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Prevalence of Vitamin-D Deficiency in HIV Positive Patients and its Correlation with Occurrence of Opportunistic Infections and CD4 Counts

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Abstract
The vitamin D endocrine system plays an essential role in calcium homeostasis and bone metabolism required for optimal absorption of dietary calcium and phosphate. Apart from role in calcium homeostasis other biological actions include induction of cell differentiation, inhibition of cell growth, immunomodulation and control of other hormonal systems. Several studies have demonstrated low serum vitamin 25(OH)D levels in populations across India. Very high percentage of HIV -infected people live in areas of the world where nutritional deficiencies are also highly prevalent, and the interactions between HIV and nutritional status have been documented. In last few years there have been reports suggesting an increased prevalence of osteopenia and osteoporosis in HIV positive patients [7,8,9]. Middle-aged persons with human immunodeficiency virus (HIV) infection are at risk for numerous co-morbidities typically seen including metabolic syndrome, osteoporosis and fractures, insulin resistance, diabetes, cardiovascular disease, cognitive impairment and opportunistic infection .Many of which have also been associated with vitamin D deficiency.
HIV infection and exposure to certain antiretroviral might contribute to altered levels of 25(OH) vitamin D.Vitamin D insufficiency or deficiency may increase the incidence of opportunistic infection in HIV patients. Vitamin D level in HIV patients and its co- relation with opportunistic infection and immunological status are not yet well defined. Therefore present study is designed to find the correlation between HIV status and vitamin D levels.

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Aim and Objective

To Measure the Vitamin-D Levels in HIV Positive Patients (PLHA) and Controls, then to Correlate with Opportunistic Infections, CD4 Count and Duration of Art.

Background

The prevalence of low 25(OH)D levels <20 ng/mL [50 nmol/L]) is approximately36% in otherwise healthy young adults aged 18 to 29 years. Prevalence of inadequate 25(OH) D levels is high in post menopausal women and especially

those with osteoporosis and a history of fracture. More than 90% of HI V-infected people live in areas of the world where nutritional deficiencies are also highly prevalent, and the interactions between HIV and nutritional status have been widely documented. Deficiencies of several micronutrients have been associated with increased rates of progression to AIDS and HIV - related mortality.[5]

Materials and Methods

This study was carried out in Department of General Medicine at Sri Ramachandra Medical College and Research Institute, a tertiary care referral hospital in Chennai India. The study was approved by Institutional Ethics Committee and a written informed consent was obtained from all patients prior to inclusion. The study subjects included 50 HIV positive patients and HIV non reactive individuals who are (aged more than 18 years) admitted to the hospital or following ART clinic in Sri Ramachandra Medical College and Hospital.

Inclusion Criteria

- ART naive HIV positive patients attending ART clinic at Sri Ramachandra Medical College
- Both, Male & Female patients will be included
- Age> 18 yrs
- Patients willing to give informed consent

Exclusion Criteria

- Patients on Vitamin D supplementation in last one month.
- Pregnant women

Methodology

All patients were subjected to detailed history and clinical examination using a pre-designed Performa. Data concerning the patients' age, gender, date of diagnosis of HIV status, drug intake, occupation. Patients categorized in different clinical stages as per NACO guidelines. Blood samples were collected on the day of interview. Fasting venous samples of all study subjects were drawn in calcium free test tubes. Serum was separated in a refrigerated centrifuge at 800 x g for 15 minutes at 4°C. CD4 count done by FACS count method. The complete hemogram, serum urea, creatinine, bilirubin, aspartate aminotransferase, and alanine aminotransferase levels, coinfections like HbsAg and HCV was included were checked to examine liver and renal function.

The samples were stored at -70°C until analyzed, and were protected from light during processing. All serum concentrations of 25(OH) D were measured by chemiluminescent micro particle immunoassay in the Nuclear Medicine laboratory of Sri Ramachandra Medical College and Research Institute. The sensitivity of the method is <1.5 ng/ml. Vitamin D deficiency was defined as deficiency < 20 ng/ml, insufficiency is 21-30 ng/ml, sufficiency is >30 ng/ ml.

Statistical Analysis

Analysis was performed using SPSS. Quantitative data were expressed as means ± SD. Qualitative data expressed as percentage. Difference between the two means was performed using Student "t" test (if data was normally distributed), or Mann-Whitney U test (if data was not normally distributed). Correlations were examined using Spearman's rank correlation test. Chi- square test was used to assess differences in the proportion between qualitative data.

