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Viral load and CD4 cell count in HIV patients receiving antiretroviral therapy from eastern Nepal

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Abstract
HIV is one of the major public health problems in Nepal, fuelled by several socio-economic factors. Antiretroviral (ARV) drugs have become the cornerstone of HIV (Human Immunodeficiency Virus) care and treatment. The aim of this study was to characterize the relationship for viral load response to ART with baseline CD4 cell count and base line viral load at Kirant Yakthung Chumlung Rehabilitation Center (KYC) based at Dharan city of Eastern, Nepal from July to December 2016. A total of 213 blood samples were collected through random sampling method. The collected blood was mixed properly by inverting the tubes 6-8 times immediately after collection and transferred to the laboratory for analysis within 30 minutes. CD_4 count, hemoglobin level and serum albumin level determination were performed on the same day of collection within 2-3 hours of receipt. The remaining blood specimen in the vacutainer tube was centrifuged within 6 hours of collection to get plasma and kept in two aliquots of five hundred micro litres (500 µL) and frozen at -70˚C used for plasma viral load analysis. The enumeration of CD4 lymphocytes numbers was carried out by SP flow cytometry (Trucount) on a FACS Calibur flow cytometer. HIV RNA levels were measured in plasma prepared from blood that had been collected in k3 EDTA containing tube and stored at -70˚C. HIV viral RNA levels in patient's plasma were detected by quantitative real-time PCR (q-PCR) Rotor gene using Qaigen kit. Among total 213 patients,123 (57.7%) were male and 90 (42.3%) were female. 101 having viral load 35-400 copies/mL showed CD4 count 200-500 cell/mm^3 and 1 showed viral load below 34 and between 10001-100000 with CD_4 count less than 200 cell/mm^3.There was no significant association between viral load and CD4 count with P value 0.118 (P> 0.05). 77(36.1%), 30(14.0%),72(33.1%), 22(10.3%) and 11(5.16%)were under ART from 5-6 years, below 2 years, 3-4 years, 7-9 years and 8 years and above respectively. 54 were under ART treatment from 5-6 years having CD4 count 200-500. None of them showed CD4 count less than 200 whom were under treatment from 8 years and above. There was significant association between CD4 count and duration of ART treatment with the P value 0.052. 45 were under 5-6 years of ART therapy with viral load 35-400 whereas none of them showed 100001 and above viral load in the same duration. In 7-8 years of treatment and above 8 years, 1 showed viral load above 34. There was no signification between viral load and duration of treatment. HIV viral load can be reduced by using ART treatment that leads to suppression of viral load to undetectable level, having a CD4 cell count in the normal range to maintain patients for life. Its use has led to a marked reduction in AIDS (Acquired Immune Deficiency Syndrome) related morbidity and mortality.

Keywords: human immunodeficiency virus, CD4 cell, viral load, antiretroviral therapy

Introduction

Human immunodeficiency virus (HIV) is a T-lympotropic RNA virus belonging to the member of genus lentivirus of the family retroviridae [1] leaving a patient vulnerable to a host of life-threatening opportunistic infections, neurological disorders or unusual malignancies [2] massive dysfunction of the immune system leading (CD4) lymphocytes, macrophages, dendritic cells which breaks down the body's immune system to severe immunodeficiency condition known as Acquired immuno deficiency syndrome (AIDS) [3].

HIV is a spherical virus enveloped by a lipid bilayer about 90–120 nm in size (Fisher, 2007) belong to a family of primate lentiviruses of two types including the HIV-1 and HIV-2 [4]. The single stranded RNA has an outer icosahedral shell and an inner cone-shaped core enclosing the ribonucleo proteins, p7 and three viral enzymes protease, reverse transcriptase and integrase [5]. The viral envelope is composed of the lipid bilayer containing proteins from the host cell consisting a cap made of three molecules known as glycoprotein gp120, and a stem consisting of three gp41 molecules anchoring into the viral envelope [6]. HIV/AIDS is considered a pandemic actively spreading from other primates to humans in west-central Africa in the early-to-mid 20th century [7].

