Original Article

HIV AIDS

Inter-Relationship of Viral Load and CD4+ Cells in Patients Suffering from Acquired Immuno Deficiency Syndrome (AIDS): Update from Punjab, Pakistan

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ABSTRACT

Objective: Purpose of current study was to evaluate the effect of antiretroviral drugs (Three regimen) Tenofovir, Lamivudine and Efavirenz to HIV patients presented in D.G. Khan Zone in regard to CD4 level and viral loads before start of drugs and after one year treatment.

Study Design: Comparative study

Place and Duration of Study: This study was carried out at the Institute of molecular biology and biotechnology (IMBB), and Centre for research in molecular medicine (CRiMM), The University of Lahore-Pakistan during May 2013 to Feb. 2014.

Materials and methods: Seventy five (75) patients suffering from HIV and twelve (12) control individuals were selected for the study from Dera Ghazi Khan during 2013-2014. Rapid testing and ELISA screening were performed for identification of presence/absence of virus and antibodies respectively. Viral load and CD4+ absolute count were also evaluated by PCR and Multiset software respectively. All the analytical work was performed at the Institute of molecular biology and biotechnology (IMBB), and Centre for research in molecular medicine (CRiMM), The University of Lahore-Pakistan.

Results: Statistically highly significant difference (P= 0.00) was observed regarding viral load before and after the treatment in HIV patients receiving combination therapy, ART (antiretroviral therapy). The viral load in control and HIV patients before and after the treatment was (0.00, $3.22x10^4$) and (0.00, $1.61x10^2$) respectively. The CD4+ cells levels in control and HIV patients before and after the treatment was (330.67, 186.29) and (171.92, 372.64) respectively. Inverse correlation was also recorded between viral loads (After) and CD4+ levels (After), (Viral Load Vs CD4+, r=-.328**).

Conclusion: It is concluded that from one year antiviral therapy in AIDS patients the viral load decrease from $3.22x10^4$ to $1.61x10^2$ and CD4+ count increased from 186.29 to 372.64 with no significant complications hence improve the AIDS patients' lives and minimize the spread of infection.

Key Words: Antiretroviral drugs, ART, Tenofovir, Lamivudine, Efavirenz, ELISA, CD4+, PCR, viral load, HIV AIDS.

INTRODUCTION

HIV is one of the major health problems which infect about 40 million people worldwide including 2.3 million children. Highly active Antiretroviral Therapy has reduced morbidity and mortality in HIV infected adult and children. WHO (2009)[1] stated that antiretroviral drugs are those drugs that are used for treatment and prevention of HIV infection. They work by stopping viral replication. There are different classes of antiretroviral drugs; Nucleoside reverse transcriptase inhibitors, Non nucleoside reverse transcriptase inhibitors, protein inhibitors and some other drugs are also available that inhibit viral process to make copies of itself. Paul and Deeks (2010)[2] stated that there are seventeen drugs that are used for treatment against HIV. They stated that anti retroviral therapy has changed HIV from fatal disease into chronic illness. They concluded that although complete cure against HIV is not possible but continued and combination ART therapy has increased life ling suppression of HIV replication. Despite of all this some limitations are still there and treatment success needs long time drug adherence.

MATERIALS AND METHODS

Patients form both genders, ages groups include children up to 60 years of age. The study was conducted from May 2013 to Feb. 2014. Seventy five (75) patients suffering from HIV and thirteen (13) control individuals were selected for the study from Dera Ghazi Khan. All the analytical work was

performed at the Institute of molecular biology and biotechnology (IMBB), and Centre for research in molecular medicine (CRiMM), The University of Lahore-Pakistan.

Following criteria was adopted to start anti-retro viral therapy.

1. Patient was a confirmed case of HIV/AIDS by rapid testing, by ELISA screening of detection of anti bodies and viral load by PCR.
2. CD4 absolute count. CD4 absolute count should be less than 350 cells/micro ml (normal range 450-1100)
3. Clinical Staging of the patients was determined.
4. Incidence of repeated opportunistic infection along with repeated Diarrhea.

Exclusion criteria:

1. Age above 70 years
2. CD4 absolute count more than 350 cells/micro ml.
3. Patient not willing to start antiretroviral therapy.

Methodology:

Rapid testing: 3CC of venous blood was taken and centrifuged, serum was separated and put on rapid kit along with buffer if 2 lines are appearing patient was positive. In case of negative only control line appeared on device. The first line was for control and second line was for patient (test).

ELISA Screening: Antibodies were detected by sandwich method of labeled prefilled antigen-antibody mixture and were compared with cut off ratio and determined the reactivity.

Viral Load by PCR: PCR facility was provided by Centre for Research in Molecular Medicine (CRiMM) and Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore, Lahore.

CD4 absolute Count: The absolute CD4 & CD8 count (absolute number of positive cellular events in sample compared to beads events) was determined by Multiset software and expressed in cells/ µl.

