Quantitation of HIV1 RNA viral load and its correlation with CD4 cell count in Hepatitis C virus and human immunodeficiency virus co-infected patients attending RIMS hospital Imphal

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Abstract

Background: HCV-HIV co-infection leads to more rapid progression to cirrhosis, end stage liver disease and hepatocellular carcinoma but some literature opined that overall survival of HIV positive persons is not affected by the presence of HCV. This study was conducted with the objective to quantify HIV1 RNA viral load in HIV-AIDS coinfected individuals & its correlation with CD4 cell count.

Methodology: Hospital based Cross sectional study was conducted among HIV-HCV co-infected individuals from Nov 2015 to Oct 2017.HIV1 RNA viral load was measured by the Cobas TaqMan HIV1 Test for manual specimen preparation and Cobas TaqMan 48 Analyzer (Roche Diagnostics, USA) for automated amplification and detection. CD4 cell count was measured by Fluorescent Activated Cell Sorter count system (Becton Dickinson's immunocytometry system, San Jose, CA,USA). The correlation between HIV viral load and CD4 cell count was determined by Pearson correlation test (r_p) and probability value of < 0.05 was considered statistically significant.

Results: Among 47 HIV-HCV co-infected individuals analysed, maximum number i.e., 45(95.74%) have HIV viral load <10,000 copies/ml while only 2(4.26%) have ≥10,000 copies/ml. There was a positive good correlation (r_p=0.611) between HIV viral load and CD4 cell count, but it was not statistically significant. **Conclusions:** Majority of these individuals having low HIV viral load have low CD4 cell count, some as low as 25 cells/ul. All the subjects were on ART but none on antiviral therapy for HCV. So, in these circumstances, even though they are taking ART, they are still prone to opportunistic infections **Keywords:** HIV-HCV Co-Infection, HIV1 RNA viral load, CD4 cell count, correlation

Introduction:

Human immunodeficiency virus (HIV) is the aetiological agent of Acquired Immunodeficiency Syndrome (AIDS).[1]Within three to six weeks of exposure to HIV, infected individuals generally develop a brief acute syndrome characterised by flu-like symptoms and associated with high levels of viremia in the peripheral blood.[2]Quantitative measurement of HIV viremia in the peripheral blood have shown that higher virus levels may be correlated with increased risk of clinical progression of HIV disease and that reduction in plasma virus level may be associated with decreased risk of clinical progression.[3]So, the measurement of human immunodeficiency virus type 1 (HIV1) RNA levels in plasma (viral load) is presently one of the most reliable clinical tools for predicting HIV disease progression [4]for determining the need to initiate or change antiretroviral therapy [5] and for evaluating the efficacy of newly developed antiretroviral drugs.[6]The CD4 cell count is also the most important laboratory indicator of immune function in HIV infected individuals. It is also the strongest predictor of subsequent disease progression and survival according to finding from clinical trial and cohort studies.[7] The more severe and life-threatening complications of HIV infection occur in patient with CD4 cell counts less than 200 cells/ μl.[8] Thus, both the CD4 cell count and PVL play a very important role in assessing the immune status of an HIV/AIDS patient. Because of shared routes of transmission, the prevalence of HCV is especially high among persons infected with HIV. In the United States and Europe, about 16% of HIV infected persons also have HCV infection.[9] In HCV and HIV co-infection, there is more rapid progression to cirrhosis, end stage liver disease and hepatocellular carcinoma.[10]Some workers opine that overall survival of HIV positive persons is not affected by the presence of HCV.[11]So, in order to preliminary assess HCV-HIV co-infection leads to more complications, this study was conducted to quantify HIV1 RNA viral load in Hepatitis C Virus co-infected HIV/AIDS individuals and its corelation with CD4 cell count.

Materials and Methods:

A hospital based cross sectional study was conducted at the Department of Microbiology, RIMS, Imphal over a period of two years from November2015 to October 2017. The study population consisted of all HCV and HIV co-infected individuals except age less than 15 years attending the institutional FACS count centre.Using mean CD4 count of 116.3 cells/ml

and Standard deviation of 87.7 cells/ml[12], sample size was calculated using the formula n $=\frac{s^2}{e^2}$, where n = sample size, s = standard deviation,e = standard error. Taking the margin of error, L =25 the adequate sample size for the proposed study is 47 HCV and HIV co-infected individuals.

