# Vitamin D Status in treatment naive HIV seropositive patients and its correlation with CD4 cells count

Authors
**Dr Benrithung R Ngullie[1], Dr Rahul Kumar[2*], Dr Rajesh Manocha[3], Dr VPS Punia[4], Dr Prabal Rajvanshi[5], Dr Sunil Ranga[6]**

[1]Senior Resident, Department of Medicine, VMMC & Safdarjung Hospital, New Delhi
[2]Assistant Professor, Department of Medicine, Dr.S.N. Medical College, Jodhpur, Rajasthan
[3,4,5]Professor, Department of Medicine, VMMC & Safdarjung Hospital, New Delhi
[6]Professor, Department of Pathology, VMMC & Safdarjung hospital, New Delhi
*Corresponding Author
**Dr Rahul Kumar**
Assistant Professor, Department of Medicine, Mahatma Gandhi Hospital, Dr.S.N. Medical College Jodhpur, Rajasthan.342001, India
Email: *dr.rahulvmmc@gmail.com*. Mobile- 9654955425

**Abstract**
**Introduction:** *Human Immunodeficiency Virus (HIV) infection is characterized by a progressive deterioration in immune function. Abnormalities in vitamin D status and metabolism might be an important concern in HIV patients. CD4 counts are the principal surrogate marker in assessing the degree of immune deficiency in HIV infected persons.*
**Objective:** *This study was performed to evaluate and compare vitamin D levels in treatment naïve HIV seropositive patients and its relationship to CD4 cells count.*
**Material and Methods:** *50 cases and 50 controls of both sexes between the age of 18-70 years attending OPD of ART clinic and Department of Medicine were recruited and serum Vitamin D level and CD4 cell count were measured between January 2013 to December 2015.*
**Results:** *The mean and standard deviation values in the cases for Vitamin D were 10.9188 and 5.46304 respectively and for CD4 counts, mean and standard deviation were 294.8400 and 152.07790. The mean and standard deviation values in the controls for Vitamin D were 16.7388and 16.16334 respectively and for CD4 counts, mean and standard deviation were 1052.0400and 273.76237.*
**Conclusion:** *Vitamin D had a statistically significant positive correlation with CD4 count in cases but had a negative correlation with CD4 count in controls. All patients with HIV had Hypovitaminosis D. Vitamin D levels were found to be lower in older age groups and directly proportional to duration of sun exposure.*
**Keywords:** *Vitamin D, CD4 count, HIV.*

## Introduction

The Human Immunodeficiency Virus (HIV) infects human T cells, causing a disease that progressively leads to a dramatic deterioration of the immune function. According to NACO, 2011 India is a country with low HIV prevalence but it

has the third largest number of people living with HIV/AIDS. Vitamin D acts as a major prohormone. It has numerous physiological functions within the body. Newer evidence suggests that it also plays a major role regulating the immune system, perhaps including immune responses to viral infection. Though vitamin D's anti-viral mechanism has not been fully established, it may be linked to vitamin D's ability to up-regulate the anti-microbial peptides LL-37 and human beta defensin-2.[1] Vitamin D has been shown to have significant effects on immune system components.Vitamin D successfully promoted monocyte maturation, which is defective in HIV-infected patients, in an *in vitro* study.[2] Vitamin D, via receptors on lymphocytes and monocytes, stimulates macrophage, dendritic cell and NK cell activity. Also, the 1α-hydroxylase activity responsible for vitamin D activation has been found in macrophages.[3] HIV-1 replication and consequent macrophage cell infection has been decreased following *invitro* vitamin D pre treatment, for which one of the proposed mechanisms is decreased CD4 cell receptor presentation following vitamin D exposure. Defective migration capacity of the monocytes in HIV-infected patients improved after vitamin D incubation in another *in vitro* study. Displacement of nuclear factors of activated T cells resulted in suppression of cytokine related genes, Th1 proliferation and consequently decreased IFN-γ and IL-2. Also, increased Th2-related cytokines, like IL-4, improving innate immunity and mediating cathelicidin production (as a membrane antiviral agent), have been proposed as vitamin D immunomodulatory effects.[4] CD4 counts are the principal surrogate marker in assessing the degree of immune deficiency in HIV infected persons and they are also used as a surrogate marker for the improvement of HIV/AIDS patients after initiation of ARV [WHO2005geneva]. Vitamin D deficiency can be caused by several factors, including decrease in skin synthesis of previtamin-D with skin pigmentation and aging,