RESULTS

Data presented in Table-1 shows statistically highly significant difference (P=.000) was observed regarding viral load before and after the treatment in HIV patients receiving combination therapy, ART (Tenofovir-Lamivudine-Efavireinz). The viral load in control and HIV patients before and after the treatment was (0.00, $3.22x10^4$) and (0.00, 1.61 $x10^2$) respectively. The data regarding CD4+ cells depicted in table 01 also shows that with combination therapy, ART (Tenofovir-Lamivudine-Efavireinz), the levels of CD4+ cells was increased and a highly significant difference was recorded among control and ART treated patients. The CD4+ cells levels in control and HIV patients before and after the treatment was (330.67, 171.92) and (186.29, 372.64) respectively. Data in Table-2 (Pearson Correlation, Two Tailed**)** shows that a highly significant inverse relationship between viral load (Before) and CD4+ cell levels (Before) (Viral Load Vs CD4+, r = -.113**). Likewise inverse correlation was also recorded between viral loads (After) and CD4+ levels (After), (Viral Load Vs CD4+, r = -.328**).

Table No.1: Comparison of viral load and CD4+ among control and patients

	Group	n	Mean±SD	P value
Viral load (before)	Control	12	0.00±0.00	.000
	Patient	75	$3.22x10^4$±0.70	
Viral load (after)	Control	12	0.00±0.00	.000
	Patient	75	$1.61x10^2$±0.43	
CD4+ (before)	Control	12	330.67±77.07	.000
	Patient	75	186.29±29.02	
CD4+ (after)	Control	12	171.92±49.04	.000
	Patient	75	372.64±53.41	

Viral load: copies/mm CD4+: cells/mm^3

Table No.2: Pearson Correlation

	Viral load (before)	Viral load (after)	CD4+ (before)	CD4+ (after)
Viral load (before)	1	.161	.113	.036
	.	.137	.299	.739
Viral load (after)		1	.502**	-.328**
		.	.000	.002
CD4+ (before)			1	-.159
			.	.141
CD4+ (after)				1

**. Correlation is significant at 0.01 level (2-tailed)

DISCUSSION

US department of Health and Human source (2012)[3], reported that there are six classes of antiretroviral drugs; NNRTIs, NRTIs, Protease Inhibitors, Fusion Inhibitors, CCRS Antagonistism and integrase inhibitors. Treatment of HIV with three or more drugs from two different classes at right time will lead to undetectable viral load within three to six months. They also stated that physician should consider following points before stating ART; viral load, HIV related illness, CD4 cell count, opportunistic infections and pregnancy. Kelley et al. (2009)[4] concluded that these days antiretroviral therapy is potent, convenient and very well tolerated. They stated that if treatment with ART is started before advance stage of disease, they reduce plasma HIV RNA concentration to undetectable level. Although immunological response varies in different patient, most that begin therapy before advanced immunodeficiency e.g. CD4 count below

200cell/ul, show robust and sustained CD4 T-cell count again.

Zolopa et al., (2009)[5] stated that HIV treatment should be started with CD4 cell count below 350cells/ul but patient suffering from other opportunistic infections or cancer treatment can be stated with CD4 count above 350cells/ul. Yeni et al., (2004)[6] stated that treatment of HIV with HAART should be started in all symptomatic patients and in asymptomatic patient with CD4 count less than 200cell/ul. In asymptomatic patients with CD4 count more than 200cells/ul decision to start ART be made with rate of decrease of CD4 cells. U.S department of Health and Human resource, (2012)[3], concluded in his study that progression rate are consistent in those patients starting High antiretroviral therapy at CD4 cell count below 200cell/ul and are inconsistent in patient with CD4 cell count above 350cell/ul. Johnson et al., (1999)[7], reported that lamivudine, common name 3TC, is very good rug against HIV. It is very rapidly absorbed up to >80% and distributed in different extra vascular compartments. The drug is 5-60% excreted in urine and its half life is about 3 hours. Lamivudine has very little interaction with other drugs.

HIV/AIDS Statistics and Epidemiology Section (2009)[8] stated that potency of ART suppressed viral load in 90% patients. Cao et al., (2013)[9] stated that treatment of HIV-1with tenofovir is related with renal impairment. They concluded that tenofovir is not metabolized by enzyme CYP450, so is not eliminated from kidneys. This leads to decrease in GFR and renal toxicity. Scherzer et al., (2012)[10] concluded that tenofovir treatment lead to increased risk of three kidney diseases; proteinuria, rapid decline in kidney functions and development of CKD and this risk is irreversible.

CONCLUSION

It is concluded that from one year antiviral therapy in AIDS patients the viral load decrease from $3.22x10^4$ to $1.61x10^2$ and CD4+ count increased from 186.29 to 372.64 with no significant complications hence improve the AIDS patients' lives and minimize the spread of infection.

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