Study tools:

1) HIV1 RNA viral load was measured by the Cobas TaqMan HIV1 Test using the high pure system viral nucleic acid kit for manual specimen preparation and Cobas TaqMan 48 Analyzer (Roche Diagnostics, USA) for automated amplification and detection. HIV1 RNA can quantitated over the range of 34-10,000,000 copies/ml.Clinical specificity is 100%.[13]

2) CD4 cell count was measured by Fluorescent Activated Cell Sorter (FACS) count system (Becton Dickinson's immunocytometry system, San Jose, CA,USA).[14]

All HIV and HCV co-infected individuals attending the FACS Count centre, Department of Microbiology, RIMS were requested to participate in the study. Those willing patients were made to fill up the questionnaire including data on demographic and risk factors,apart from the particulars of the patient and their presenting complaints. All the data collection forms were given a unique study number or code to remove any patient identifiers.

Procedure of sample collection and processing:

The skin over the cubital fossa was cleaned with spirit (70% alcohol) and air dried. Ten ml of venous blood sample was collected in vacutainer tube containing EDTA (Ethylenediaminetetraacetic acid) as anticoagulant. 50µl whole blood for CD4 cell count estimation was immediately separated by reverse pipetting. The remaining amount of blood sample was centrifuged at 800-1600x g for 20 minutes at room temperature to separate the plasma. Separated plasma was transferred into a sterile, 2.0 ml polypropylene screw cap tube. 500µl of plasma was used for viral load quantification. The collection of blood sample and estimation of HIV1 viral load and CD4 cell count were done on the same day, as far as possible. For few samples where HIV1 viral load quantification could not be performed on the day of sample collection, the plasma obtained was stored at 4°C and processed within 6 days (These specimens were placed at room temperature for 15-30 minutes before use). All the samples were processed according to the manufacturer's instructions.

Statistical Analysis:

Statistical Package for the Social Sciences (SPSS) version 21was used for data entry and statistical analysis. The correlation between HIV1 RNA viral load and CD4 cell count was determined by Pearson correlation (r_p) and probability value of < 0.05 was considered statistically significant.

Ethical considerations: Ethical approval was obtained from the Research Ethics Board (REB), RIMS, Imphal. Informed written consent was taken from all the willing participants

before the study was undertaken. Privacy and confidentiality were maintained in all cases by coding the patients.

Results:

A total of 47 HIV-HCV co-infected individuals fulfilling the inclusion criteria were analysed in this study. The age range in the study group was 33 to 57 years (mean age 46 ± 8.6 years). Majority of the cases (76.60%) belonged to the age group of 15 to 49 years (Fig. 1). As shown in Fig.2 higher number (53.25%) of the study population falls in the lower–middle (III) strata of the Kuppuswamy's Socioeconomic Status Scale. Of the total 47 HIV-HCV co-infected subjects, 42 (89%) were males and 5 (11%) were females. Based on history of the participants, most common route of acquiring HIV infection was IDU (87%) out of which 32% gave history of unprotected sexual exposure. Other transmission routes include history of blood transmission, unprotected sex with no history of IDU and use of unsterlized needle. Intravenous drug use is exclusively seen among the males while only unprotected sexual exposure is seen among the females only (Table 1). Most of the patients had their HIV status known for long i.e, more than 60% of them had HIV for more than 10 years. A majority of 45 (95.74%) co-infected individuals of the study population detected HCV infection within $\leq$ 1 year of HIV diagnosis while only 2 cases (4.26%) came to know their HCV status after 1 year, the longest duration being 5 years (Table 2). A total of 27 (58%) co-infected subjects of the study population was asymtomatic. Among symptomatic cases, generalized weakness 3 (6%), diarrhoea 7 (15%), oral thrush 5 (11%), tuberculosis 3 (6%) and liver cirrhosis 2 (4%) were present (Figure 3). In this study, baseline HIV1 RNA PVL and CD4 cell count ranged from Target Not Detected (TND) i.e., less than (<) 34 copies/ml to 32,000 copies/ml and 25 cells/µl to 1063 cells/µl respectively. Overall mean and standard deviation (SD) of HIV1 RNA PVL and CD4 cell count in the study population were 1175.63±5230 copies/ml &388.28±203 cells/µl respectively. The mean HIV1 RNA PVL was 1280.26±5527.19 copies/ml in males but only 40.00±23.64 copies/ml in females. The mean CD4 cell count was 368.09± 202.60 cells/µl in males and 557.80± 123.67 cells/µl in females (Table 3). Out of the 29 study cases with <10000 copies/ml of HIV 1 RNA plasma viral load, 19 subjects have CD4 cell count ≤500 cells/µl (65.52%) and 10 subjects (34.48%) above 500 cells/µl respectively. While only 2 cases with CD4 cell count ≥500 cells/µl had viral load between 10000 to 50000 copies/ml. There were 16 Target Not Detected cases whose CD4 cell count ≤500 cells/µl in 75% of the cases and 25% above 500 cells/µl (Table 4). A good positive correlation between HIV 1 RNA plasma viral load and CD4 cell count of 47 HIV-HCV co-infected individuals was found (Pearson Correlation (r) = +0.611) but it was not statistically significant (p value = 0.533).