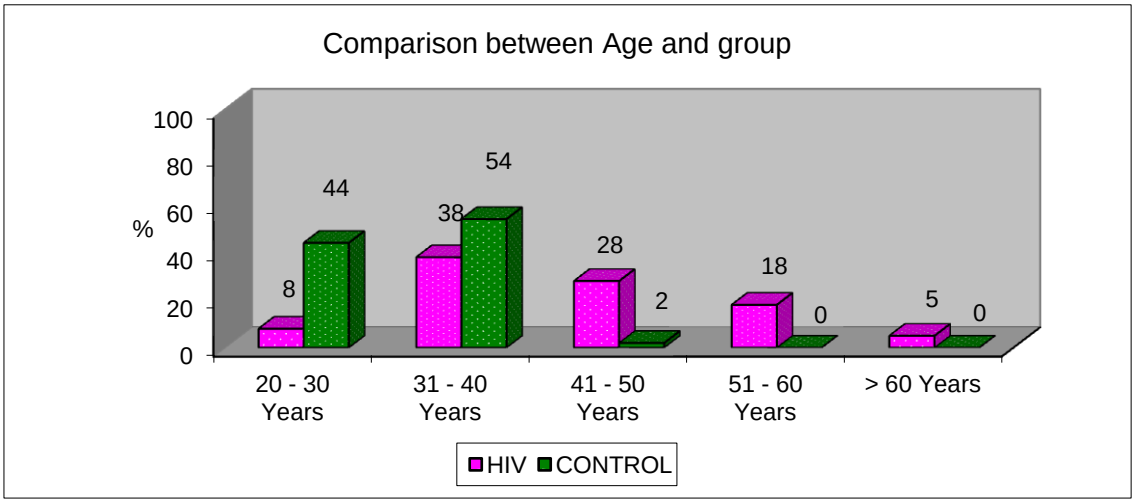
Results

A total of 50 HIV patients and 50 non reactive individuals were included in the study for comparing vitamin D levels and its correlation with opportunistic infections and CD4 count

Table 1 T-Test

GROUP	N (100)	Mean (Age)	Std. Deviation	Std. Error Mean	P Value
HIV	50	44.08	11.998	1.697	0.000
CONTROL	50	30.66	6.249	0.884	

Age	GROUP		
	HIV	CONTROL	TOTAL
20-30 Years	4 (8.0%)	22 (44.0%)	26 (26.0%)
31-40 Years	19 (38.0%)	27 (54.0%)	46 (46.0%)
41-50 Years	14 (28.0%)	1 (2.0%)	15 (15.0%)
51-60 Years	9 (18.0%)	0 (0%)	9 (9.0%)
> 60 Years	4 (8.0%)	0 (0%)	4 (4.0%)
Total	50 (100.0%)	50 (100.0%)	100 (100.0%)



In our study age groups were compared in which 4 (8%) patients was in HIV and 22 (44%) was in control. In age group 31-40 years 19 (38%) was HIV patients 27 (54%) was control. In 41-50 years 14 (28%) was HIV, 1 (2%) was in control. 51 – 60 years 9 (18%) patients was HIV and 0 was in control. In > 60 years 4 patients (8%) was HIV, 0 was in control.

Table 2 Crosstabs

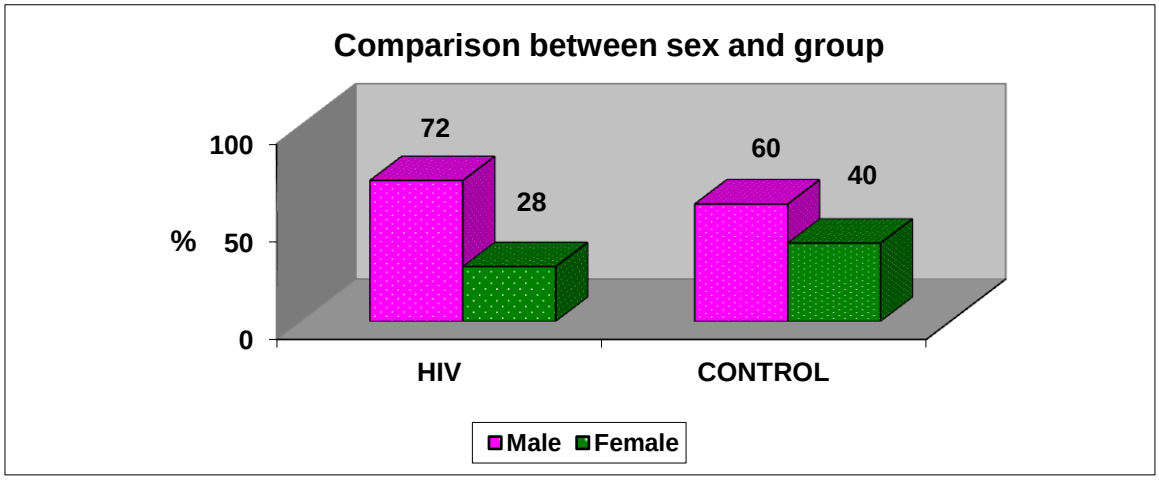
SEX	GROUP		TOTAL	P Value
	HIV	CONTROL		
Male	36 (72%)	30 (60%)	66 (66%)	0.205
Female	14 (28%)	20 (40%)	34 (34%)	
Total	50 (100%)	50 (100%)	100 (100%)	