Nepal's first cases of HIV/AIDS were reported in 1988 and the disease has primarily been transmitted by intravenous drug use and unprotected sex **[8]** since then this disease has become major public health concern [9]. HIV enters the body through mucosal tissues and blood infecting different immune cells including T-cells (CD4 cells) and finally becomes established in lymphoid tissue and remain latent for long periods. Moreover active viral replication is associated with further infection of cells and progression to AIDS [5].CD4 cell count and viral load (HIV RNA) count are the common laboratory markers that are regularly used for HIV/AIDS patient management in addition to predicting disease progression and treatment outcomes in immuno-compromised [10]. HIV positive patients continue to have raise in CD4 cell count for several years after initiation of

anti-retroviral therapy (ART) [11].The decrease in CD4 cell count in HIV infected patients indicates their increase in viral load so, the status of CD4 cells in the patient body provides one of the benchmarks against the progression of HIV/AIDS. In HIV positive patients, CD4 count and HIV viral load is used to monitor and initiate ART [12].

The normal CD4 count is 250-300 cell/mm^3 blood, and is used to stage the patient's disease, determine the risk of illnesses, assess prognosis, and guide decisions about when to start antiretroviral therapy [13].As of the end of 2019, 25.4 million people with HIV (67%) were accessing antiretroviral therapy (ART) globally [8]. ART treatment for HIV decelerate the virus progression butCD4 cell count monitoring sometimes fails to predict virological failure resulting in unnecessary switch of treatment lines causing drug resistance and limitations of treatment options[14]. The aim of this study included to determine the CD4 cell counts in HIV-infected patients, to determine the oral manifestations and to correlate the values of CD4 cell counts with the patients [15].

Materials and Methods

The study was retrospective cross sectional conducted at Kirant Yakthung Chumlung Rehabilitation Center (KYC) based at Dharan city of Eastern, Nepal. After getting consent of patients and hospital authority, this study was carried for the duration of six months from July to December 2016.

Collection of sample

A total of 213 blood samples were collected through random sampling method since it was a purposive study from HIV infected people from viral load testing and CD4 count. HIV seropositive volunteers visiting KYC who were under ART treatment therapy for CD4 monitoring were requested to participate in the study during counseling for informed verbal consent. HIV seropositive adult individuals of both sex and social classes under treatment were included in the study. Pregnant women, Neonates, children <18 and the volunteers who did not agree were excluded from the study.

Sample collection and handling

Venous blood specimen was collected in tripotassium ethyl enediaminetetra acetic acid (k3 EDTA) vacutainer tube (5 mL) to full draw. The tubes labeled with patient's identification code number, name, age, sex and address were kept confidential. The collected blood was mixed properly by inverting the tubes 6-8 times immediately after collection and transferred to the laboratory for analysis within 30 minutes. CD4 count, hemoglobin level and serum albumin level determination were performed on the same day of collection within 2-3 hours of receipt. The remaining blood specimen in the vacutainer tube was centrifuged within 6 hours of collection to get plasma and kept in two aliquots of five hundred micro litres (500 µL) and frozen at -70˚C used for plasma viral load analysis. The samples were rejected if they were clotted, hemolysed or frozen [16].

CD4$^+$ T cell count

The enumeration of CD4 lymphocytes numbers was carried out by SP flow cytometry (Trucount) on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA USA). Trucount tube containing twenty microliter (20 µL) monoclonal antibodies, fifty microliter (50 µL) well-mixed whole blood and four hundred and fifty microliter (450 µL) of FACS lysing solution was pipetted, capped, vortexed and incubated 15 minutes for analysis. The CD4 T cell was computed on the flow cytometer by the Multi set software (BD Biosciences) using the formula: CD4 T cell= sample bead count/50 and result of each patients were recorded to obtain absolute count [16].

The process was carried out at B.P. Koirala Institute of Health Science (BPKIHS) Dharan, Nepal.

