Assessment of HIV Disease Progression before and after Initiation of Anti-retroviral Therapy (ART) by CD4 & CD8 T-lymphocyte Count and Viral Load Assay.

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Abstract:

Backgrounds: As there is no published data regarding the response to Anti-retroviral therapy (ART) among HIV patients from Bangladesh, the present study was designed to determine the immunological and virological responses of HIV infected Bangladeshi adults starting ART. **Objectives:** To monitor the changes of CD4 and CD8 T-lymphocyte count and Viral load (VL) before and after three and six months of starting ART. **Methods:** 20 symptomatic HIV infected patients with CD4 T-lymphocyte count of <350 cells/µl of blood were initiated ART.CD4 and CD8 T-lymphocyte counts were estimated by Flowcytometer and VL was determined by real-time PCR technique. **Results:** The mean CD4 T-lymphocyte count among the study patients were 177±127 cells/µl before initiation of ART. After ART initiation, their mean CD4 count increased significantly to 368±181 and 452±183 cells/µl after three and six months respectively (P<0.0001).The mean CD8 T-lymphocyte counts were 901±650 cells/µl before initiation of ART, which increased to 1085±393 and 1121±372 cells/µl after three and six months respectively after ART initiation (P>0.05). Before ART initiation, the mean VL was 5.25±1.19 log10 (copies/ml) among the study population which became undetectable in 15 (75%) patients after three months of ART and in another 2 (10%) patients after 6 months of ART initiation. **Conclusion:** Our study concluded that, ART is effective in slowing the progression of HIV infection to AIDS with good immunological and virological outcome among the ART initiators.

Key words: HIV, CD4 and CD8 T-lymphocyte count, Viral load, Anti-retroviral therapy.

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Introduction:

Human Immunodeficiency Virus (HIV) causes AIDS (Acquired Immune Deficiency Syndrome) which is a chronic, potentially life threatening condition. It damages the human immune system by damaging the body's ability to fight against opportunistic infections and tumors.[1] HIV infects CD4 T-lymphocytes which are the vital cells of the human immune system along with macrophages and dendritic cells.[2] These lead to lowering of the number of CD4 T-lymphocytes through mechanisms causing direct viral killing of infected cells, increased rates of apoptosis in infected cells and by killing of infected CD4 T-lymphocyte cells by CD8 cytotoxic T-lymphocytes through reorganization of infected cells. Thus, both CD4 and CD8 T-lymphocyte count serves as the major laboratory indicator of immune functions of people living with HIV (PLHIV), whereas, CD4 T-lymphocyte estimation is the key factor for deciding when to initiate ART.[3] The rate of virus replication and assessing the risk of disease progression by determining HIV RNA viral load in response to ART initiation is an important measure of efficacy testing. VL monitoring showed a significant association between a decrease in plasma viraemia and improved clinical outcome.[4] Therefore, VL determination serves as a surrogate marker for treatment response and is useful for predicting clinical progression.[5, 6]

The Government of Bangladesh (GOB) is taking all possible measures to halt the epidemic along with prevention among the targeted groups and is offering care, support and treatment to the identified HIV/AIDS patients. To ensure standard and rational treatment, the National AIDS/STD program (NASP) developed the first National ART guideline in 2006 following the WHO ART guideline of the same year. According to the current National ART guidelines, HIV positive individuals with CD4 count

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≤350 cells/mm^3 or HIV positive individual with WHO clinical staging 3 or 4, irrespective of CD4 cell count is used for the categorization and initiation for ART.[7] ART initiation and follow up solely depends on CD4 T-lymphocyte count by flowcytometry and viral suppression following ART initiation is detected by VL estimation by real-time PCR technique. Till date, there is no published data regarding the treatment response among the PLHIV of Bangladesh. This is the first report on HIV disease progression among HIV/AIDS patients from Bangladesh by estimating CD4 and CD8 T-lymphocyte count and VL before and after anti-retroviral therapy (ART) initiation.