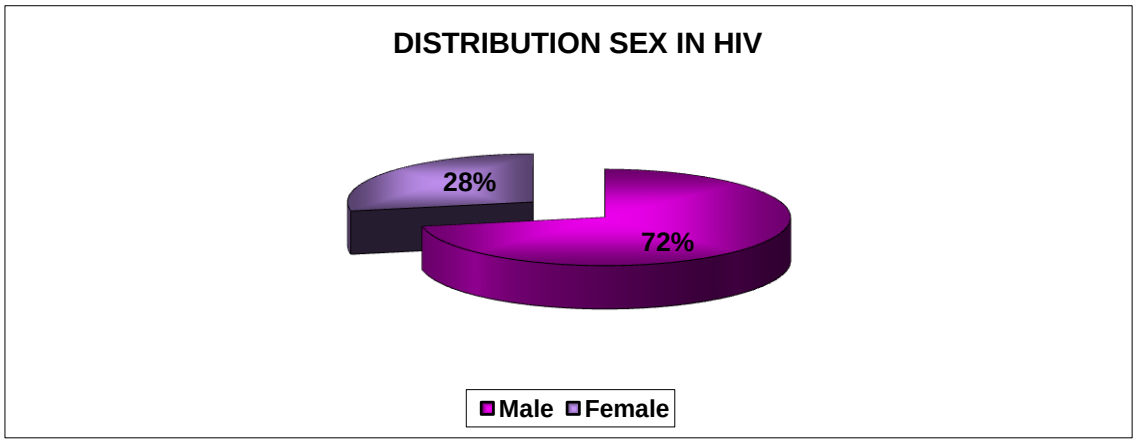
Chi-Square Tests

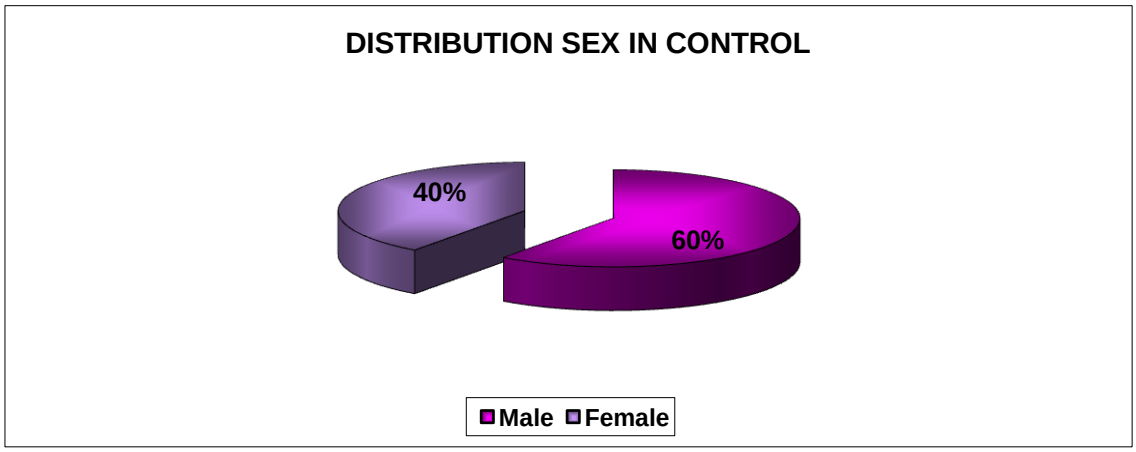
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.604[b]	1	.205		
Continuity Correction[a]	1.114	1	.291		
Likelihood Ratio	1.611	1	.204		
Fisher's Exact Test				.291	.146
Linear-by-Linear Association	1.588	1	.208		
N of Valid Cases	100				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 17.00.







In our study HIV group contains 36 (72%) males, 14 (28%) females whereas in control group 30 (60%) was male and 20 (40%) was females.

We compared 25 (OH)2 Vitamin D level in HIV patient of which 37 (74%) patients were vit D deficient (< 20 ng/ml) and in the control group 27 patients (54%) are vit D deficient.

Table 3 T-Test

GROUP	N (100)	Mean (25 OH ng/ml)	Std. Deviation	Std. Error Mean	P Value
HIV	50	16.126	7.2032	1.0187	0.024
CONTROL	50	20.494	9.7632	1.3807	

Independent Samples Test

25(OH)	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	.756	.387	-2.546	98	.012	-4.3680	1.7158	-7.7730	-.9630
Equal variances not assumed			-2.546	90.152	.013	-4.3680	1.7158	-7.7767	-.9593

