent definitions for deficiency/insufficiency, as well as variations in the populations studied, contributes to this discrepancy. Overall, rates of low 25(OH) D levels are high. This may be partly due to traditional risk factors for low 25(OH) D levels such as lack of exposure to UV-B (Ultraviolet-B) radiation, age, and darker skin pigmentation [23] or due to disease pathology.

Serum vitamin D levels were compared between subjects who received different regimes for HIV treatment. Serum vitamin D levels were found to be higher in subjects who were on TLE regime (mean=43.45 ± 10.1) compared to subjects with ZLN regime (mean=32.55± 9.8). The difference was found to be significant on assessment by students-t test ($p=0.01$). Data on the association of low 25(OH)D levels with ART is conflicting but overall suggests an association may be present between type of ART and Vit D deficiency.

Nonnucleoside-reverse transcriptase inhibitor (NNRTI)-based cART has most consistently been associated with low 25(OH)D levels [24] albeit not in all cross-sectional studies. Within the class of NNRTIs, an association with low vitamin D status has been reported for efavirenz but not for nevirapine. Among the NRTIs, zidovudine has been associated with lower 25(OH)D levels [25]. Tenofovir has not been associated with vitamin D deficiency or insufficiency [26].

Status of serum vitamin D was assessed in subjects with variable duration of treatment. No significant difference was found in frequency distribution in subjects with various duration of treatment ($p=0.7$). Association of vitamin D levels with duration of treatment was also assessed. No significant association was found between Vitamin D levels and duration of treatment ($p=0.419$) This is in contrast to the findings of The Italian ICONA Cohort [20] which demonstrated an association of hypovitaminosis D with duration of ART and Conrado T et al [22]

who found independent association between Vitamin D and prolonged use of ART.

Status of serum vitamin D in subjects with differing CD4 counts was assessed. It was found that the three groups were matched for age ($p=0.4$) and sex ($p=0.2$) but the difference in frequency distribution of status of vitamin D was not statistically significant ($p=0.6$). Further, serum vitamin D levels were compared between subjects with varying CD4 count. It was found that serum vitamin D levels were higher in subjects with CD4 count >500 (mean= 42.1 ± 29.1) compared to those with CD4 count 200-500 (mean= 34.6 ± 18.6) and CD4 count <200 (mean= 35.1 ± 21.7). Although the difference failed to achieve statistical significance ($p=0.7$), this is in coherence with the findings of Ezeamama et al [27].

Salawu et al have earlier reported a significant correlation between serum vitamin D level and CD4 counts [28]. Coelho et al have also reported an association between improved CD4 recovery and vitamin D repletion suggesting a potential benefit of vitamin D supplementation on immunologic recovery during HIV treatment [29]. Many other studies have also reported a

positive correlation between Vitamin D levels and CD4+ counts [30].

One major limitation of the study was a skewed age distribution with maximum subjects in the 31-50 years age group. A small sample size due to resource constraints also acted as a limitation that could have caused correlations to miss statistical significance. In the absence of pre-HIV and pre-ART Vitamin D levels, the causal attribution could not be conclusively inferred. More detailed studies are required to conclusively discern the interplay between HIV and Vitamin-D.

Conclusion

There appears to be a greater prevalence of subnormal levels of Vitamin D among PLHIV-on-ART as compared to non-diseased population. ZLN regime appears to have a further negative impact on Vitamin D levels and therefore, monitoring and supplementation should be considered for such patients and for others on long term NNRTI therapy. There may be a possible correlation between Vitamin D levels and CD4+ counts. More studies are required to further establish the causes, role, and implications of Vit D deficiency in HIV patients.

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References

1. WHO. Progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. Accountability for the global health sector strategies 2016–2021: actions for impact. Geneva: *World Health Organization*. 2021 (Accessed 19 June 2022). Available from: <https://www.who.int/publications/i/item/9789240027077>
2. National AIDS Control Organisation & ICMR-National Institute of Medical Statistics (2021). India HIV Estimates 2020: Technical Brief. New Delhi: NACO, Ministry of Health and Family Welfare, Government of India (Accessed 19 June 2022). Available from: http://naco.gov.in/sites/default/files/India%20HIV%20Estimates%202020__Web_Version_0.pdf
3. Legeai C, Vigouroux C, Souberbielle JC, Bouchaud O, Boufassa F, Bastard JP, Carlier R, Capeau J, Goujard C, Meyer L, Viard JP. ANRS-COPANA Cohort Study Group. Associations between 25-hydroxyvitamin D and immunologic, metabolic, inflammatory markers in treatment-naïve HIV-infected persons: the ANRS CO9 «COPANA» cohort study. *PLoS One*. 2013; 8(9):e74868.
4. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007; 357:266-281.
5. Norman AW, Bouillon R. Vitamin D nutritional policy needs a vision for the future. *Exp Biol Med*. 2010; 235:1034-1045.
6. Souberbielle JC, Body JJ, Lappe JM, Plebani M, Shoenfeld Y, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice. *Autoimmunity Rev*. 2010; 9:709-715.
7. Adams JS, Hewison M. Update in vitamin D. *J Clin Endocrinol Metab*. 2010; 95:471-478.
8. Ng K, Meyerhardt JA, Wu K, Feskanich D, Hollis BW, et al. Circulating 25-hydroxyvitamin D levels and survival in patients with colorectal cancer. *J Clin Oncol*. 2008; 26:2984-2991.
9. Coelho L, Cardoso SW, Luz PM, Hoffman RM, Mendonça L, Veloso VG et al. Vitamin D3 supplementation in HIV infection: effectiveness and associations with antiretroviral therapy. *Nutr J*. 2015; 14:81.

10. Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. *Faseb J*. 2001; 15(14):2579e85.
11. Qurban R, Saeed S, Kanwal W, Junaid K, Rehman A. Potential immune modulatory effect of vitamin D in HIV infection: A review. *Clin Nutr ESPEN*. 2022;47:1-8
12. Rodríguez M, Daniels B, Gunawardene S, Robbins GK. High frequency of vitamin D deficiency in ambulatory HIV-Positive patients. *AIDS Res Hum Retroviruses*. 2009; 25(1):9-14.
13. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr*. 2005; 135(2):317-22.
14. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: *World Health Organization*. 2007. Available from: www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf
15. Sofi NY, Jain M, Kapil U, Seenu V, Ramakrishnan L, Yadav CP et al. Status of serum Vitamin D and calcium levels in women of reproductive age in national capital territory of India. *Indian J Endocrinol Metab*. 2017; 21:731-733.
16. Bawaskar PH, Bawaskar HS, Bawaskar PH, Pakhare AP. Profile of Vitamin D in patients attending at general hospital Mahad India. *Indian J Endocrinol Metab*. 2017; 21:125130.
17. Siddiqee MH, Bhattacharjee B, Siddiqi UR, Meshbahur Rahman M. High prevalence of vitamin D deficiency among the South Asian adults: a systematic review and meta-analysis. *BMC Public Health*. 2021; 21(1):1823.
18. Rattan R, Sahoo D, Mahapatra S. Prevalence of Vitamin D deficiency in adults in the coastal regions of Odisha, India. *IOSR J Pharm Biol Sci*. 2016; 11:49-52.
19. Zhang L, Tin A, Brown TT, Margolick JB, Witt MD, Palella FJ Jr, Kingsley LA, Hoofnagle AN, Jacobson LP, Abraham AG. Vitamin D Deficiency and Metabolism in HIV-Infected and HIV-Uninfected Men in the Multicenter AIDS Cohort Study. *AIDS Res Hum Retroviruses*. 2017; 33(3):261-270.
20. d'Arminio Monforte A, Diaz-Cuervo H, De Luca A, Maggiolo F, Cingolani A, Bonora S, Castagna A, Girardi E, Antinori A, Lo Caputo S, Guaraldi G, Cozzi-Lepri A; ICONA Foundation Study Group. Evolution of major non-HIV-related comorbidities in HIV-infected patients in the Italian Cohort of Individuals, Naïve for Antiretrovirals (ICONA) Foundation Study cohort in the period 2004-2014. *HIV Med*. 2019; 20(2):99-109.
21. García Aparicio AM, Muñoz Fernández S, González J, Arribas JR, Peña JM, Vázquez JJ, Martínez ME, Coia J, Martín Mola E. Abnormalities in the bone mineral metabolism in HIV-infected patients. *Clin Rheumatol*. 2006; 25(4):537-539.
22. Conrado T, Miranda-Filho Dde B, Ximenes RA, Albuquerque Mde F, Lacerda HR, Ramos RC, Araújo PS, Montarroyos U, Bandeira F. Vitamin D Deficiency in HIV-Infected Women on Antiretroviral Therapy Living in the Tropics. *J Int Assoc Physicians AIDS Care (Chic)*. 2011; 10(4):239-245.
23. Rodríguez M, Daniels B, Gunawardene S, Robbins GK. High frequency of vitamin D deficiency in ambulatory HIV-Positive patients. *AIDS Res Hum Retroviruses*. 2009; 25:9-14.
24. Adeyemi OM, Agniel D, French AL, Tien P, Weber K, Glesby MJ, et al. Vitamin D deficiency in HIV-infected and un-infected women in the US. *J Acquir Immune Defic Syndr*. 2011; 57:197-204.
25. Fox J, Peters B, Prakash M, Arribas J, Hill A, Moecklinghoff C. Improvement in vitamin D deficiency following antiretroviral regime change: Results from the MONET trial. *AIDS Res Hum Retroviruses*. 2011; 27:29-34.
26. Dao CN, Patel P, Overton ET, Rhame F, Pals SL, Johnson C et al. Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D Levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. *Clin Infect Dis*. 2011; 52:396-405.
27. Ezeamama AE, Guwatudde D, Wang M, Bagenda D, Kyeyune R, Sudfeld C, Manabe YC, Fawzi WW. Vitamin-D deficiency impairs CD4+T-cell count recovery rate in HIV-positive adults on highly active antiretroviral therapy: A longitudinal study. *Clin Nutr*. 2016; 35(5):1110-1117.
28. Salawu AA, Oloyede TW, Oke EO, Oladibu OT, Ojedokun SA, Oiwoh SO, Oreagba KI. Vitamin D Level in Patients Receiving Highly Active Antiretroviral Therapy in LAUTECH Teaching Hospital, Ogbomoso. *West Afr J Med*. 2022; 39(1):70-75.
29. Coelho L, Cardoso SW, Luz PM, Hoffman RM, Mendonça L, Veloso VG, Currier JS, Grinsztejn B, Lake JE. Vitamin D3 supplementation in HIV infection: effectiveness and associations with antiretroviral therapy. *Nutr J*. 2015; 14:81.
30. Teichmann J, Stephan E, Lange U, Discher T, Friese G, Lohmeyer J et al. Osteopenia in HIV infected women prior to highly active antiretroviral therapy. *J Infect*. 2003; 46(4):221-227.

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