low sunlight exposure, obesity, and insufficient vitamin D intake. In HIV-positive patients, factors causing deficiency is linked to the virus itself, and also to the use of antiretroviral drugs. Studies show that vitamin D stimulates maturation of monocytes to macrophages and activated monocytes and macrophages produce 1,25 $(OH)_2D$ dependent antimicrobial peptide, cathelicidin. Studies show that cathelicidin have the ability to inhibit HIV replication in CD4 and macrophages.[5] To date, the relationship between vitamin D sufficiency and CD4 T cell count remains unclear, although most studies have shown a positive association. A tangible knowledge of vitamin D status among HIV positive Indian patients is not known yet. The purpose of the present study is not just to provide vitamin D status in HIV positive Indian patients but also to compare median vitamin D levels in these patients with their CD4 counts.

**Material and Methods**

This cross sectional study was conducted over the period of 24 months in ART Clinic and Department of Medicine, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, After taking approval from Ethics Committee. Study sample comprised of total 100 patients, out of which 50 cases and 50 controls of both sexes between the age of 18-70 years attending OPD of ART clinic and Department of Medicine. This was a cross sectional study, conducted on treatment naive HIV seropositive patients and healthy controls. Patients were allocated to two different groups.

1. Patients with diagnosis of HIV(Cases)
2. Patients without HIV infection.(Controls)

**Inclusion Criteria:** Patients who are willing to participate in study , Cases and controls of both sexes, between 18-70 yrs of age, Treatment naive HIV seropositive patients.

**Exclusion Criteria:** Patients taking vitamin D supplement, Drugs-Anticonvulsants, Flurbiprofen, Gabapentin, Heparin, Hydroxychloroquine, Indapamide, Oral Corticosteroids, Estrogens,

Digoxin etc. Known case of osteomalacia, osteoporosis, Chronic renal disease, Chronic liver disease, Pregnant/ lactating women.

**Investigation:** Complete haemogram, Kidney function tests, Liver function test, Serum calcium and phosphorous and alkaline phosphatase. HIV positive cases were diagnosed by the presence of HIV1 and HIV2 antibodies by E/R/S (Elisa/rapid/simple) using NACO approved kits, following NACO guidelines ELISA. CD4 count were determined by using flowcytofluorimetry involving fluorescence activated cell sorter technique (FACS). Samples were collected by using standard vein puncture under strict aseptic precautions without using tourniquet. 2ml blood was allowed to clot and then centrifuged to separate serum. This serum was used to estimate the level of 25-hydroxy vitamin-D using ELISA method [25-OH vitamin-D ELISA kit DL. D Diagnostika GMBH, EA 300/96 kit]. A deficiency in vitamin D levels were considered as below 20ng/ml, insufficiency if the levels were between20 to 30ng/ml and normal values were considered as above 30ng/ml.[6]

**Statistical Analysis:** Data was simultaneously entered into proforma and was updated during review at 3 months. It was entered into Microsoft excel (MS Office XP) and Master Chart prepared.

The data were analyzed using SPSS software version 18.0. Statistical analysis of data among groups was done, performed by Nominal data (such as gender, Age groups) were presented as number and percentages. Continuous data (such as age, lab values) were expressed as mean, standard deviation and range. *Chi-Square test* was applied as appropriate for comparison of nominal data. P value of 0.05 was considered as statistically significant. (Confidence interval of 95% was taken into account).

