

Microbiology

STUDY OF CD4 T-CELL COUNT AND HIV VIRAL LOAD IN HIV PATIENTS UNDERGOING ANTIRETROVIRAL THERAPY VISITING NATIONAL PUBLIC HEALTH LABORATORY, TEKU, KATHMANDU, NEPAL

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Abstract

Infection with Human Immunodeficiency Virus (HIV) is a global burden affecting large population throughout the world. Regular monitoring of CD4 cell count and viral load is necessary to evaluate treatment success in patients undergoing antiretroviral treatment (ART). This study was carried out with the aim of determining the CD4 T-cell counts and viral load on patients in antiretroviral therapy. The cross sectional descriptive study was conducted on 175 HIV infected individuals on ART who visited at National Public Health Laboratory (NPHL), Teku, Kathmandu, Nepal for their viral load estimation. The study was carried over a period of six month from June 2013 to November 2013. Three to five ml of venous blood was collected from each patient and processed in parallel to determine CD4 T-cell count according to manufacturer's protocol. HIV viral load in patient's serum was detected by real time PCR (Rotar gene) using QAigen kit. Prevalence of HIV was found higher in sexually active age group 15-49 years (82.86%) and males were predominant (54.86%). Regarding CD4 and viral load, correlation was statistically significant ($P=0.008$). 60% of the patients had undetectable viral load and 24% were suffering from AIDS as they had CD4 count less than or equal to 200 copies/ μ l. Mean viral load in female was found drastically lower (13938 copies/ml) than in male (37526 copies/ml) while CD4 counts were not significantly different (336 for male and 396 for female). In HIV patients under ART, the progression to AIDS is slower but regular monitoring of CD4 count and viral load is necessary to predict the stage of infection and to find viral burden in the patient.

Keywords: *HIV, ART, Viral load, CD4 count*

Introduction

Human immunodeficiency virus (HIV-1 and HIV-2), RNA virus, belonging to the member of genus lentivirus of the retroviridae family (Kazushu *et al.* 2008) predominantly infects helper T (CD4) lymphocytes, macrophages, dendritic cells causing massive dysfunction of the immune system leading to severe immunodeficiency condition known as Acquired immunodeficiency syndrome (AIDS) (Weinberg and Pott 2003). According to WHO, there were approximately 35 million people worldwide living with HIV/AIDS in 2013. Of these 3.2 million were children (less than 15 years old), 2.1 million of individuals worldwide become newly infected with HIV in 2013. This includes 240,000 children (less than 15 years). UNAIDS

report shows that 19 million of the 35 million people living with HIV today do not know that they have the virus (UNAIDS 2014). Although HIV/AIDS is in pandemic stage there is neither vaccine nor proper medication to cure this infection (Arts *et al.* 2012). In the 1990s, introduction of Anti- Retro Virals (ARVs) brought new hope to people living with HIV. Administration of ARVs causes dramatic decline in AIDS related morbidity and mortality (Amirayan-Chevillard *et al.* 2002) by reducing plasma human immunodeficiency virus load (Connor *et al.* 1994) restoring CD4 T cells numbers and greatly decreasing the incidence of OI's (Weinberg and Pott 2003). Viral load, which can also be named as viral titre or viral burden, can be calculated by estimating the amount of virus in

an involved body fluid and that also provides the severity of a viral infection in infected patient. Viral load is one of the standard tools to monitor treatment response in patients who are taking ART and in conjunction with CD4 cell count, to access HIV progression in those places where there is access of viral load monitoring. In some situations, the viral load may factor into decisions to initiate or change ART (Palella *et al.* 2003). Change in viral load is also very important measurement. Change in viral load is a very important measurement as a falling count indicates improvement and suppression of HIV infection and a rising count indicates that the infection is getting worse or has developed resistance to the drugs that have been used in the therapy (Saahene 2009). CD4 lymphocyte cells (also called T-cells or T-helper cells) are the primary targets of HIV. The CD4 count and the CD4 percentage mark the degree of immunocompromised. The CD4 count is the number of CD4 cells per microliter (μL) of blood. It is used to stage the patient's disease, determine the risk of opportunistic illnesses, assess prognosis, and guide decisions about when to start antiretroviral therapy (AETC NRC 2012).