Discussion:

In this study, maximum number (77%) of the study populations were from age group15-49 years while only 23% belonged to age group $\geq$ 50 years and a majority of 42 (89%) individuals were males and 5(11%) were females.The UNAIDS/WHO,[15] NACO [16] data also showed that 93% and 86.3% of People Living with HIV/AIDS (PLHA) belonged to adults age group of 15-49 years, respectively.

More than half of the study subjects (58%) were asymptomatic for HIV orHCV related problems. The symptomatic cases presented with generalized weakness(6%), diarrhoea (159%), oral thrush (11%), tuberculosis (6%) and liver cirrhosis(4%). In this study,those 2 cases (4%) giving history of liver cirrhosis had detectedboth HIV and HCV more than 20 years before the study was done. Likewise, SPDPonamgi*et al* [17] also found in their study that an estimated 20% of people withchronic HCV infection will progress to cirrhosis over a 20-50-year interval.

There was good positive correlation between HIV1 RNA viral load and CD4 cell count of 47HCV and HCV co-infected individuals (Pearson Correlation=+0.611), but it was not statistically significant (pvalue = 0.533). In this study, most of the cases had low HIV RNA plasma viralload along with low CD4 cell count of which one case had 25 cells/µl and 6 caseswith CD4 cell count below 200 cells/µl even though all their HIVI RNA viral loadwere low. The reason for this positive correlation might be due to co-infection withHCV which possibly could have altered the immune mechanism. So, in thesecircumstances, even though the HIV-HCV co-infected individuals were on ART, itdidn't guarantee prevention of opportunistic infections occurring mostly below CD4cell count 200 cells/µl. So, there is a need for alertness for prevention for OIs as wellas early treatment for OIs to avoid further worsening of health.

This is contrasting to the findings in the study by García F *et al* [18] where they found a negative correlation between HIV plasmaviral load and CD4 cell count in HIV mono-infected individuals.However, this positive correlation between HIVI RNA viral load and CD4cell count is also supported by study by Greub G *et al*[19].In a study by Mohammad M. Sajadi*et al*[20], they observed that chronic HCVviremia was found to be associated with both decreased CD4 cell count and elevatedlevel of immune activation, suggesting that the mechanism for reduced CD4 cellcount was related to immune activation.

Limitation:

Since it was a cross-sectionalstudy there may be recall bias regarding disease duration. HCV RNA viralload was not done and so correlation between HCV viral load with CD4 count and/or HIVI RNA viral load could not be done.

Conclusion:

Majority of these individuals having low HIV1 RNA viral load have low CD4 cell count, some as low as 25 cells/ul. All the study subjects were on ART but none on antiviral therapy for HCV. So, in these circumstances, even though they are taking ART, they are still prone to opportunistic infections.

Where there is a scarcity of information on HIV-HCV co-infection,particularly in a state like Manipur where HIV prevalence is highest in the countryas well as intravenous drug use is rampant, this study will provide a useful insight to researchers working onHIV-HCV co-infection.Finally, further studies of HIV-HCV co-infection are needed to explorefurther detail the current prevention strategies and the therapeutic management ofthis condition

References:

1. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, *et al*.Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired

   immune deficiency syndrome (AIDS). Science 1983; 220:868-71.

2. Gaines H, Sydow MAV, Stedingk LVV. Immunological changes in primary

   HIV-1 infection. AIDS1990;4:995-9.

3. O'Brien WA, Hartigan PM, Martin D, EsinhartJ, Hill A, Benoit S, *et al*. Changes in plasma HIV-1 RNA and CD4 lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDs. N Engl J of Med 1 996;334:426-31.

4. Coombs RW, Welles SL, Hooper C, Reichelderfer PS, D'Aquila RT, Japoyr AJ, *et al*. Association of plasma Human Immunodeficiency Virus type 1 RNA level with risk of clinical progression in patients with advanced infection. J Infect Dis 1996;174:704-12.