**Observation & Results**

This cross sectional study was conducted on treatment naïve HIV patients and healthy controls who fulfilled the inclusion and exclusion criterion. Written informed consent were obtained from the patients enrolled in the study and they were followed up.

Table 1.is showing that among cases 46(92%) out of 50 had vitamin Deficiency and 4 were found to have Vitamin D insufficiency. None of the cases had sufficient Vitamin D levels. In Control groups 36(72%) out of 50 had Vitamin D deficiency, 7 had Vitamin D insufficiency whereas 7 individuals in control group had sufficient Vitamin D levels.

**Table 1** Vitamin D level among case and control group

|  | <20ng/dl –deficient | 20-30ng/dl insufficient | >30ng/dl-sufficient |
|---|---|---|---|
| Cases | 46(92%) | 4(8%) | 0(0%) |
| Controls | 36(72%) | 7(14%) | 7(14%) |

**Table 2** Age distribution of 25(OH) Vitamin D levels in study group.

| Age(in years) | <20ng/dl deficiency (study group) | 20-30ng/dl insufficiency (study group) | >30ng/dl normal level (study group) |
|---|---|---|---|
| Group 1(20-30) | 9(81.8%) | 2(18.18%) | 0 |
| Group 2(31-40) | 13(100%) | 0 | 0 |
| Group 3(41-50) | 21(95.45%) | 1(4.55%) | 0 |
| Group 4(50 and >) | 3(75%) | 1(25%) | 0 |
| Total | 46 | 4 | |

Table-2.is showing that out of 50 patients in study group, 46 patients were 25(OH) vitamin D deficient i.e (value <20ng/dl) and 4 patients had vitamin D insufficiency (value >20<=30). Out of 46 patients of Vitamin D deficiency 9 were in age group 20-30,13 were in age group 31-40,21 were in age group 41-49, 3 were in age group 50 and above. Among 4 patients of Vitamin D insufficiency 2 were in age group 20-30,1 in age group 41-50 and 1 in age group 50 and above.

Table 3 is showing that in control group 36 patients are Vitamin D deficient, 7 patients have Vitamin D insufficiency, and 7patients have adequate Vitamin D levels. Out of 36 patients who are Vitamin D deficient 4 were between age group of 20-30 years, 11 were between age group of 31-40 years, 9 were between age group of 41-50 years, 12 were above 50 years. Out of 7 patients who had Vitamin D insufficiency, 2 were in age group 20-30 years, 2 were between age group of 31-40 years, 3 in age group of 41-50 years and 0 were above 65 years. Normal Vitamin D levels were found in 4 patients of 20-30 years and all the other age groups had 1 patient each. The Statistical analysis revealed that correlation is significant at the 0.01 level(2 tailed) in cases. In controls correlation was significant at the 0.05 level (2 tailed). In cases out of 50 patients 46 had Vitamin –D deficiency out of which 37(80.4%) belonged to urban region and only 9 (19.6%) were from rural background. 4 patient had Vitamin D insufficiency out of which 1(25%) was from rural background & other 3(75%) were from urban region. In control group 36 out of 50 had Vitamin D deficiency. 6(16.7%)were from rural backgrounds 30 (83.3%) were urban background. 7 had Vitamin D insufficiency out of which

2(28.6%)were from rural background 5 (71.4%) from urban region. 7 had sufficient Vitamin D levels among which 4 were from urban region & 3 from rural background. No statistical significance (P>0.05) was found between distribution of Vitamin D deficiency and insufficiency among rural and urban population. In study group out of 46 patients of Vitamin D deficiency 44(95.65%) had inadequate sun exposure and 2(4.4%)had adequate sun exposure. 4 patients who had Vitamin insufficiency 3 (75%) had adequate & 1(25%) had inadequate sun exposure. In control group out of 36 individuals with Vitamin D deficiency 30(83.3%) had inadequate sun exposure & 6(16.7%) had adequate sun exposure. Out of 7 individuals from control group who had adequate Vitamin D level 4 had inadequate sun exposure 3 had adequate. The difference between Vitamin D level and sun exposure was found to be statistically significant (p<0.05). 30 min of sun exposure daily can provide 90–100% of the RDA of vitamin D.[86] The amount of sun exposure that causes minimal redness of the head and hands, roughly 6% of total body surface area, will yield 600–1000 IU of vitamin D. This is considered adequate sun exposure.

