Int.J.Curr.Microbiol.App.Sci (2023) *12*(03): 81-88

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 3 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1203.011

Study of the HIV-1 RNA Levels and CD4+ Cells Counts in HIV Infected Patients

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Keywords

HIV, CD4 cells counts, viral load, HIV RNA Levels, TND, ART

Article Info

Received:
02 February 2023
Accepted:
25 February 2023
Available Online:
10 March 2023

A B S T R A C T

HIV infection is a global pandemic. There is an inverse relationship between CD4 counts and degree of immunosuppression. The present study aim to assess the use of viral load (HIV RNA) and CD4 cell counts in the monitoring of HIV progression and its correlation. This period of 20 months study was conducted on 300 seropositive patients attending ICTC in Department of Microbiology at the tertiary care hospital, fulfilling inclusion and exclusion criteria. CD4 counts were estimated at baseline (Pre ART) and after 6 months and 12 months. Viral load test had done at 6 and 12 months of Post ART. This study didn't show any significant comparison between HIV RNA levels and CD4 cell counts after 6 months of ART treatment, but after 12 months there was significant negative linear correlation. 12 months of Post ART, TND number of patients increased from 152 patients to 215 patients. Patients who had VL>1000 copies/ml decreased from 27 patients to 10, only 1 patient showed static CD4 counts but achieved TND of viral load. We can conclude that combined use of CD4 and Viral load prognostic markers should prove useful in individual patient management and in the design and evaluation of therapeutic trials.

Introduction

HIV infection is a global pandemic**.** Human Immunodeficiency Virus (HIV) was first recognized in June 5th 1981, in five homosexual men in Los Angeles. Inlate (1982), the first cases of AIDS-like illness were reported in transfused patients.

To describe the recently identified syndrome, the CDC coined the term "AIDS." (CDC, 1981) Since the discovery and isolation of the human immunodeficiency virus (HIV) forty years ago, AIDS has killed millions of people worldwide. HIV remains a global health crisis and the world must reckon with the 1.5 million new HIV infections and 680 000 deaths from AIDS-related causes that occurred in 2020. (UNAIDS epidemiological estimates, 2021)

The most commonly used parameters to monitor the efficacy of antiretroviral treatment are viral load and CD4 T-cell counts. There is an inverse relationship

between CD4 count and degree of immunosuppression. Higher the HIV-1 viral particles in the blood, faster the CD4 cell depletion and faster the progression towards AIDS (Gupta and Gupta, 2004; Nasi *et al.*, 2017).

In March 2017, the Government of India accepted the WHO Test and Treat Policy.CD4 count cut-off point for ART initiation moving from less than 200 cells/mm3 in 2004 to less than 350 cells/mm3 in 2011 and then to less than 500cells/mm3 in 2016. The current recommendation to Treat All, regardless of the clinical stage or CD4 counts (National AIDS Control Organization, 2021).

In addition, plasma viral load significantly affects both the spread of HIV and the effectiveness of treatment. Every six months, or more frequently if antiretroviral therapy is changed, HIV RNA levels should be checked. (Georgina *et al.*, 2013)

The main aim of this study to assess the use of both viral load (HIV RNA) and CD4 cell count in the monitoring of HIV /AIDS progression. And also to explore Plasma Viral Load and it's correlation with CD4 cell counts.

The objectives of this study include Patients will be confirmed for HIV-1 sero-positivity according to NACO guidelines.

Materials and Methods

Research setting

All HIV-infected patients visited ICTC and ART Clinic at M.B.Goverment Hospital, Udaipur were screened for eligibility and enrolled for this study.

Inclusion Criteria

HIV seropositive confirmed cases as per NACO guideline, but not yet taking ART were included in this study**.**

Exclusion Criteria

HIV seropositive but on ART treatment patients, Below 15 years of age and Pregnant female were excluded. Permission for this study was obtained from Institutional Ethics Committee.