5. Holodniy M, Katzenstein DA, Israelski DM, Merigan TC. Reduction in plasma Human Immunodeficiency Virus ribonucleic acid after dideoxynucleoside therapy as determined by the polymerase chain reaction. J Clin Invest 1991;88:1755-9.

6. Schooley RT. Correlation between viral load and outcome in clinical trials of antiviral drugs. AIDS 1995;9(2):15-9.

7. Ribaudo H, Lennox J, Currier J. Virologic failure endpoint definition in clinical trials: Is using HIV-1 RNA threshold <200 copies/nil better than <50 copies/ml? An analysis of ACTG studies. Paper presented at 16th Conference on Retroviruses and Opportunistic Infection; Montreal, Canada: 2009.

8. Havlir DV, Bassett R, Levitan D. Prevalence and predictive value of intermittent viremia with combination HIV therapy. JAMA2001;286(2):171- 9.

9. Sherman KE, Rouster SD, Chung R, Rajicic N. Hepatitis C prevalence among patients infected with human immunodeficiency virus: a cross-sectional analysis ofthe US adult AIDS Clinical Trials Group. Clin Infect Dis 2002;34:831-7.

10. Romeo R, Rumi M, Donato M, Cargne P, Vigano M, Mondelli B. Hepatitis C is more severe in drug users with Human Immunodeficiency V. infection. J Viral Hepat 2000;7:297-301.

11. Duming JIM, Nelson M. WV and hepatitis with co-infection. Int 1 Clin Pract 2005;59:1082-92.

12. Antonello VS, Silva MC, Kliemann DA, Santos BR, Tovo CV. Immune restoration in human immunodeficiency virus and hepatitis C virus co-infected patients after highly active antiretroviral therapy. Braz J Infect Dis 2013;17(5):551-4.

13. Roche Molecular Diagnostics. COBAS®TaqMan® HTV-1 test, v2.0 for use with the high pure system for in vitro diagnostic use. 2013. Available at: https:// COBAS® TaqMan® HCV Test v2 (roche.com). Accessed July 20, 2016.

14. Becton Dickinson and Company. BD PACS Count system user's guide for use with BD FACS Count CD4 reagents. 2008. Available at: http://www. bdbiosciences.comidsiab/others/339011.pdf. Accessed October 13, 205.

15. Joint United Nations Programme on AIDS. Global summary of AIDS epidemic. 2014. Available at http://wvv-wainaids.org/ sites/default/files/media asset/20150714epicore_en.pdf. Accessed April 23, 2017.

16. National AIDS Control Organisation. State HIV epidemic fact sheets. July 2014. Available at: http://naco.gov.in/sites/default/files/STATE_HIV_EPIDEMIC_FACT_SHEET.pdf. Accessed December 21, 2016.

17. Ponamgi S, Rahamathull S, Kumar YN, Chandra M, Lakshmi N, Habibullah CM, *et al*. Prevalence of hepatitis C virus (HCV) co-infection in HIV infected individuals in south India and characterization of HCV genotypes. Indian J Med Microbiol 2009;27:12-6.

18. Garcia F, Vidal C, Gatell .11v1, Miro JM, Soriano A, Pumarola T. Viral load in asymptomatic patients with CD4+ lymphocyte counts above 500x 106/1. AIDS 1997;11(1):53-7.

19. Greub G, Ledergerber B, Battegay M. Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus co-infection: the Swiss HIV Cohort Study. Lancet 2000;356:1800-5.