**Table 3** Age distribution 25(OH) Vitamin D levels in control group

| Age(in years) | <20ng/dl-deficiency(Controls) | 20-30ng/dl-insuffiency(controls) | >30ng/dl normallevel(controls) |
|---|---|---|---|
| Group 1  (20-30) | 4 | 2 | 4 |
| Group 2  (31-40) | 11 | 2 | 1 |
| Group 3  (41-50) | 9 | 3 | 1 |
| Group 4  (50 and >) | 12 | 0 | 1 |
| Total | 36 | 7 | 7 |

**Table 4** Distribution of CD4 count of patients with deficient, insufficient and sufficient vitamin D levels in cases

| | <20ng/dl-deficiency | 20-30ng/dl-insufficiency | >30ng/dl-sufficiency |
|---|---|---|---|
| <200 | 19 | | |
| 200-500 | 23 | 1 | |
| >500 | 4 | 3 | |

Table 4 is showing that among cases all patients with CD4<200 had vitamin D <20ng/dl(100%). In patients with CD4 count 200-500, 23(95.8%) were

deficient and 1(4.1%) were insufficient. In patients with CD4>500, 5(71%) had deficiency and 2(28.5%) had insufficiency.

**Table 5** Correlations of vitamin D and CD4 among cases

|  | Mean | Std. Deviation | N |
|---|---|---|---|
| Vitamin D | 10.9188 | 5.46304 | 50 |
| CD4 count | 294.8400 | 152.07790 | 50 |

|  |  | VITD | CD4 |
|---|---|---|---|
| VITD | Pearson Correlation | 1 | .923[**] |
|  | Sig. (2-tailed) |  | .000 |
|  | N | 50 | 50 |
| CD4 | Pearson Correlation | .923[**] | 1 |
|  | Sig. (2-tailed) | .000 |  |
|  | N | 50 | 50 |

**. Correlation is significant at the 0.01 level (2-tailed).



**Correlation Between Vitamin D levels and CD4 counts among Cases**
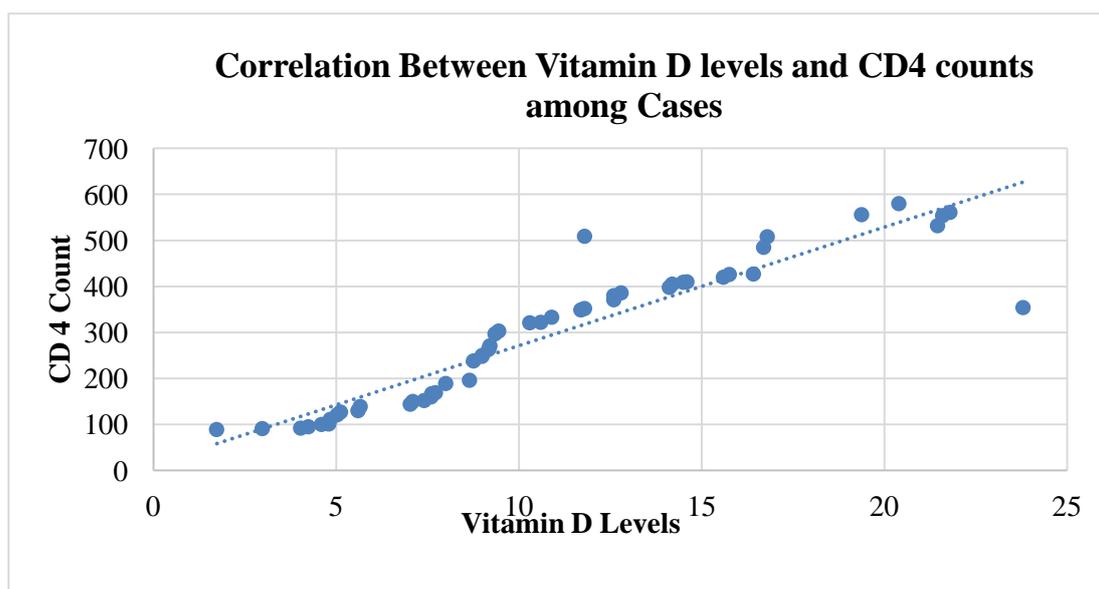
**Table 6** Correlations of vitamin D and CD4 among controls