As the CD4 count in HIV infected patients decreases their viral load increases so, the status of CD4 cells in the patient body provides one of the benchmarks against the progression of HIV/AIDS. In HIV infected persons, CD4 count and HIV viral load is used to monitor and initiate ART (Sharma *et al.* 2009). Viral load has been seen lower in female as compared to male whereas CD4 count has been seen similar in both the gender so, this changing pattern of viral load in case of gender is a new subject of study (Farzadegan *et al.* 1998; Sterling *et al.* 2001).

Materials and Methods

Study site

This study was carried out in National Public Health Laboratory Teku, Kathmandu, Nepal in mutual collaboration with department of microbiology GoldenGate International College from July to November 2013. A total of 175 blood samples were collected from HIV patients under ART referred to NPHL for CD4 T cell count and HIV viral load testing.

Sample collection and handling

Venous blood was collected in K3 EDTA vacutainer tube (depending upon tube capacity 3-5

ml) to a full draw. The tube was labelled with patient's identification number, date, time of collection and the name of the collecting personnel. The tube labels were doubled checked for accuracy with the sample request forms before sending it to the testing lab. The blood was mixed properly by inverting the tube 6-8 times immediately after collection. Formation of small clots may affect accuracy of the count and ability to run the instrument. Sample was transported to the laboratory within 30 hours of phlebotomy. Samples were transported and stored at ambient temperature (20-25°C) for CD4 count. Samples were processed within a maximum of 48 hours of collection and were rejected if they were clotted, haemolysed or frozen, and for viral load the remaining blood specimen in the vacutainer tubes were centrifuged within six hours of collection to get plasma. The plasma was then kept in two aliquots of 500 μL and frozen at -70°C. This plasma was used for the measurement of viral load analysis.

CD4 T cell count

The enumeration of CD4 lymphocyte numbers was carried out in the blood collected into EDTA containing tubes by SP flow cytometry (TruCOUNT) on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). Tru-COUNT reagent containing monoclonal antibodies for CD3, CD45 and CD4 labelled with fluorescent dyes (PE, per CD and FITC, respectively) was mixed with anticoagulant whole blood and red blood cells were lysed with lysing solution before enumeration. BD multiSET software (BD-Biosciences) was used to determine the absolute CD4 count.

Viral RNA extraction and viral load (HIV RNA levels) measurement

HIV-1 RNA was extracted by using QIAamp viral RNA Mini kit (QIAGEN GmbH, Hilden, Germany) following the manufacturers protocol. The eluted RNA was stored at -80°C until RNA quantification. HIV-1 RNA was quantified by real time PCR using artus HI Virus-1 RG RT-PCR kit (QIAGEN GmbH, Hilden Germany) and Corbett Rotar Gene 6000 Real time PCR system. The PCR conditions were set for denaturation 95°C/ 30 sec, annealing 50°C/60 sec and elongation 72°C /30 seconds. A range of standard (10, 100, 1000, 10,000 IU/ ml) provided with kits were used to develop standard curves for the quantification of the viral load copies per ml.

Data recording and analysis

All the data were recorded by using Microsoft Excel 2007 and then transferred to SPSS software for analysis. Double entry of data i.e. in computer as well as on hard copy was made.

The result obtained were all analyzed using standard statistical technique using SPSS version 19. The mean was calculated using excel whereas level of significance was calculated by SPSS. The relation was considered statistically significant if $p \leq 0.05$.

Results

A total of 175 patients under ART referred to National Public Health Laboratory, Teku, Kathmandu for CD4 Count and Viral load testing were included in this study from June 2013 to November 2013.

Sex wise distribution of HIV patients

Among the 175 study population, 96 patients (54.85%) were male and 79 patients (45.14%) were female.

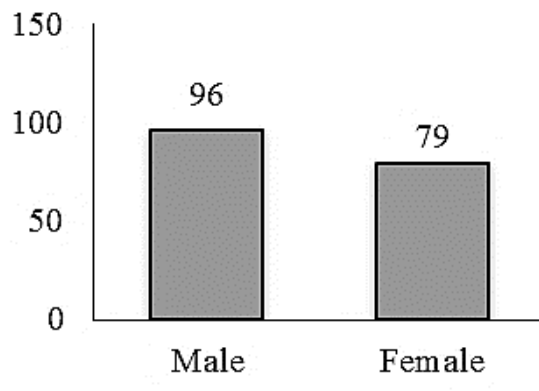


Figure 1. Sex wise distribution of HIV patients

Age wise distribution of HIV patients

The age of the patients included in the study ranged from 2 to 69 years. Majority of the seropositive cases were adults with sexually active age group.