A Prospective Observational study was conducted on 300 HIV-seropositive ART patients of ≥15 years of age attending the ICTC, Department of Microbiology at a tertiary care hospital from November 2020 to June 2022. Blood samples were collected after proper counselling and after obtaining written informed consent from each patient. Then 3 to 4 ml of blood was withdrawn aseptically from each client and testing for anti HIV antibodies was done as per Strategy III of NACO Guidelines. The client was reported seropositive if all the three HIV tests (E/ R/ S) were reactive. In this study, the CD4 counts were estimated at baseline, and after 6 months and 12 months of ART. CD4 counts were measured on SysmexPartecCyflow R Counter CyView TM 2.11) by CD4% easy count kit. HIV RNA level was measured by quantitative Polymerase Chain Reaction. RNA extraction and then HIV-1 Viral load was done by Abbott Real Time HIV-1 viral load kit on m2000rt/sp instrument, Viral load tested at 6 months and 12 months of Post ART. Software called MedCalc 16.4 was employed to perform the statistical analysis or to determine correlation between CD4 count & HIV-1 viral load.

Results and Discussion

This study was conducted on 300 HIV serologically confirmed cases. Out of 300 cases, 31 patients were lost to follow up (LFU) during study. So after 12 months, remaining 269 patients were followed-up; 157 were male, 112 were female. Number of patients after 6 months of treatment were 176 (65.42%) patients, who had achieved TND (Target not detected), 55(20.44%) had <1000 copies/ml and 38 (14.12%) had >1000 copies/ml. After 12months of Post ART the number of patients decreased from 55 to 20 (7.4%) who had viral load <1000 copies/ml and 15(5.57%) from 38 patients who had viral load >1000 copies /ml. there was a clear cut increase in TND patients from 176 to 234 (86.98%).

Graph 1 showed stratification of patients between HIV RNA levels and CD4 cell counts after 6 months of treatment. 55 patients had viral load <1000 copies/ml among them maximum 41 (74.54%) patients had less than < 500 counts, 14 (25.45%) patients had >500 CD4 cells counts. 176 patients had achieved TND, out of them 124 (70.45%) patients had less than <500 CD4 cells counts and only 52 patients (29.55%) had >500 CD4 cells counts. 38 patients had >1000 copies/mL, out of them 24 (63.15%) patients had < 500 CD4 cells counts and 14 (36.84%) had >500 CD4 cells counts.

we found that patients who had less than < 500 CD4 cells counts and suppressed viral load (VL<1000 copies/ml+ TND) were 165(87.30%) patients and unsuppressed Viral load (VL>1000 copies/ml) were 24(12.69%) patients. Patients of CD4 cells counts ≥ 500 cells and suppressed viral load were 66(82.5%) patients and unsuppressed viral load were 14 (17.5%) patients. It was statistically Non – significant (p >0.05) by chi square test. We can conclude that treatment has not achieved it's full impact on the disease after 6months of ART treatment. It is important to do the viral load testing at 6months as CD4 cells counts is not indicating clear cut regarding treatment impact on the disease. This study concordant with Laxmeshwar *et al.*, (2020), He reported CD4 cells counts < 500 and suppressed viral load were 1448 (80%), unsuppressed viral load were 359 (20%) patients.CD4 cells counts ≥ 500 and suppressed viral load were 1923 (96%), unsuppressed were 81(4%) patients after treatment.

Graph 2 showed stratification of patients between HIV RNA levels and CD4 cells counts after 12 months of treatment. 20 patients had VL<1000 copies/ml among them 11 patients had > 500 CD4 cells counts and 9 patients had <500 CD4 cells counts. 234 patients had achieved TND, out of them 122 (52.13%) patients had less than <500 CD4 cells counts and 112 patients (47.86%) had >500 CD4 cells counts. 15 patients had VL >1000 copies/ml, out of them 9 patients (60%) had < 500 CD4 cells counts and 6 patients (40%) had >500 CD4 cells counts.