|  | Mean | Std. Deviation | N |
|---|---|---|---|
| Vitamin D | 16.7388 | 16.16334 | 50 |
| CD4 count | 1052.0400 | 273.76237 | 50 |

|  |  | VITD | CD4 |
|---|---|---|---|
| VITD | Pearson Correlation | 1 | -.068 |
|  | Sig. (2-tailed) |  | .637 |
|  | N | 50 | 50 |
| CD4 | Pearson Correlation | -.068 | 1 |
|  | Sig. (2-tailed) | .637 |  |
|  | N | 50 | 50 |

**Correlation Between Vitamin D levels and CD4 counts among Controls**
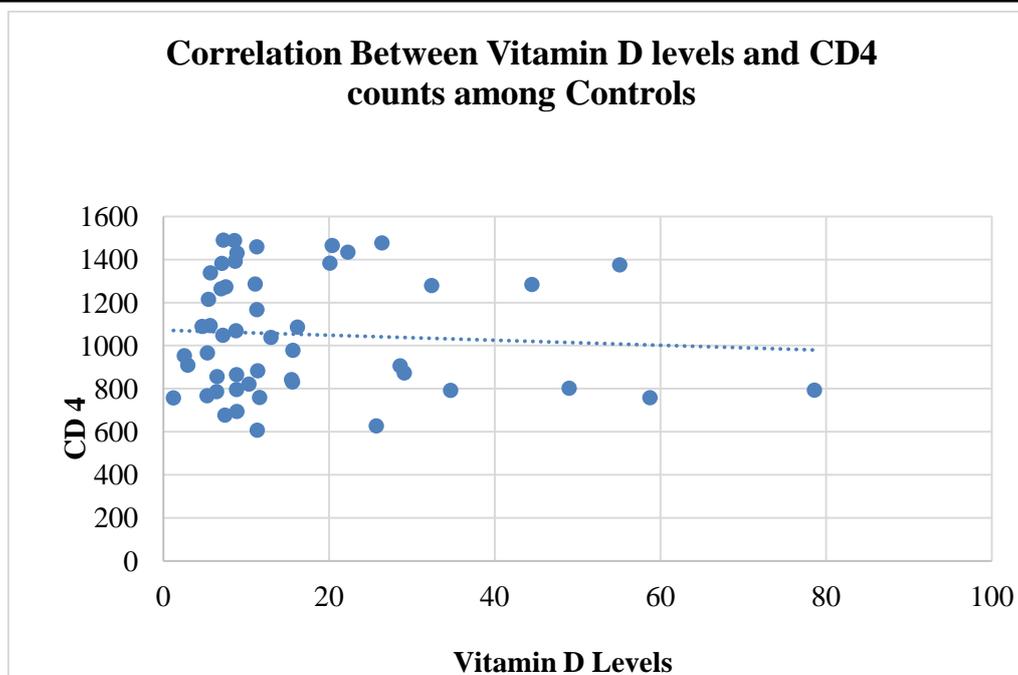


## Discussion

This study evaluated and compared vitamin D levels in treatment naive HIV seropositive patients and its relationship to CD4 levels. Suboptimal levels of vitamin D have frequently been reported in the different HIV-infected populations, regarding patients' age, gender, geographical location and related risk factors. Vitamin D has been shown to have significant effects on immune system components. Several studies have focused on the immunomodulatory effect of vitamin D in association with its receptors, related mechanisms and eventually viral replication and disease progression. An association between serum vitamin D and activated CD4 cell count has been reported in some other studies.[7] Opportunistic infections are the most common cause of morbidity and mortality in HIV positive patients. Significant reduction in duration of diarrhea, pneumonia and consequently hospitalization was reported in vitamin D-supplemented HIV positive childrens.[8] Earlier studies show that 25(OH)D3 levels were significantly lowered in NNRTI-treated patients, risk increases if ART use for ≥3 years[9]. Vitamin D deficiency although common before HAART, its frequency increased after initiation of therapy. In the cases, none of the patient had sufficient Vitamin D levels. 46 patients had vitamin D deficiency and 4 had insufficiency. Lower vitamin D levels were seen in older age groups in both cases and controls. Vitamin D deficiency was significantly higher in HIV positive patients as compared to age and sex matched healthy controls and hence such patients would benefit from vitamin D supplementation given at initiation of ART as it would offer a safe and effective means of augmenting the immune restoration response to treatment. out of 50 cases 20 (100%) females and 25(83.3%) males had Vitamin D levels <20ng/dl (Vitamin D deficiency) and 0 females and 5 males (16.6%) had Vitamin D insufficiency. Serum 25(OH) vitamin D levels of females was lower as compared to males as males had higher duration of sun exposure and higher body surface exposer to sun. In our study group out of 50 cases 10 were from rural background and 40 from urban area. Among 