Methods:

A total of 33 serologically confirmed (by both rapid test and ELISA) HIV infected patients were purposively recruited for the study from the Ashar Alo Society (AAS), Dhaka, which is a non-government organization (NGO) involved in management of PLHIV of Bangladesh. The selection was based on their preliminary review of medical record files, their physician's clinical evaluation and most recent available (within six months) CD4 T-lymphocyte count <350 cells/µl of blood. Informed written consent was taken from all the patients and ethical clearance (BSMMU/2010/12167-A) for the study was taken from the Institutional Review Board (IRB) of BSMMU. The study was conducted from January 2011 to December 2011.

This was a longitudinal cohort study, consisting of PLHIV to estimate their CD4 and CD8 T-lymphocyte count and VL before initiation of ART and to monitor their responses after three months and six months of ART.

Before initiation of ART, all patients underwent a complete clinical evaluation and necessary baseline laboratory investigations to avoid drug related toxicities. After baseline evaluation, study subjects were initiated ART which consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) by medical consultants of AAS according to the NASP ART guideline 2011.[7]

Blood samples were collected from PLHIV attending the Ashar Alo Society (AAS), Mohammadpur, Dhaka. All laboratory work was performed at the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU). CD4 and CD8 T-lymphocyte count was performed by Flow cytometer (BD FACScount, USA) on the same day of sample collection. HIV-1 RNA isolation steps were performed with INSTANT Virus RNA Kit (Germany) and HIV-1 RNA was quantified with a commercially available kit (RoboGene® HIV-1 Quantification Kit, Germany) according to the manufactures' instructions.

The results of the study were recorded systematically. Statistical data analysis was done using SPSS 17.5 software and P value of <0.05 was considered as significant. Sequential measurements of CD4 & CD8 T lymphocyte count and VL of the same patient at different points were evaluated with the Paired T test.

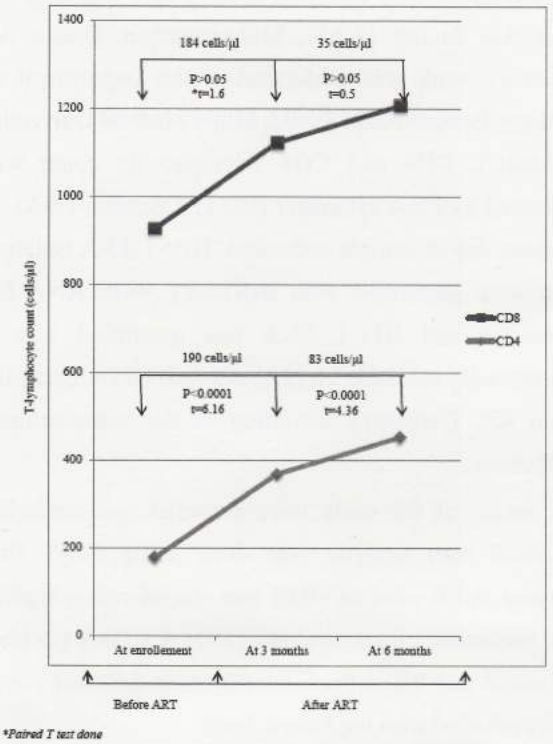
Results:

Out of the 33 HIV infected patients, 18 (54.5%) were males and 15 (45.4%) were females. The age range of the study population was 19 to 60 years (mean ±SD; 33.52±9.13 years). The mean age (mean±SD) of the males and females were 34.48±9.83 and 37.72±8.82 years respectively.

Table-I

CD4 and CD8 T-lymphocyte count of study patients before ART initiation (n=33)

T-Lymphocytes (cells/µl of blood)		Symptomatic HIV infected patients (n=33)
CD 4	<100	15 (45.46%)
	101-200	6 (18.18%)
	201-350	12 (36.36%)
	351-500	0
	>500	0
CD8	<300	6 (18.19%)
	301-600	10 (30.30%)
	601-900	9 (27.27%)
	901-1200	2 (6.06%)
	1201-1500	1 (3.03%)
	1501-1800	2 (6.06%)
	>1800	3 (9.09%)



*Paired T test done

Fig-1: *Changas in mean CD4and CD8 T-lymphocite count before and after ART initiation among HIV infected study patients.*

Follow up of HIV infected patients after ART initiation:
The clinical status, adherence to ART and development of any unwanted drug related toxicities among the study population were evaluated by medical consultants of AAS. During the study period, among the 33 patients, 6 patients dropped out after 3 months of ART initiation and another 7 patients dropped out after 6 months of ART initiation. As such, the final results were compiled on 20 patients who completed the whole study period.