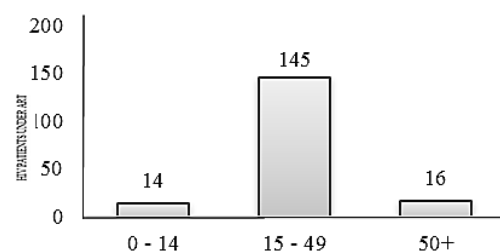


Figure 2. Age wise distribution of HIV patients

A significant number (14) of the children were found to be infected with HIV while 16 patients were old aged.

Sample distribution according to ART period

Out of 175 total study population, 60% (105 patients) had been taking ART for one to five years, and the least percentage, 0.57% (1 patient) was taking ART for more than eleven years.

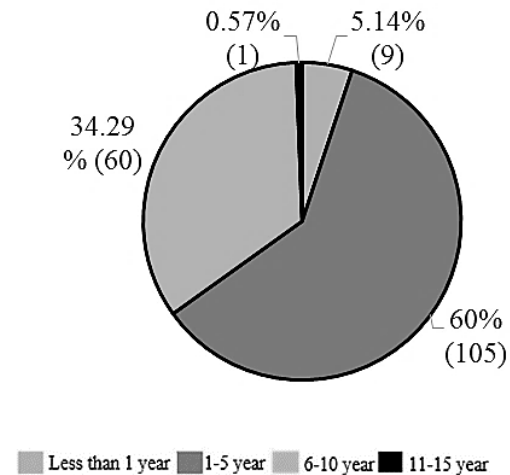


Figure 3. Sample distribution according to ART period.

Relationship between CD4 T-Cell and viral load

In this study, 32 patients (the highest number) were found in two groups with CD4 count between 200 and 350, and CD4 count between 350 and 500, both having viral load less than or equal to 400. 1 patient (the lowest number) had viral load more than 1,000 with CD4 count greater than 500. The co-relation between CD4 T-cell count and viral load was found to be statistically significant ($p=0.008$).

Table 1. Relationship between CD4 and viral count.

CD4 Count (copies/ μ l)	Viral Load (copies/ml)			Correlation (P)
	≤ 400	400-1,000	>1,000	
≤ 200	10	3	29	0.008
200-350	32	4	21	
350-500	32	3	6	
>500	31	3	1	

Mean CD4 count for different viral load groups

The mean CD4 of the patients with viral load less than or equal to 400 (undetectable) showed highest value, whereas greater than 1,000 showed the least. So, as the viral load decreased, the mean CD4 increased and vice versa.

Table 2. Mean CD4 count for different viral load groups.

Viral Load (copies/ml)	Mean CD4 Count (copies/ μ l)
≤ 400	439
400-1,000	381

Mean viral load for different CD4 count groups

The mean viral load of patients with CD4 count less than or equal to 200 was found to be the highest, while lowest mean of viral load was seen in CD4 count group greater than 500. Hence, as the CD4 count increased, the mean viral load decreased and vice versa.

Table 3. Mean viral load for different CD4 count groups.

CD4 Count (copies/ μ l)	Mean Viral Load (copies/ml)
≤ 200	87,176
200-350	16,316
350-500	2,269
>500	546

Sex wise mean of CD4 and viral load

The mean of CD4 count of male and female did not show significant difference, but the mean of viral load of male was found remarkably higher than of female.

Table 4. Sex wise mean of CD4 count and viral load

Sex	Mean of CD4 Count (copies/ μ l)	Mean of Viral Load (copies/ml)
Male	336	37,526
Female	396	13,938

Discussion

Nepal is considered as a concentrated epidemic zone for HIV infection with about 0.20% prevalence in adult population (UNAIDS/Nepal

2013). Although prevalence is lower than overall prevalence in South-East Asia (0.7%), the diagnosis and monitoring of HIV infection is to be scaled up throughout the country. As per NCASC guideline, the CD4 count is being used as a basis for initiation of ART (NCASC 2012). The validity of this approach in comparison to viral load has not been studied much in Nepal. HIV viral load estimates are being carried out in very few patients because of limited resources.