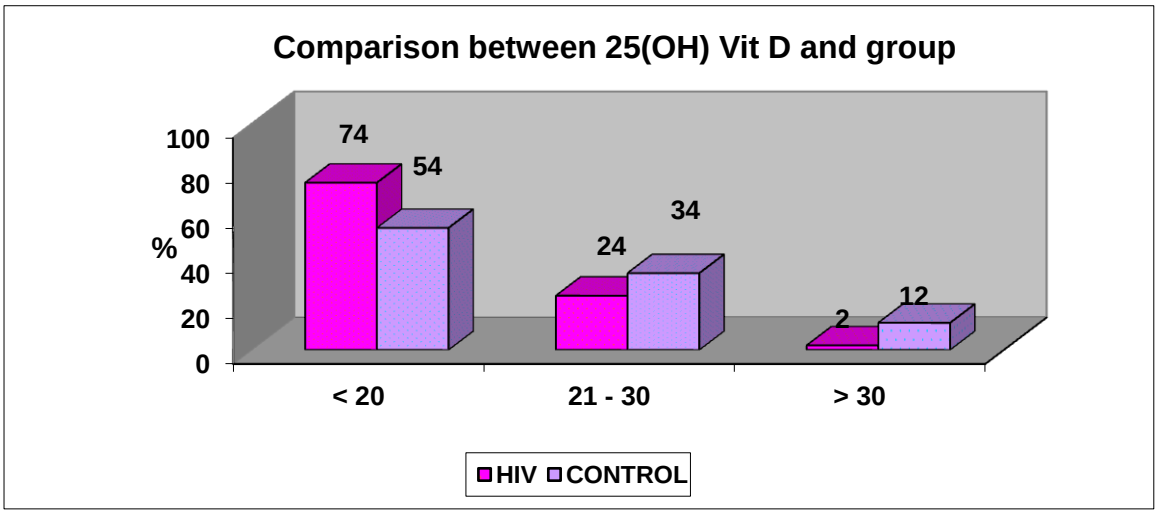
Crosstabs

25 (OH) Vit D (ng/ml)	GROUP		TOTAL	P Value
	HIV	CONTROL		
< 20	37 (74%)	27 (54%)	64 (64%)	0.05
21-30	12 (24%)	17 (34%)	29 (29%)	
> 30	1 (2%)	6 (12%)	7 (7%)	
Total	50 (100%)	50 (100%)	100 (100%)	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.996[a]	2	.050
Likelihood Ratio	6.398	2	.041
Linear-by-Linear Association	5.784	1	.016
N of Valid Cases	100		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 3.50.



In our study we compared vitamin D levels in HIV group and control group of, HIV patients had vitamin D deficiency (< 20 ng/ml), compared to controls which was statistically significant, P value being 0.024 using chi square test.

Crosstabs

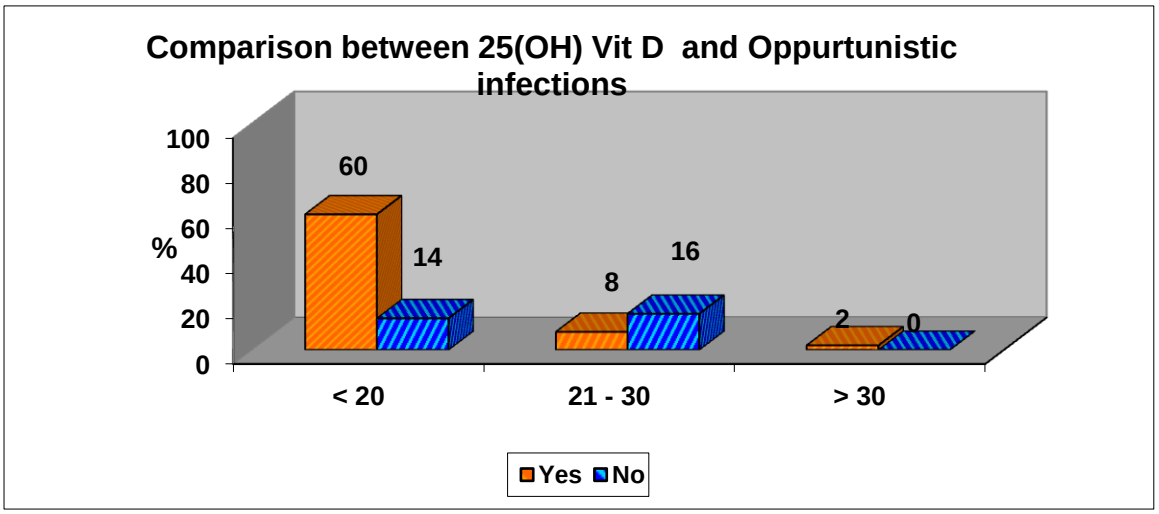
Table 4 Oppurtunistic infections * 25(OH) Vit D

25 (OH) Vit D (ng/ml)	Opportunistic Infection		Total	P Value
	Yes	No		
< 20	30 (60%)	7 (14%)	37 (74%)	0.006
21-30	4 (8%)	8 (16%)	12 (24%)	
>30	1 (2%)	0 (0%)	1 (2%)	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.275[a]	2	.006
Likelihood Ratio	9.917	2	.007
Linear-by-Linear Association	5.578	1	.018
N of Valid Cases	50		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is .30.



In our study oral candidiasis most commonly found opportunistic infection when we compared 25 (OH)2 Vitamin D levels with opportunistic infections in HIV patients, they had vitamin D deficiency (< 20 ng/ml), which was statistically significant of P value being 0.108.