CD4 and CD8 T-lymphocyte count of HIV infected patients and their response to ART:

Before ART initiation, the mean CD4 T-lymphocyte count was 177±127 cells/µl of whole blood (range: 2 to 349). After 3 months of ART initiation, the mean CD4 T-lymphocyte count increased to 368±181 cells/µl (range: 90 to 750), with a mean increase of 190±138 cells/µl than baseline values (P<0.0001). The mean CD4 T-lymphocyte count increased further to 452±183 cells/µl (range: 157 to 875) after 6 months of ART with a mean increase of 83±85 cells/µl than that of the 3rd month (P<0.0001) (Fig.-1). Before ART initiation, the mean CD8 T-lymphocyte count were 901±650 cells/µl (range: 9 to >1800). After 3 months of ART initiation, the mean CD8 T-lymphocyte count increased to 1085±393 cells/µl (range: 350 to >1800), with a mean increase of 184±513 cells/µl than baseline values. After 6 months, the mean CD8 T-lymphocyte count increased further to 1121±372 cells/µl (range: 451 to >1800) with a mean increase of 35±316 cells/µl than that of 3rd month (P>0.05) (Fig.-1).

Table-II

HIV-1 RNA Viral load before and after 3 and 6 months of ART initiation

Study patients	HIV viral load (Log10 copies/ml)		
	<0.48	0.48 to 9.70	Mean VL among the virus positive patients
Before ART (n=20)	0	20 (100%)	5.25±1.19*
3 months after ART (n=20)	15 (75%)	5 (25%)	3.40±1.57*
6 months after ART (n=5)	2 (40%)	3 (60%)	3.54±0.25

VL <0.48 Log_{10} copies/ml (<3 copies/ml) denoted VL below the level of detection

VL =0.48 to 9.70 Log_{10} copies/ml (3 to 5 X 10^9 copies/ml) denoted virus positive

* Paired t test done, P <0.0001

VL status among HIV infected patients and their response to ART:

Before ART initiation, the mean VL was 5.25±1.19 log_{10} (copies/ml) (range: 2.19 to 6.98). After 3 months of ART initiation, 15 (75%) patients attained undetectable VL (P<0.0001). The mean VL of the remaining 5 patients with detectable viraemia was 3.40±1.57 log_{10} (copies/ml) (range: 1.44 to 5.83). After 6 months of ART, only these 5 patients with detectable viraemia were re-tested and among them, 2 (10%) patients were found to attain undetected VL status (Table II).

Discussion:

Estimation of CD4 and CD8 T-lymphocyte count is one of the measures to ascertain the immune competence of HIV infected patients throughout the broad spectrum of HIV

disease. This should be obtained during the initial evaluation of all HIV infected patients for staging the disease, monitoring of progression and initiation of therapeutic regimen depending on the CD4 level. Thus, serial CD4 T-lymphocyte measurements are more informative than individual values because they reflect trends over time. In case of VL, earlier detection of virological failure allows both targeted adherence intervention and better preservation of the efficacy of second line regimens.[8]In Bangladesh, PLHIVs receiving ART have very limited facilities for monitoring CD4 T-lymphocyte count. Moreover, their treatment response by measuring their VL is not available. The present study is the first of this kind to observe the treatment response at different times after starting ART among HIV infected patients by monitoring both CD4 and CD8 T-lymphocyte count and VL detection.