Viral Load (HIV-RNA level)

HIV RNA levels were measured in plasma prepared from blood that had been collected in k3 EDTA containing tube and stored at -70˚C.HIV viral RNA levels in patient's plasma was detected by quantitative real-time PCR (q-PCR) Rotor gene using Qaigen kit. The process was carried out at National Public Health Laboratory (NPHL) Kathmandu, Nepal.

Data recording and statistical analysis

All the data collection on CD4 count, viral load assessment level determination were recorded by using Microsoft excel 2007 and then transferred to 17 version of statistical package for social sciences (SPSS). The statistical significance was tested by Chi-square test and Pearson's Co-relation Test wherever applicable using SPSS statistical software 17.

Result and Discussion

All of the samples regarding HIV cases were monitored, studied, and conducted at Kirant Yakthung Chumlung Rehabilitation Center, Nepal from July 2016 to December 2016. A total of 213 blood samples were collected from HIV infected people for HIV viral load testing and CD4 count taking ART. Among them higher number of populations were male 123 (57.7%) and female population 90 (42.3%) as shown in the pie-chart (Fig 1).

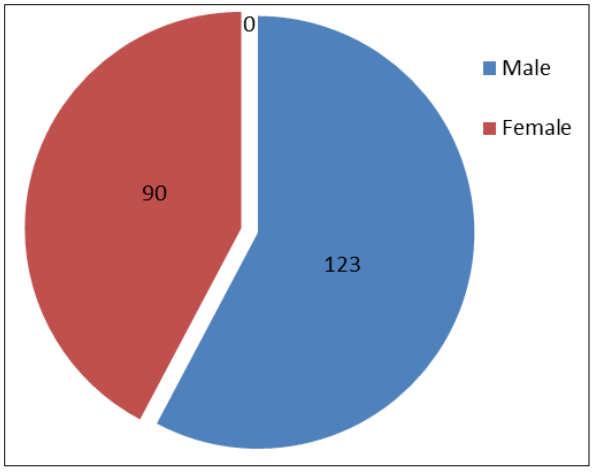

Fig 1: Sex wise distribution of population

Among 213 HIV positive individuals taking ART were taken in this study, male 123 (57.7%) were male and 90 (42.3%) were females. The high prevalence in male population is because they stay outside and travels more for seeking job opportunities than female population and comparatively involves more in unsafe sexual practice than females [17]. Moreover the other reason in

male dominated country like Nepal is that females cannot express their HIV related health problems freely due to it being a sexually transmitted disease and also inconvenience due to social stigmas [18]. Research concludes that the country's HIV epidemic is mainly concentrated among Female Sex Workers (FSWs), Male Sex Workers, Trans-genders and their Clients (MTCs) and Injecting Drug Users (IDUs), who form the most vulnerable or Most-at-Risk Population groups (MARPs) [19].

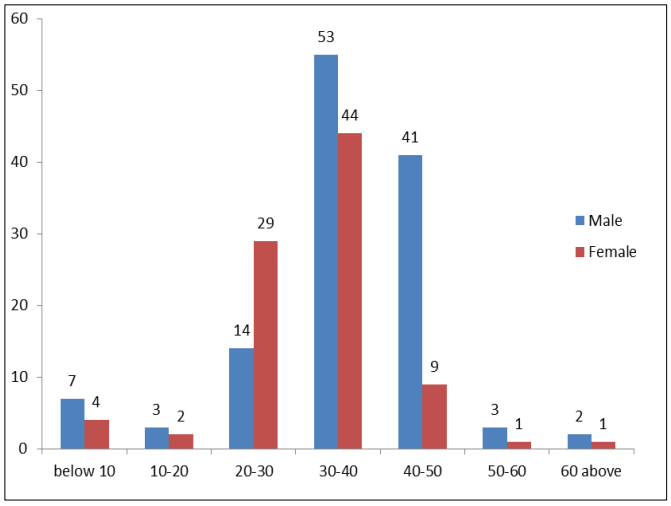

Fig 2: Age and sex wise distribution of population

A total 97(45.5%) belongs to age group 30-40 years, 50(23.4%) belongs to age group 40-50 years, 43(20.20%) belongs to age group 20-30. Similarly 13(6.1%) belongs below 10 age group and 4(1.8%) belongs to age group 50-60, whereas only 3(1.4%), above 60 years. Highest number of male population and female population i.e. 53(24.8%) and 44(20.65%) respectively were in age group 30-40 years as shown in the bar diagram (fig 2). This finding is in harmony with the other studies done in Nepal and India [12].