Among 175 HIV seropositive individuals were taken in this study, 96 were males and 79 were females. The high prevalence in male population is because they stay out more than female population and involve more in unsafe sexual practice than females (Kapoor *et al.* 2004). The other reason is that females cannot express their HIV related health problems freely due to it being a sexually transmitted disease and also due to social stigmas (NCASC 2012).

In this study, among the three age groups, highest prevalence of 145 patients (82.86%) was seen in sexually active adults (15-49 age group), followed by elderly (≥ 50) with 16 patients (9.14%) and least prevalence of 14 patients (8%) was seen in children (0-14) age group. This finding is in harmony with the other studies done in Nepal and India (Dhungana 2004; Ukey *et al.* 2005; Neupane *et al.* 2013).

60% of the patients were under 1-5 years of treatment, 34.29% were under 6-10 years of treatment, 5.14% were under less than one year of treatment and 0.57% had experienced 11-15 years of treatment. Thus, we found 94.59% were taking ART for more than one year. This figure is slightly higher than the report of NCASC (2012), according to which 84.7% of the people with HIV infection were taking ART for more than one year which might be due to the improving treatment of HIV/AIDS in Nepal.

In this study, the co-relation between CD4 T-cell count and viral load was found to be statistically significant ($p=0.008$). CD4 cell count status of HIV patients provides one of the benchmarks against the progression of HIV /AIDS (Sharma *et al.* 2009). CD4 cells decrease with the severity of HIV progression (Lyten 2006). Plasma viral load strongly predicts the rate of decrease in CD4 T-lymphocyte count and progression to AIDS and death, but the prognosis of HIV infected persons is more accurately defined by combined measurement of plasma HIV-1 RNA and CD4 T-lymphocytes. Gautam *et al.* (2008) showed that all

patients with CD4 count less than 50 cells/ μ l had viral load more than 100,000 copies/ml. According to another study conducted in UK found that the viral load data fluctuated according to the changes in CD4 count during natural infection. They found that viral load and CD4 count were negatively correlated using statistical approach (Masel *et al.* 2000).

The mean values of CD4 count at different levels of viral load showed the decreasing trend with the increase in viral load. In people with viral load less than or equal to 400, mean of CD4 count was 439, with viral load between 400 and 1,000, it was 381 and with viral load greater than 1,000, it was 219. The mean viral load of patients with CD4 count less than or equal to 200 copies/ μ l was found to be the highest, while lowest mean of viral load was seen in CD4 count group greater than 500 copies/ μ l. Hence, as the CD4 count increased, the mean viral load decreased. The mean in the decreasing trend showed negative correlation between viral load and CD4 count.

The mean viral load remained lower in female (13,938 copies/ml) than in male (37,526 copies/ml). However, the mean CD4 count did not differ significantly according to sex i.e. male had 336 copies/ μ l and female had 396 copies/ μ l. Our study was also supported by Prins *et al.* (1999) and Govender *et al.* (2014) where female had higher CD4 count than male and which suggests delay in initiation of therapy in female than in male. Our study has further been supported by Farzadegan *et al.* (1998) and Sterling *et al.* (2001), where female had lower mean viral load than male.

So, understanding the mechanism responsible for sex difference may provide insight into HIV pathogenesis. Although the biological mechanism remains unclear, the data suggest that the current recommendation for HIV viral load threshold to initiate ART should be revised downward towards female (Homayoon *et al.* 1998). Sex affects viral load and CD4 count at various stages of diseases, where females develop AIDS at higher CD4 counts and lower viral loads than males (Sarishen *et al.* 2014).

Conclusion

The high prevalence of HIV was seen in male than in female. Similarly, sexually active age group (15-49) had the highest number of patients. As the CD4 count declined in patients under ART, their viral load increased and vice versa. It was also seen that

the mean viral load in female was significantly lower than in male, whereas, in both the sexes, CD4 count was similar. Significant statistical relation between CD4 T-cells and HIV viral load in patients under ART was established.

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