Patients had CD4 cells counts < 500 cells and suppressed viral load (VL <1000 copies/ml + TND) were 131 (93.57%) and unsuppressed viral load (VL >1000copies/ml) were 9 (6.42%) patients. Patients of CD4 cells counts ≥ 500 cells and suppressed viral load (VL <1000 copies/ml + TND) were 123 (95.34%) patients, unsuppressed viral load (VL >1000copies/ml) were 6 (4.65%) patients at 12 months.

It was statistically significant (p<0.05).it shows clearly that treatment has achieved it's full impact on the disease after 12 months. It also shows that long term treatment improves CD4 cells counts and decrease viral load as viral load suppression level increased from 231 (85.87%) patients at 6 months to 254 (94.42%) patients after 12months of ART. This indicates good immune response.

Our findings were similar to Abdullahi *et al.*, (2009). Shidhaye *et al.*, (2021) were also observed CD4 cells counts < 500 and suppressed viral load were 2067 (86.7%), unsuppressed viral load were 318 (13.3%) patients. patients of ≥ 500 CD4 cells counts and suppressed viral load in 261 (92.2%) and unsuppressed Viral load in 22 (7.8%) patients, so total 87.3% patients had achieved suppression level and 12.7% had not achieved-suppression level. This findings are concordant with our study's findings.

Table 1 showed, number of patients with increasing trend of CD4 counts were 224 (83.27%). Out of them 152 had achieved TND and 45 patients had VL<1000 copies/ml and 27 had VL>1000 copies/ml at 6 months.

TND number of patients increased from 152 patients to 215 patients after 12 months of treatment. Patients who had VL>1000 copies/ml decreased from 27 patients to 10. It was statistically highly significant (p value <0.001).

Table.1 Increasing trend of CD4 cells counts with viral load at 6months and 12 months

No. of patients with increasing trend of CD4 counts		TND	<1000VL	>1000VL
At 6months	n=224	152 (67.85%)	45 (20.09%)	27 (12.05%)
At 12months	n=241	215 (89.21%)	16 (6.64%)	10 (4.15%)

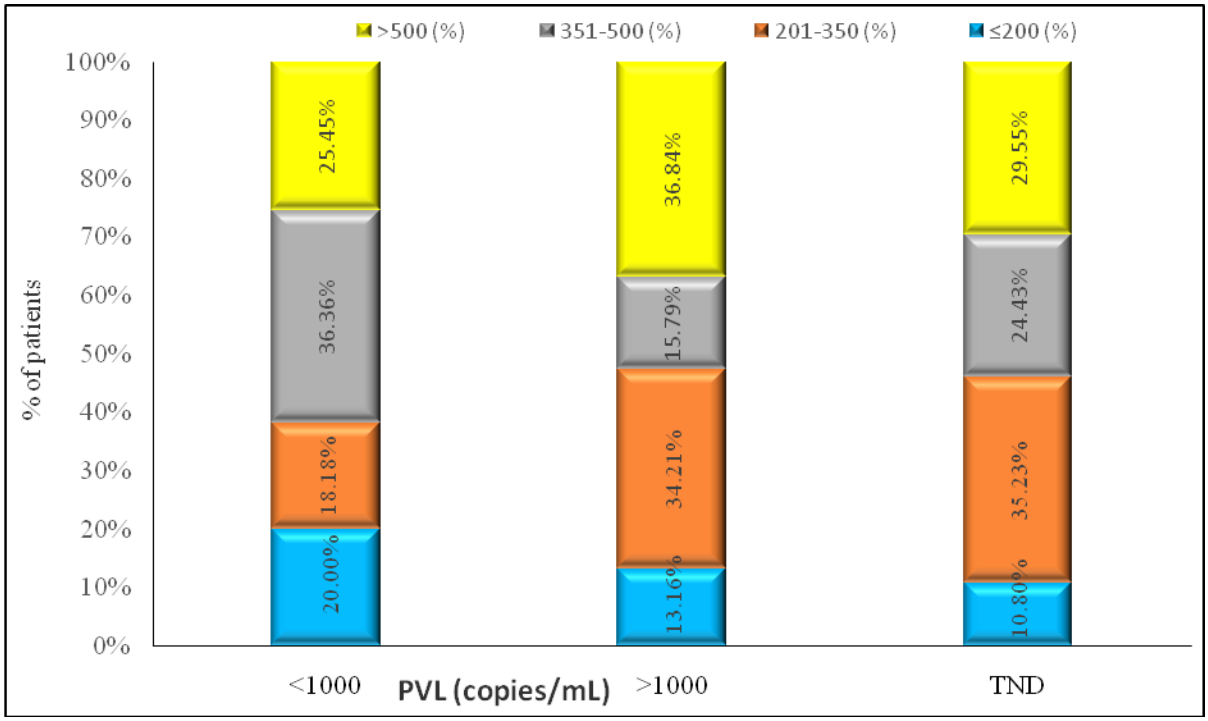
Chi square- 31.83, D.F.-2, P <0.001(HS)