According to the estimates by World Health Organization (WHO) and UNAIDS, by the last of 2019, approximately 38 million people were living with HIV/AIDS globally **[20]** among these, 36.2 million were adults and 1.8 million were children (<15 years old) [8].

HIV continues to be a major global public health issue considered as the concentrated epidemic for HIV infection with about 0.33% prevalence in adult population in a country like Nepal which is lower than overall estimated prevalence of worldwide living with HIV [21]. Nepal is considered as a concentrated epidemic zone for HIV infection with about 0.20% prevalence in adult population [22]. CD4 T lymphocyte count is an essential marker in guiding clinical decisions and monitoring in case of HIV infection [23].

Viral load monitoring predicts deaths of HIV/AIDS patients better than CD4 cell count monitoring. Assessment of the fitted models shows that viral load monitoring is a better predictor of HIV/AIDS progression than CD4 cell count [24].

Table 1: Association between viral load and CD4 count

Viral load copies/ml	CD4 cell/mm^3			Total	P-value
	Less than 200	200-500	500 and above		
Below 34	1	32	3	36	
35-400	17	101	27	145	
401-10000	5	13	2	20	0.118
10001-100000	1	7	0	8	
100001 and above	0	4	0	4	
Total	24	157	32	213	

Out of 213, 101(47.45%) patients having viral load 35-400 copies/ml showed CD4 count 200-500 cell/mm^3 & least only 1 showed viral load below 34 copies/mL and between 10001-100000 with CD4 count less than 200 cell/mm^3.There was no significant association between viral load and CD4 count with P value 0.118 (P> 0.05).

In this study, a total 101(47.45%) of patients were having CD4 count 200-500 cell/mm^3 showed viral load 35-400 copies/ml. 157(77.70%) patients were having CD4 count 200-500 cell/mm^3 with viral load 35-400 copies/ml only 1 showed viral load below 34 and between 10001-100000 with CD4 count less than 200cell/mm^3.There was no significant association between viral load and CD4 count with P value 0.118 (P> 0.05).Our result is strongly supported by the finding of John W. Mellors et al, suggested that the plasma viral load is not the better predictor of number of CD4 T cells. Moreover, the study indicates that greater the virus proliferation higher the exhaustion of capacity of the immune system to recover CD4 T cells [25].

14.0% of the patients were below two years of treatment, 33.8% were under 3-4 years of treatment, 36.1% were above 5-6 years of treatment, above 7-8 years of treatment and 5.16 % had experienced above 8 years of treatment. Thus, we found 85.36% were taking ART for more than two year. This figure is slightly higher than the report of [26], 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 [12].

Table 2: Distribution of population accordance of duration of ART used

Duration of Treatment	Count of population	%
Below 2 years	30	14.0
3-4 years	72	33.8
5-6 years	77	36.1
7-9 years	22	10.3
8 and above	11	5.16
Total	213	100

Highest number of patients 77 (36.1%) were under ART from 5-6 years, 30(14.0%) below 2 years, 72 (33.8%) from 5-6 years. Similarly 22(10.3%) from 7-9 years whereas least of the population i.e. 11(5.16%) were under ART from 8 years and above.

Table 3: Relationship between CD4 count and duration of ART treatment

Duration of treatment	CD4 cell/mm^3			Total	P-value
	Less than 200	200-500	500 and above		
Below 2 year	4	31	5	40	0.052
3-4 year	10	51	6	67	
5-6 year	7	54	11	72	
7-8 year	3	15	4	22	
8 and above	0	6	6	12	
Total	24	157	32	213	

Highest number of population 54 were under ART treatment from 5-6 years having CD4 count 200-500. None of them showed CD4 count less than 200 whom were under treatment from 8 years and above. All duration of treatment, major population showed CD4 count 200-500. There was significant association between CD4 count and duration of ART treatment with the P value 0.052.