20. Sajadi MM, Pulijala R, Redfield RR, Talwani R. Chronic immune activation and decreased CD4 counts associated with Hepatitis C Infection in HIV-1 Natural Viral Suppressors. AIDS 2012; 26(15):1879-84.
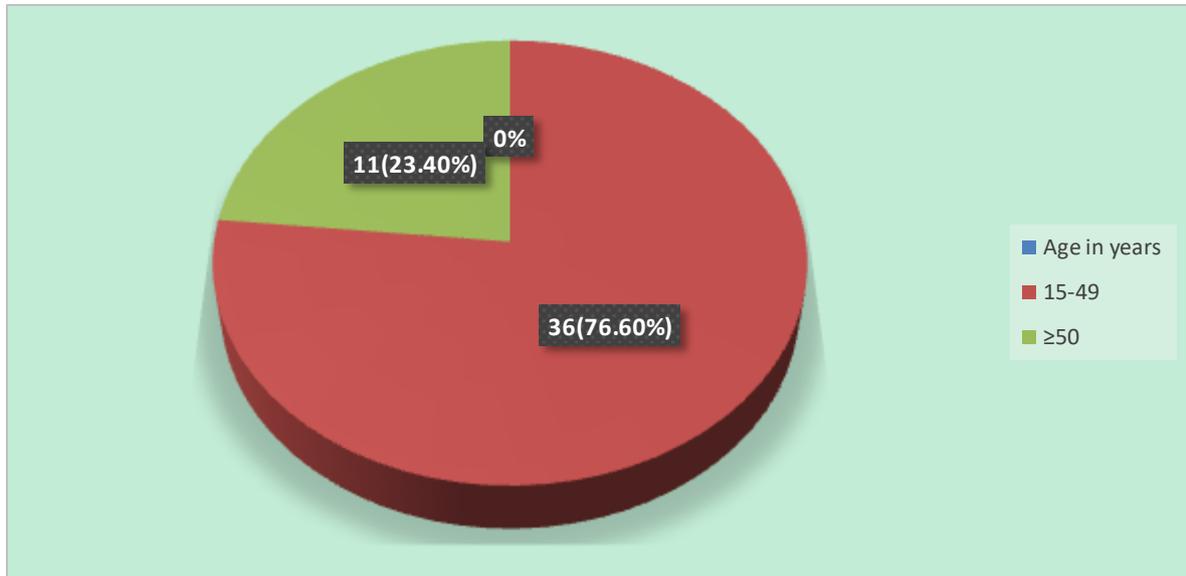
Figure 1: Age distribution of the study population
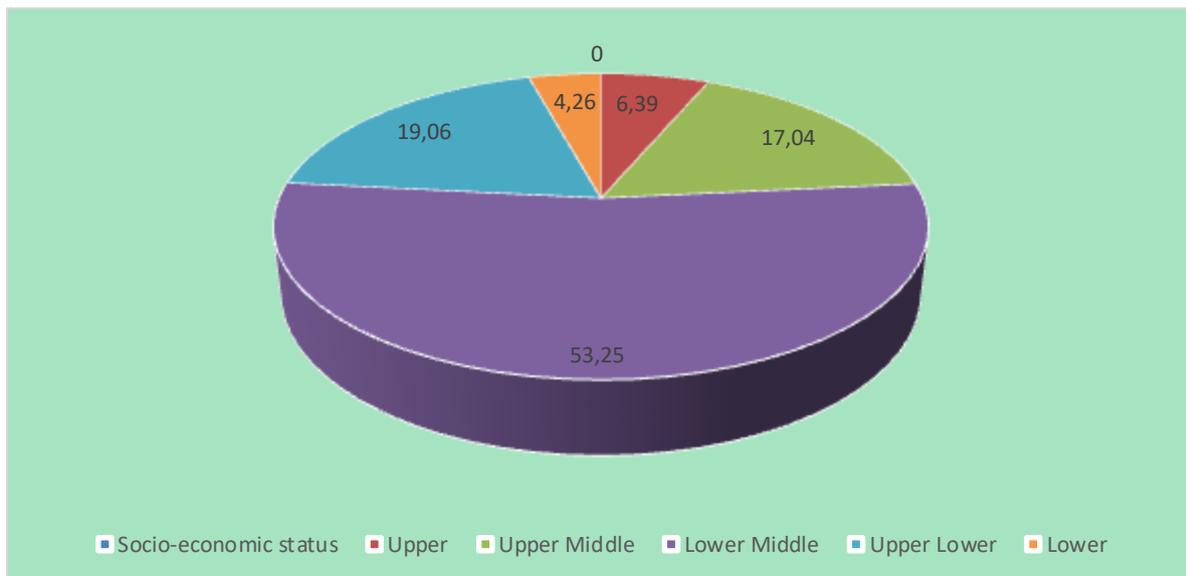
Figure 2: Socio –economic status of the study subjects

Table 1: Gender distribution of different risk factors

Risk Factors	Male	Female	TOTAL (%)
Blood transfusion	1	1	2 (4.3%)
Intravenous drug use	26	0	26 (55.3%)
Only unprotected sexual exposure	0	3	3 (6.4%)

Use of unsterilized needle	0	1	1 (2.1%)
Unprotected sex + Intravenous Drug use	15	0	15 (31.9%)
TOTAL	42	5	47

Table 2: Duration of diagnosis of study subjects.