Table.2 Decreasing trend of CD4 cells counts with viral load at 6months and 12 months

CD4 Decrease in no. of patients		TND	<1000VL	>1000VL
At 6months	n=45	24(53.33%)	10(22.22%)	11(24.44%)
At 12months	n=27	18(66.67%)	4 (14.81%)	5 (18.52%)
Static at 12 months	n=1	1 (100%)	-	-

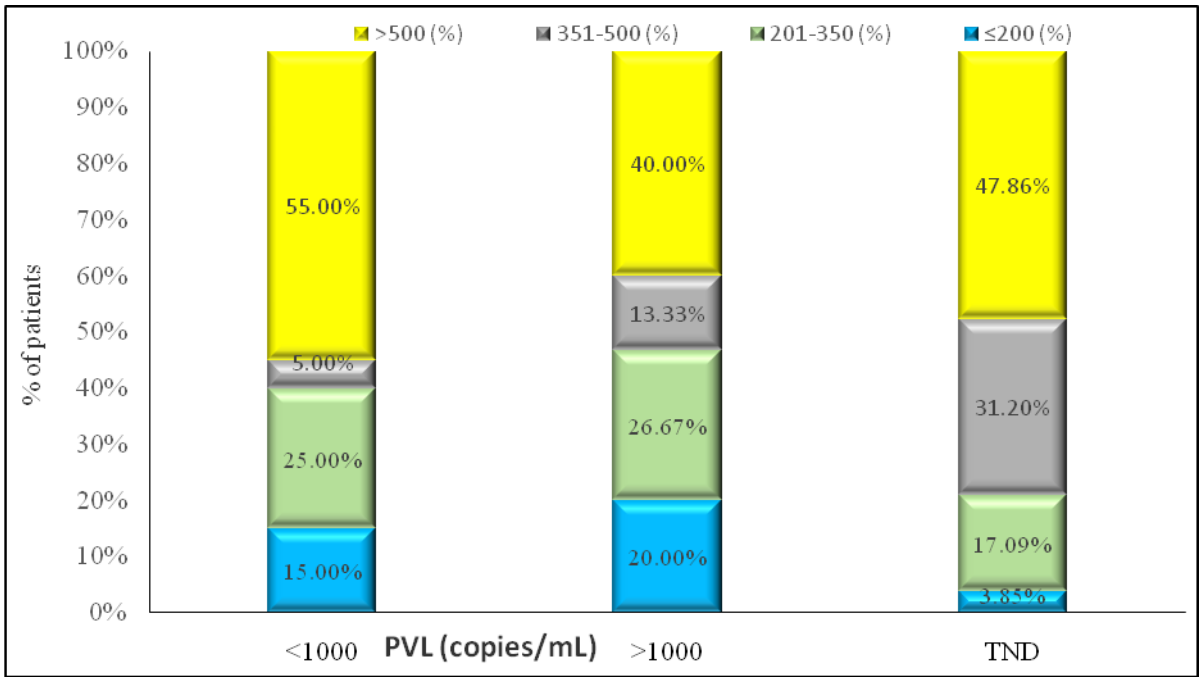
Chi-square 1.97; D.F.-4; P =0.74(NS)

Graph.1 Stratification of patients by plasma viral load (PVL) and CD4 cells counts after 6months of treatment.