Our study observed a significant increasing trend of CD4 T-lymphocyte among the patients after ART initiation. Before ART initiation, the mean CD4 T-lymphocyte counts was 177±127 cells/µl, which increased to 368±181 cells/µl after 3 months of ART initiation, with a mean increase of 190±138 cells/µl, which was highly significant (P<0.0001). This increased trend continued after 6 months of ART initiation although the rate was less (mean increase of 83±85 cells/µl). Similarly, CD8 T-lymphocyte count also increased after ART initiation, but was not statistically significant. It is suggested that, at initial period, ART is efficacious in reducing VL rapidly and the immune system of the body quickly generates CD4 T-lymphocytes to combat HIV infection.[9]

A study from India observed an increase in median CD4 T cell of 256 cell/µl among symptomatic treatment naïve patients at first visit after ART initiation with NRTIs and NNRTI or Protease inhibitors (PI), as compared to median baseline 179 cells/µl.[10] A similar study from the UK reported a mean increase of CD4 cell count of 207 cell/µl at 48 weeks among treatment naïve symptomatic patient when Lopinavir-Ritonavir based ART was initiated.[11] Another study from Nepal, among 20 HIV patients with mean CD4 counts <50 cells/µl, reported a mean increase of 173.7 cells/µl after 6 months of ART initiation with Zidovudine (AZT)/ Stavudine + Lamivudine (3TC) + Nevirapine (NVP).[12]Similar studies from India have documented a mean CD4 gain of 118.8 cells/µl among patients on ART (AZT+3TC + Ritonavir), and 152 cells/µl after a mean duration of 4.3 months and 4.7 months of ART respectively.[13,14] In a study from the Caribbean, an increase of CD4 T-lymphocyte count of 124.6 cells/µl was reported after 6 months of ART with NRTIs +PI or NNRTI.[15] When compared to other studies, [10,12,15] the net gain of CD4 T-lymphocyte count among the treatment naïve patients after initiation of ART was by and large more in our study. It is important to mention here that, in case of all the earlier mentioned studies, ART was initiated when CD4 count was below 200 cells/µl according to the WHO ART guideline 2006.[16] However, in our study, ART was initiated when CD4 count was below 350 cells/µl by following the current WHO ART guideline of 2010.[17] Therefore, more informative comparisons may be done when published data based on current guideline are compared with data of our study.

The mean time period to reach undetectable level of VL varied among our study patients after receiving ART. A total of 15 (75%) patient attained undetectable level (VL <3 copies/ml) after 3 months of receiving ART. Among the 5 patients with detected VL, 2 patients attained undetectable VL on retesting after 6 months of ART. Previously, a study from the UK reported a median VL reduction of more than 2 $\log_{10}$ in patients receiving three drug combinations (AZT+ 3TC+Indinavir) at week 8 with 90% of patients attaining undetectable levels (<500 copies/ml) at 24 weeks.[18] In a study from India, 13 out of 15 patient who had started triple drug combination (2NRTIs+ NVP/indinavir) attained undetectable VL (<400 copies/ml) after a mean period of 5 months of ART.[19] Similar observations have been made by other investigators. [20-22] The VL of our study population decreased to undetectable level much earlier than shown in other studies, as 75% of the patients in our study attained undetectable VL status after 3 months of ART, while in the other studies, most patients attained undetectable VL level

after 4-6 months of ART. [18, 19, 22]

Thus, among the 20 patients in our study, 15 (75%) patients responded well within 3 months and 2 (10%) other patients within 6 months of ART initiation. None of the patients complained of serious side effects of ART, and it was not necessary to stop treatment in any case. The lost cases could not be traced, therefore no definite outcome of their treatment could be ascertained. For a more clear understanding of the immunological and virological status among Bangladeshi PLHIVs, more structured trials with large sample size and longer duration of follow-up is worth designing and implementing in the near future.

Conclusion:

A satisfactory level of virological and immunological benefit was attained on initiation of ART in our study, with increasing trend of CD4 T-lymphocyte count and control of viral replication. Thus, it may be concluded that ART is effective in slowing the progression of HIV infection to AIDS, and increasing the survival of patients. However, further trials are required to define the optimum time for initiating ART, and the best monitoring strategies during follow up of therapy.

Study limitations:

Our study limitations included a low number of patients during the study period with a poor age sex match due to low numbers of PLHIVs in Bangladesh. Moreover, patient drop-outs during subsequent follow up resulted in failure of full evaluation.

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