According to Sharma et al, 54 were under ART treatment from 5-6 years having CD4 count 200-500. None of them showed CD4 count less than 200 whom were under treatment from 8 years and above. 157 showed CD4 count between 200-500·As the CD4 cell count status of HIV patients provides one of the benchmarks against the progression of HIV /AIDS [12] whereas decrease in CD4 count indicates the severity of HIV progression [27]. Hence, the number of CD4 count increased with the increase in duration of treatment and the trend showed positive correlation between CD4 count and duration of ART treatment with the P value of 0.052. Regarding viral load, this study involved 213 cases, 145 of the cases showed viral load between 35-400 copies/mL where these were followed by 36 cases were less than 34 copies/ml and 20 cases had viral load between 401-10000.Most of the population i.e. 45 was under 5-6 years of ART therapy with viral load 35-400 whereas none of the population showed 100001 and above viral load in the same duration. In 7-8 years of treatment and above 8 years, none of the population showed viral load above 34. There was no signification between viral load and duration of treatment with the p value of 0.39.

Table 4: Association between viral load and duration of ART treatment

Duration of treatment	Viral Load(copies/mL)					Total	P-value
	34	35-400	401-10000	10001-100000	1000001 and above		
Below 2years	6	30	1	3	0	40	0.39
3-4years	12	43	8	2	2	67	
5-6 years	18	45	7	2	0	72	
7-8 years	0	18	2	0	2	22	
8and above	0	9	2	1	0	12	
Total	36	145	20	8	4	213	

Total 45(21.12%) patients were under 5-6 years of ART therapy with viral load 35-400 copies/ml whereas none of the population showed 100001 and above viral load in the same duration. In 7-8 years of treatment and above 8 years, none of the population showed viral load above 34 copies/mL. There was no signification between viral load and duration of treatment. Our findings in this study have some clinical complications as well. This study mainly confined to the relationship between CD4 count and viral load for the diagnosis of effective of ART therapy. Association with CD4 count, viral load and duration of ART therapy also help us to understand the effectiveness of treatment [28].Single spot CD4 count and viral load test were done for each patients rather than finding the changing patterns. Changing in CD4 count and viral load overtime was not studied. This might be the main factor for the lack of significant relationship between CD4 count and viral load. Access to antiretroviral therapy (ART) in Africa increased dramatically over the past decade, beginning with a few thousand people and reaching five million people by mid-2010 [29].High viral load and low CD4 cell count are independently associated with mortality and changes in viral load and CD4 cell count during treatment have been associated with survival [30].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 [31]. 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 [32].

Routine monitoring of viral load and CD4 cell counts during ART, however, was adopted in well- resourced settings without Studies indicating improved survival compared with careful clinical monitoring. One recent mathematical model showed little benefit and considerable cost even during 20 years of follow-up [33].The major principle of this study was to assess and compare the use of CD4 cell count and viral load level, in analyzing HIV/AIDS progression on patients receiving antiretroviral therapy. HIV viral load estimates are being carried out in very few patients because of limited resources. The validity of this approach in comparison to viral load has not been studied much in Nepal [16].

Conclusion

Altogether, 213 ART taking patients were the participant in this study with the higher prevalence of HIV was seen in male than female. Similarly, sexually active and productive age group (30-40) had the highest number of patients more prominent to HIV infections. The progression and outcome of HIV depends on the factors like baseline health, nutritional status, environment,

endemic disease and access to therapy. Though CD4 T cells and viral load are the crucial parameter for HIV progression, these parameters are not related and do not affect the status of each other. Insignificant correlation between CD4 count and HIV viral load was established by this study. However, there is a need to maintain patients on treatment for life and this call for a long term prospective of ART that leads to suppression of viral load to undetectable level, having a CD4 cell count in the normal range. CD4 count and HIV viral load cannot be imply as the substitute for one another for diagnosis, HIV viral load can be reduced by using ART treatment.

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Conflict of Interest
The authors have declared that there is no conflict of interest

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