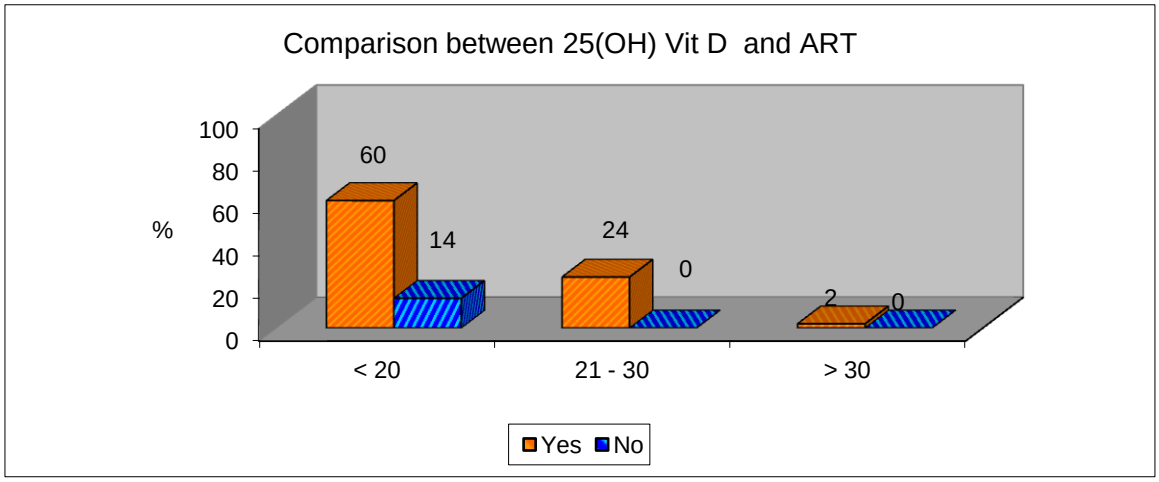
Table 5 ART * 25(OH) Vit D

25 (OH) Vit D (ng/ml)	ART		Total	P Value
	Yes	No		
< 20	30 (60%)	7 (14%)	37 (74%)	0.239
21-30	12 (24%)	0 (0%)	12 (24%)	
>30	1 (2%)	0 (0%)	1 (2%)	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.860[a]	2	.239
Likelihood Ratio	4.603	2	.100
Linear-by-Linear Association	2.588	1	.108
N of Valid Cases	50		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is .14.



When we compared 25(OH)2 vitamin D levels in patients on ART 25(OH)2 vitamin D level was deficient (< 20 ng/ml), and statistically not significant P value being 0.239.

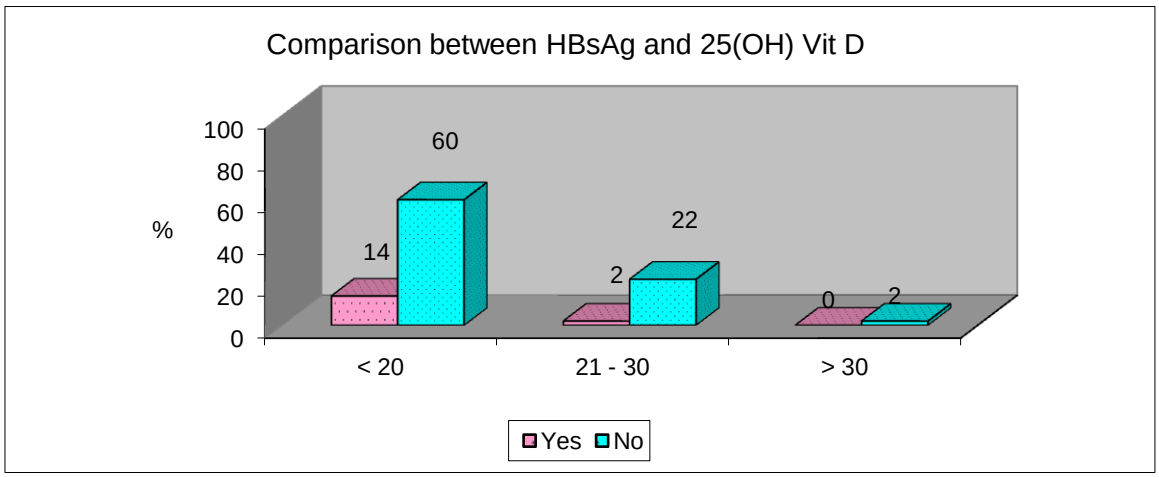
Table 6 HBsAg * 25(OH) Vit D

25 (OH) Vit D (ng/ml)	HBsAg		Total	P Value
	Yes	No		
< 20	7 (14%)	30 (60%)	37 (74%)	0.622
21-30	1 (2%)	11 (22%)	12 (24%)	
>30	0 (0%)	1 (2%)	1(2%)	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.950[a]	2	.622
Likelihood Ratio	1.190	2	.552
Linear-by-Linear Association	.928	1	.335
N of Valid Cases	50		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is .16.



When we compared 25(OH)2 vitamin D levels in HBsAg positive patients vitamin D level was statistically not significant of P value being 0.622.