Duration of diagnosis	No. of patients	Percentage (%)
A. Time of HIV diagnosis		
<10 years	18	38.30
10-20 years	27	57.45
>20 years	2	4.25
B. Duration of HCV diagnosis since HIV detection		
≤1 year	45	95.74
>1 year	2	4.26
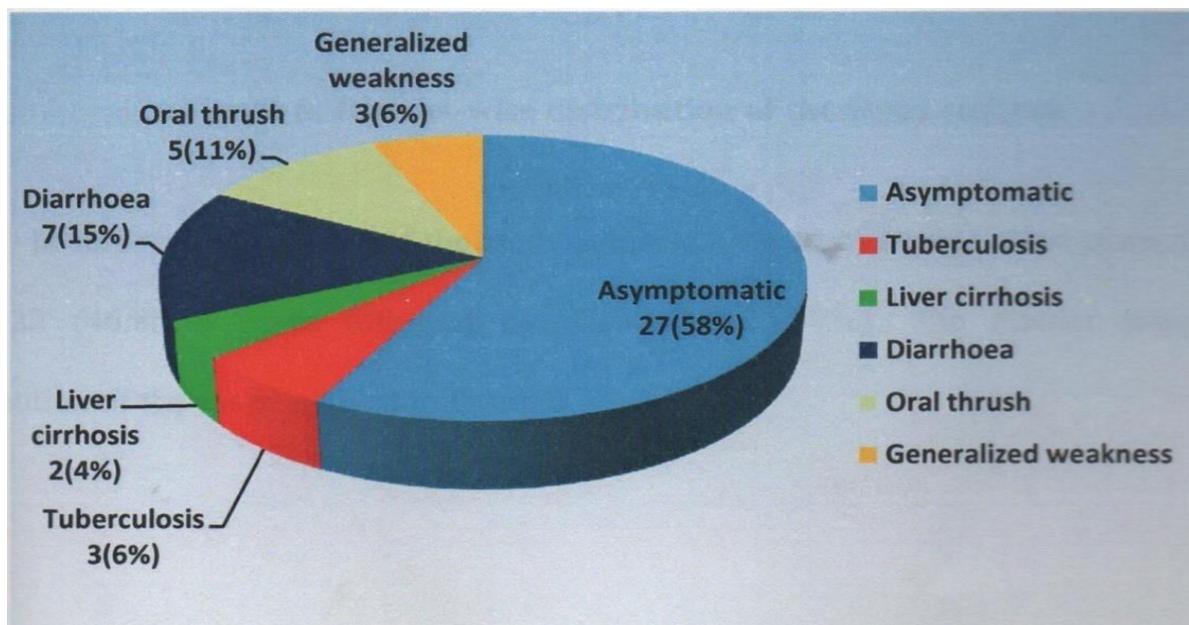
Figure 3: Clinical finding of the study subjects.

Table 3: Mean HIV1 RNA PVL and mean CD4 cell count of study Population

Variables	Total (n = 47)	Male (n = 42)	Female (n =5)

Mean PVL (copies/ml) ±SD	1175.63±5230	1280.26±5527.19	40.00±23.64
Mean CD4 cell count (cells/µl)±SD	388.28±203	368.09±202.60	557.80±123.67

HIV1 = Human Immunodeficiency Virus 1,RNA = Ribonucleic Acid,

PVL = Plasma viral load, CD4 = Cluster of differentiation 4, SD =Standard deviation

Table 4: Stratification of study population by HIV1 RNA PVL and CD4 cell count

PVL (copies/ml)	No.of cases (%)	CD4 cell count (cells/µl)	
		≤500 (%)	>500 (%)
TND	16(34.08)	12 (75)	4 (25)
<10000	29(61.76)	19 (65.52)	10 (34.48)
10000-49999	2 (4.26)	2 (100)	-
>50000	0	-	-
	47 (100)	33 (70.21)	14 (29.79)

HIV1 = Human Immunodeficiency Virus 1, Ribonucleic Acid, PVL = Plasma viral load, CD4 = Cluster of differentiation 4, TND= Target not detected

Table 5: Correlation between HIV1 RNA PVL and CD4 cell count

CORRELATIONS		
	Viral Load	CD4
Viral Load Pearson Correlation Sig. (2-tailed) N (Total cases)	1 - 47	0.611 0.533 47

CD4 Pearson Correlation	0.611	1
Sig. (2-tailed)	0.533	–
N (Total cases)	47	47