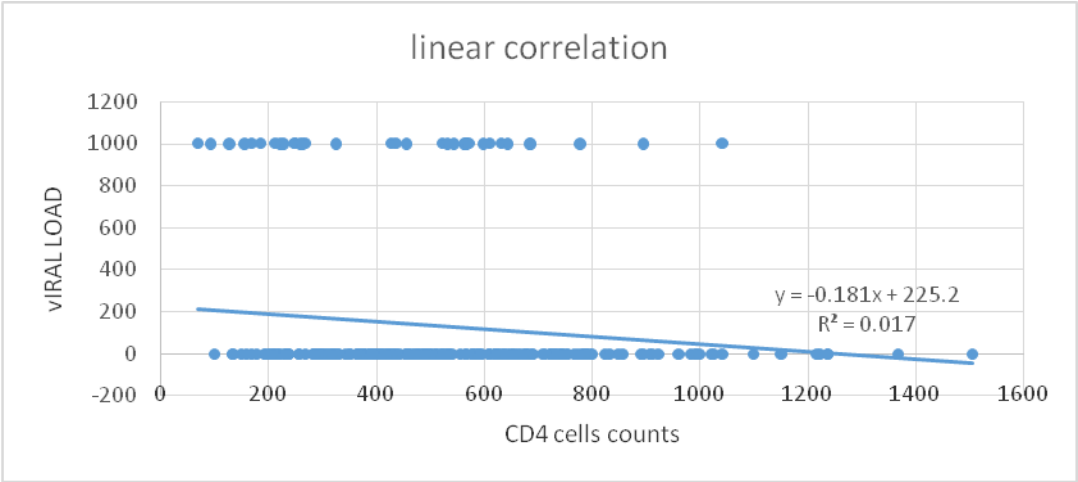
Chi Square test 11.67; DF 6; p=0.07 (NS)

Graph.2 Stratification of patients by plasma viral load (PVL) and CD4 cells counts after 12 months of treatment.



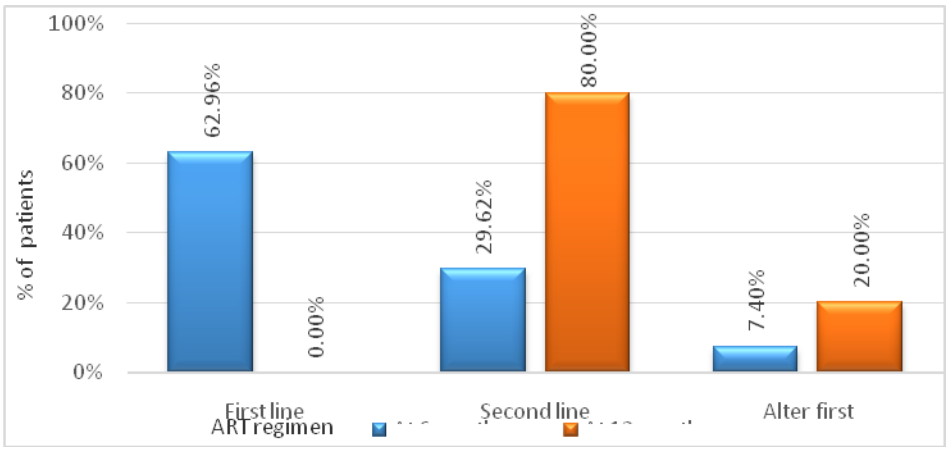
Chi Square test 17.42; DF 6; p<0.007 (S)

Graph.3 Linear Correlation of Plasma viral load and CD4 cell count (n=269).



p value-0.03

Graph.4 Number of patients with increasing trend of CD4 cells counts and viral load (>1000 copies/ml) with ART regimen at 6 months (n=27) and 12 months (n=10).