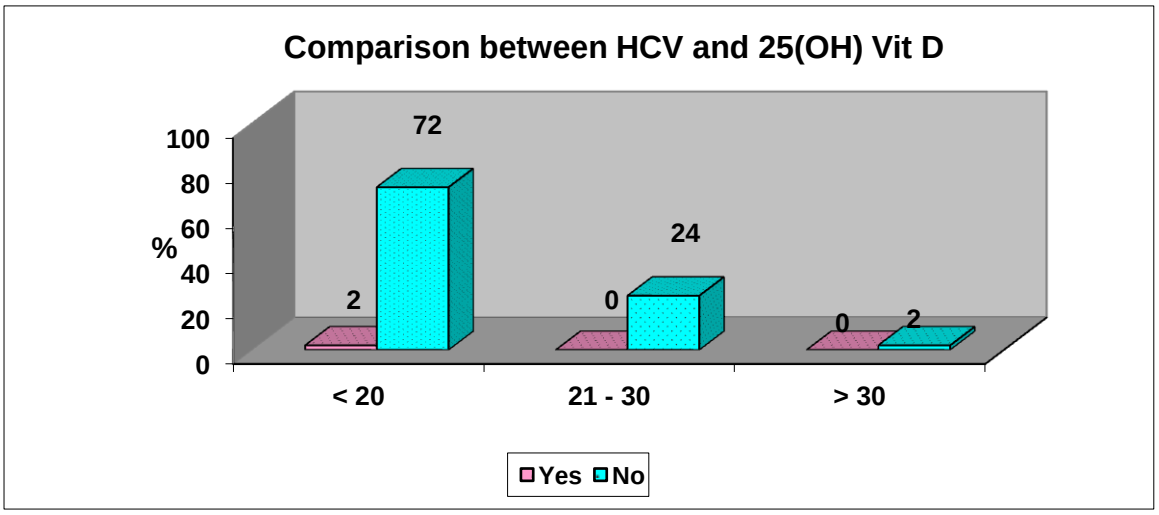
Table 7 HCV * 25(OH) Vit D

25 (OH) Vit D (ng/ml)	HCV		Total	P Value
	Yes	No		
< 20	1 (2%)	36 (72%)	37 (74%)	0.836
21-30	0 (0%)	12 (24%)	12 (24%)	
>30	0(0%)	1 (2%)	1(2%)	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.359[a]	2	.836
Likelihood Ratio	.609	2	.737
Linear-by-Linear Association	.325	1	.569
N of Valid Cases	50		

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .02.



When we compared 25(OH)2 vitamin D levels in HCV patients vitamin D level was statistically not significant P value being 0.836.