9 individuals from rural, 8 were found to have Vitamin D deficiency and 1 had Vitamin D insufficiency and among 40 from urban region, 37 had Vitamin D deficiency and 3 had insufficiency. It implies that Vitamin D deficiency is more common in urban areas. It is possibly due to less time exposure to sun, working mostly in closed indoor spaces in the urban subsets of patients. In our study it was found that majority of patient of HIV as well as

controls had inadequate sun exposure and the study concluded that Vitamin D levels were directly proportional to duration of sun exposure, as the difference between Vitamin D level and sun exposure was found to be statistically significant (p<0.05). There were individuals in both cases as well as control group with adequate sun exposure but low vitamin D levels .This could possibly be explained by the dress code and dark complexion of these individuals. In our present study, the mean and standard deviation values in the cases for Vitamin D were 10.9188 and 5.46304 respectively and for CD4 counts, mean and standard deviation were 294.8400 and 152.07790. Results show that vitamin D had a positive correlation with CD4 count which was statistically significant. The mean and standard deviation values in the controls for Vitamin D were 16.7388and 16.16334 respectively and for CD4 counts, mean and standard deviation were 1052.0400and 273.76237. Results show that vitamin D had a negative correlation with CD4 count which was statistically insignificant. Severe vitamin D deficiency was associated with low CD4 counts and increased markers of inflammation in ARV treatment naive HIV infected persons. Earlier Studies also suggests similar results, that vitamin D levels boosts the CD4 cell counts in both patients on ART as well as those not on ART.[10] . This is in agreement with the findings of Ross et al, de Luis et al. and Kim et al. who found a positive association between vitamin D with CD4 count.[11,12,13] In our study we also found a positive association between vitamin D and CD4 count but small sample size and cross sectional nature of the study were main limitations.

## Conclusion

We therefore conclude that the levels of vitamin D in treatment naive HIV seropositive patients had a positive correlation with CD4 count which was statistically significant. All patients with HIV had hypovitaminosis D. Vitamin D were found to be lower in older age groups and directly proportional to duration of sun exposure. Vitamin D replacement therapy should be considered for HIV positive patients in view of hypovitaminosis D.

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