Chi-square test =11.65; P value =0.003(S)

Table 2 showed, number of patients (n=45) with decreasing trend of CD4 counts at 6 months in which 24 patients showed a decrease in viral load to TND, whereas 11 patients had VL >1000 copies/ml. There was reduction on number of patients to 27 after 12 months out of which 18 had achieved TND and only 5 had VL >1000 copies/ml. So total 18 patients shifted from decreasing trend of CD4 cells counts to increasing trend of CD4 counts. In this study only 1 patient showed static CD4 counts but achieved TND of viral load. It was statistically Non-significant (p value =0.74).

Graph 3 as shown there was a strong negative correlation between HIV-1 plasma viral load and CD4 cell count in this study. There is Significant Negative correlation between CD4 cell count and viral load after 12 months of treatment ($p<0.05$).

This reciprocal association could be for the reason that plasma viral load strongly predicts the rate of decrease in CD4 cell count and progression to AIDS although, correct prognosis of HIV infected individual is better known when both CD4 cell and plasma viral load are used. Kumar *et al.,* (2017); Panda *et al.,* (2019); Tahir Ibraham *et al.,* (2021) and Haokip *et al.,* (2018) showed a statistically significant negative correlation between PVL and CD4 cell count in HIV seropositive patients. These studies are concordant with the present study's findings.

Graph 4 depicted number of patients with an increasing trend of CD4 cells counts and viral load (>1000copies/ml) with their ART regimen showed that there was in increased in CD4 cells counts in 27 patients at 6 months of ART along with higher viral load, more than >1000 copies/ml. Out of these 27 patients, 8 patients were switched to second line regimen of ART because of other factors associated with them (opportunistic infection, drug adherence). Remaining 17 (62.96%) were continued on same first line regimen after seeing the other parameters like adherence and opportunistic infections etc. Only for 2 (7.40%) patients there was an alteration in first line regimen.

After 12 months of ART these number of patients decreased from 27 to 10 patients who had an increase in CD4 cells counts and viral load >1000 copies/ml, out of them 8 patients were continue on second line regimen.2 patients had alter first line regimen. It was statistically significant (p value =0.003), that the factors, CD4 counts, plasma viral load, drug adherence, presence of opportunistic infections and clinical condition evaluation should be considered to decide to ART regimen for HIV positive patients.

The present study conclude that there is significant Negative linear correlation between CD4 cell count and viral load after 12 months of treatment ($p<0.05$).

Combined use of these CD4 and Viral load prognostic markers will prove useful in individual patient management and in the design and evaluation of therapeutic trials as well as the clinical conditions of patient should be correlated with CD4 counts and plasma viral load to get maximum effect of ART regimen on HIV patients. This will reduce the risk of HIV transmission and disease progression at individual and population level. This will lead to improved patient outcomes and hence be helpful to reach closer to the our 90-90-90 target.

Our findings strongly supports the WHO's recommendation that, *treating everyone, regardless of clinical stage or CD4 count* and the use of routine viral load led to early identification of treatment failures and referrals to second- and third- line regimen, while also preventing from unnecessary switches.

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How to cite this article:

Neelam Chauhan, Anshusharma, Shiv Kumar, Neera Samar and Madhubala Mishra. 2023. Study of the HIV-1 RNA Levels and CD4+ Cells Counts in HIV Infected Patients. *Int.J.Curr.Microbiol.App.Sci.* 12(03): 81-88. **doi:** https://doi.org/10.20546/ijcmas.2023.1203.011