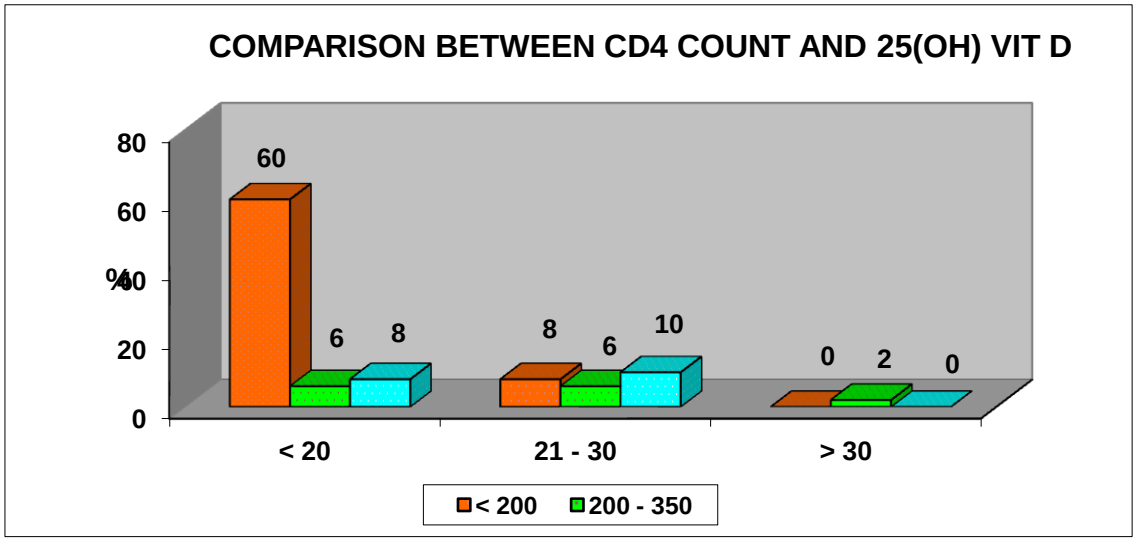
Table 8

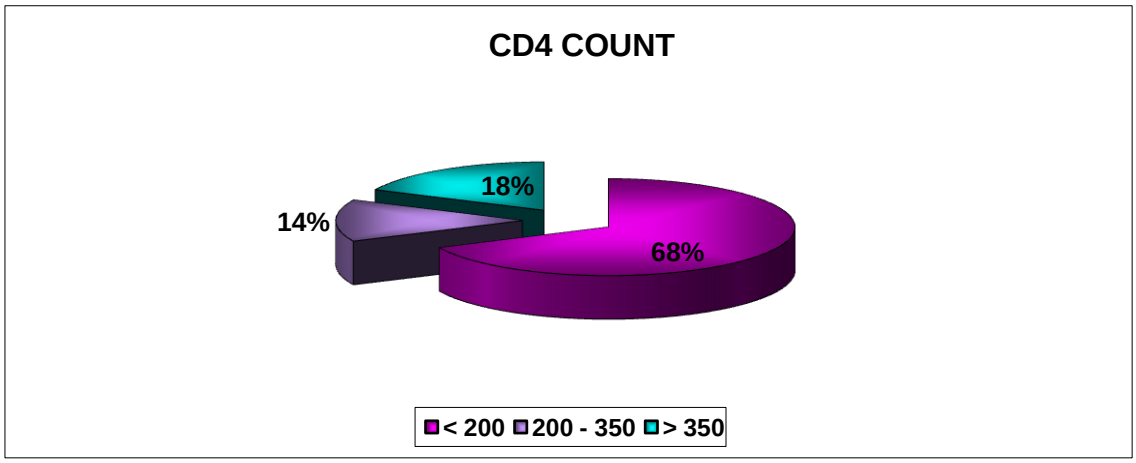
CD4	25 (OH) Vit D (ng/ml)			P Value
	< 20	21-30	>30	
< 200	60	8	0	0.015
200-350	6	6	2	
>350	8	10	0	

ANOVA

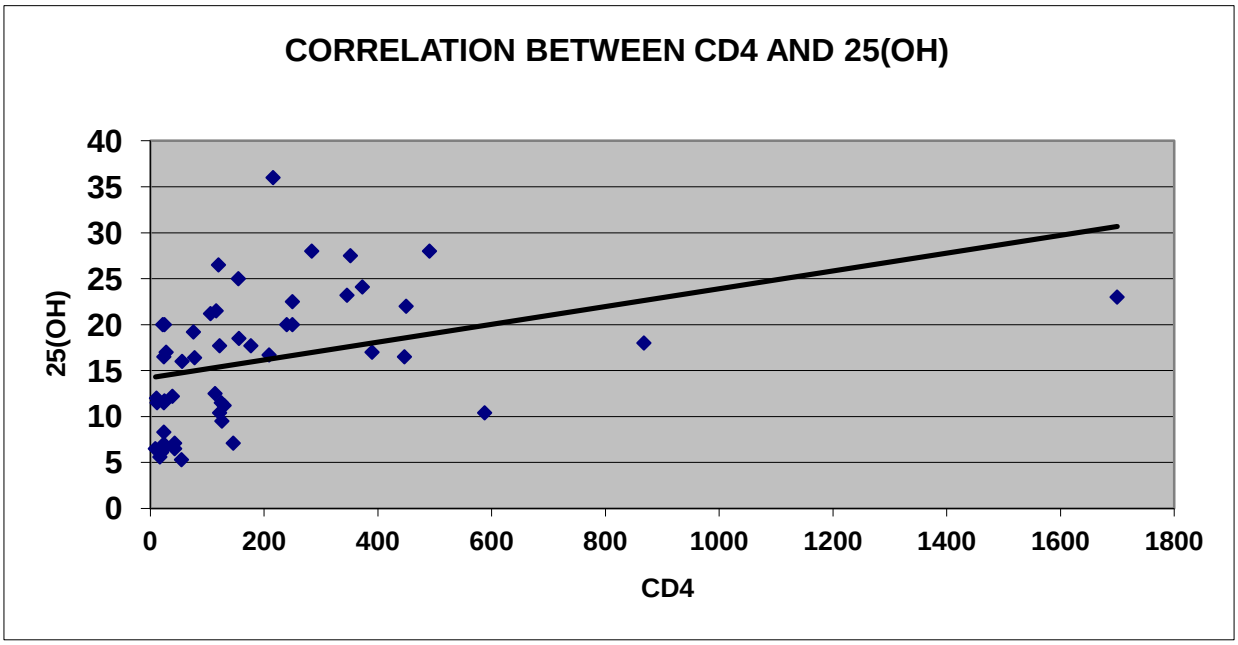
CD4

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	627284.5	2	313642.230	4.587	.015
Within Groups	3213961	47	68382.139		
Total	3841245	49			





Patients with CD4 count <100 had vitamin D deficiency (<20 ng/ml) , P value being 0.015, statistically significant.



The above scattered diagram shows 25 (OH) vitamin D is low in patient with low CD4 count.

Discussion

In our study we have taken 50 HIV positive patients and 50 HIV non reactive individuals. We have measured the 25 (OH)2 vitamin D levels in HIV positive patients (PLHA) and controls, and then correlated with opportunistic infections, CD4 count and duration of ART.

I Prevalence and Age

The key findings in our study suggest that there is a high prevalence of vitamin D deficiency in HIV positive patients (74%), when compared to HIV non reactive individuals was 54%, which is statistically siginificant (Table 3). There is trend suggesting the positive association between the vitamin D deficiency and advanced clinical stage of HIV disease, but not statistically significant.

In a similar study conducted by Christine N. Dao et al[13] showed high prevalence of vitamin D deficiency affecting >70% of HIV infected persons, however no controls were used in the study.

Age

In our study maximum number of HIV positive individuals come under the age group 31 to 40 years about 19 (38%) individuals , and in HIV negative individuals is 27(54%) comes under the age group of 31 to 40 years.

In our study the mean age group in HIV positive patients is 44.08 and HIV negative individuals is 30.66 (Table 1).There is a statistical significance of mean age group between HIV positive individuals and HIV negative controls using independent sample test.

Study conducted by Charles B Stephensen et al showed the prevalence of vitamin D insufficiency in subjects aged 12 to 29 years which was 32%. In women aged 15 to 49 years from the same survey, the prevalence of insufficiency (21 -30 ng/ml) was 42%.

II Sex Distribution

In our study, HIV positive patients contains 36 (72%) male, 14 (28%) females, Where as in HIV non reactive individuals 30 (60%) was male and 20 (40%) was females given (Table 2)..There was no statistical significance in sex distribution between HIV positive individuals and HIV negative controls.

III Opportunistic Infections

The most commonly seen opportunistic infection seen in my study was oral candidiasis, pulmonary tuberculosis, PCP pneumonia all these opportunistic infections oral candidiasis was most prevalent among HIV positive individuals. In our study there was 35 patients were Vitamin D deficient (<20 ng/ml).When we compare 25 (OH)2 vitamin D levels with opportunistic infections in HIV positive patients, they had vitamin D deficiency (<20 ng/ml). In our study w 30 patients (60%) had vitamin D deficiency (<20 ng/ml), and 5 (4%) had insufficiency (21 to 30 g/ml)

Study by Sudfeld C R et al which compared the incidence of opportunistic infections and pulmonary tuberculosis with a low vitamin D levels. Individuals with vitamin D deficiency also had features of wasting (BMI < 18.5) and > 10% of weight loss.

Our study showed that patients with low 25 (OH)2 vitamin D levels (<20 ng/ml) have increased incidence of opportunistic infections, which was statistically significant table (4)

IV CD4 Count

In our study 30(68%) patients had CD4 count (<200) ,8 patients had (12%) CD4 count 200-350 and 12(18%) persons had CD4 count >350 given in table (4). Study showed patients with CD4 count < 200 had vitamin D deficiency (< 20 ng/ml), about 60% of patients were vitamin D deficient, being statistically sifgnificant of P value being 0.015 using ANOVA method.

Study conducted by Marian Aziz et al showed the association between vitamin D levels and CD4 count with womens in advanced disease on HAART. Study showed vitamin D insufficiency may impair CD4 recovery with advanced disease on HAART therapy.

Study conducted by Nicolas J. Muller et al showed the negative correlation of hepatitis C seropositivity with 1, 25(OH)2 vitamin D but not with 25 (OH)2 vitamin D, related to an

inflammation related impairment of 1-hydroxylation if contraindicated by the unexplained negative correlation of 1, 25 (OH)2 vitamin D with CD4 cell count.

V Patients on ART

In our study there were 43 patients on ART of which 30 persons(60%) had vitamin D deficiency (<20ng/ml), 12 persons(24%) had vitamin D insufficiency (21-30) and 1 person (2%) had vitamin D suffieciency. we have compared 25(OH)2 vitamin D levels in patients on ART,25 (OH)2 vitamin D was deficient (< 20 ng/ml) among patients on ART and was not statistically significant given in Table 5 of P vaue being 0.239 using chi-square test.

Study conducted by Nicolas J Muller et al found that cART exposure had no significant impact on vitamin D levels. NRTI was associated with significantly low 25 (OH)2 vitamin D levels. Neither NNRTI are PI medication showed a significant effect on 1, 25 (OH)2 vitamin D levels in their analysis, although PI treated patients had shown low 1,25 (OH)2 vitamin D values.

Paul TV et al in an Indian study from Christian Medical College, Vellore India found high prevalence of vitamin D deficiency (74%) among HIV positive patients on HAART as compared to naïve and control group (37% in each group).[12]

VI Co-infections

HBsAg and HCV

In our study 8 patients had HbsAg positive of which 7(14%) of patients had vitamin D deficiency. There was only 1(2%) patients with HCV positive status, given in table (6 and 7).There was no statistical significance using chi-square method.

In our study there was no correlation between vitamin D levels and co-infections like HbsAg and HCV.

Limitations of Study

Less sample size is a limitation of the study.

Conclusion

- Our study revealed high prevalence of vitamin D deficiency among HIV positive patients.
- There was statistical significance between vitamin D levels, CD4 count and opportunistic infections in my study.
- HIV infected patients should be screened for vitamin D deficiency as there is high prevalence of vitamin D deficiency among these patients.
- If resources are not available to analyze the vitamin D status in these patients they should be supplemented with vitamin D to prevent the vitamin D deficiency and further complications.
- It is observed that pragmatic and statistical studies are scarcely done in India on the socially imperative subject of “VITAMIN D LEVELS IN HIV POSITIVE PATIENTS". It could be valuable in the larger interest of innumerable ailing patients if further studies are done to explore more practicable solutions to maintain the "Vitamin D levels in HIV positive patients".

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