IMMUNOLOGICAL RESPONSE TO ANTIRETROVIRAL THERAPY IN HIV-1 INFECTED PATIENTS AT MUHIMBILI NATIONAL HOSPITAL IN DAR ES SALAAM, TANZANIA

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Abstract

Background: CD4+ T-lymphocyte count is an indicator of immune status used as the eligibility criterion for initiation of antiretroviral therapy (ART) and for monitoring of immunological response to ART in HIV-infected patients in resource limited settings.

Objective: To describe the immunological response to ART in HIV-1 infected ART naïve patients in relation to age, sex, baseline CD4+ T cell counts and generic ART regimens at Muhimbili Nation Hospital, in Dar es Salaam, Tanzania.

Methods: Retrospective analysis of data from patients enrolled in the pilot ART program between July 2004 and August 2005. All were ART naïve and were mostly started on a fixed dose generic first line antiretroviral (ARV) drug combination of stavudine, lamivudine and nevirapine (triomune).

Results: As of August 2005, 1435 patients were recruited for ART, of these 1285 (89.6%) were aged 13+ years. A total of 361 patients were included in the analysis. The mean (range) age was 39 years (13 – 69 years). Two hundred and fourty seven (68.4%) were females. The overall mean baseline CD4+ T cell count was 113 cells/μl; 105 (29.1%) patients had baseline CD4+ count < 50 cells/μl. Two hundred and ninety five (81.7%) received triomune. The overall mean CD4+ T cell count increase was 23 cells/μl. CD4+ T cell count increased more rapidly within the first three months of ART (mean of 27 cells/μl per month), relatively slower thereafter and tended to plateau at 10 - 12 months. There were no significant differences in CD4+ T cell counts increase between the sexes, age groups and ART regimens.

Conclusion: Initiation of ART resulted in overall good immunological responses even among patients who had CD4+ count of <50cells/μl.

Recommendation: Scaling up the ART program country wide should be enhanced in order to provide access to care and treatment to all HIV-infected individuals.

Key words: CD4+ T-lymphocyte count, antiretroviral therapy, antiretroviral drugs

Introduction

Tanzania is one of the countries that have been highly hit by Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) pandemic in the world. In 2003, Tanzania Mainland was estimated to have about 1,820,000 people living with HIV including 960,000 males and 840,000 females.[(1)] A total of 176,102 AIDS cases have been reported from 21 regions since 1983. Recent data based on household surveys reported that 7% of Tanzanian adults aged 15-49 years are infected with HIV. Seroprevalence among women (7%) is higher than among men (6%) with a wide variation across regions.[(2)] Based on the magnitude of the pandemic and the need for antiretroviral therapy (ART), the Government of United Republic of Tanzania through the Ministry of Health and Social Welfare in collaboration with her development partners started to scale up ART in the country. The scaling up initiative was first implemented as a pilot program at Muhimbili National Hospital from July to October, 2004. This pilot program was meant to serve as the learning site for the broad program of scaling up ART over the country.

The primary goal for use of ART is to establish maximal and durable suppression of viral load, which in turn reduces the destruction of CD4+ T cells leading to restoration and/or preservation of immunologic function, improvement of quality of life and reduction of HIV-related morbidity and mortality.[(3)] Antiretroviral therapy is indicated for all patients who are in WHO stage IV clinical criteria regardless of CD4+ T cell count; WHO stage III with CD4+ T cell count $\leq$ 350 cells/μl and those with CD4+ T cell count $\leq$ 200 cells/μl regardless of symptoms.[(3)] Although there is theoretical benefit to ART for patients with CD4+ T cell counts >200 cells/μl, a major dilemma confronting patients and clinicians is that the currently available ART regimens that have the greatest potency in terms of viral suppression and CD4+ T cells preservation are, medically complex, associated with number of specific side effects and drug interactions, and pose a substantial challenge for adherence.[(3)] Furthermore, the development of mutations associated with drug resistance can render therapy less effective or ineffective. Thus, decisions regarding treatment of asymptomatic, chronically infected individuals with CD4+ T cells >200 cells/μl must balance a number of competing factors that influence risk and benefit. In addition to it being used as a determinant marker for initiation of ART, CD4+ T cell count is also used as a surrogate marker to monitor immunological response to ART as one of the indicators of progression of the disease. CD4+ T cell count is recommended at baseline and every 6 months for patients on ART.[(3)] It has been reported that HIV RNA and CD38 levels were similarly predictive of AIDS early on compared with a relatively weaker CD4 count signal.[(4)] However, later in the course of infection, CD38 level remained the strongest predictive marker and CD4 cell count registered a marked increase in prognostic power.

Previous studies have documented that ART delays clinical progression by suppressing viral replication measured by a substantial reduction in HIV RNA, allowing the immune system to reconstitute measured by an increase in CD4+ T cells.[(5-6)] However, virologic and immunologic consequences do not occur uniformly among ART users. Baseline CD4+ T cell count, history of ART, the age at the time of initiation and adherence to treatment have been found to affect immunologic response.[(5-6)] It has been reported that older age is associated with thymus degeneration and that CD4+ T cell counts in older patients do not increase as much as those in younger ones.[(7)] The larger the thymus, the more active the process of thymopoiesis, the more significant the rise in CD4+ T cells is likely to be.[(8)] It has also been reported that patients starting ART at low CD+ levels rarely attain complete immune reconstitution. In one study it was reported that low

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CD4 + T cell count at baseline was a clear factor for patients not attaining 500 CD4+ T- cells/μl after four years of treatment.[9]

Currently there are various drug combination regimens recommended for treatment of HIV-infected patients. There seems to be no great difference in efficacy among these regimens. Previous randomized, open-label, comparative trial to assess and compare the efficacy and safety of three triple combination antiretroviral therapies (Zidovudine + lamivudine + nevirapine; stavudine + didanosine + nevirapine; or stavudine + lamivudine + nevirapine) in HIV-1 infected treatment-naïve patients showed that all three-drug combinations were equally effective at suppressing viral load and increasing CD4+ T cell counts.[10] No significant differences were detected between the treatment groups in virological or immunological response or cessation of study drugs due to adverse events. The aim of the present study was to describe the immunological response to ART in HIV-1 infected ART naïve patients in relation to age, sex, baseline CD4+ T cell counts and use of generic ART regimens at Muhimbili Nation Hospital, in Dar es Salaam, Tanzania.

Materials and Methods

The newly started ART program in Tanzania provides comprehensive HIV and AIDS care and treatment to the HIV infected patients. The enrollment processes involve counseling for voluntary HIV testing, ART health education as well as adherence counseling, clinical and laboratory evaluation followed by provision of antiretroviral drugs (ARVs) free of charge and management of opportunistic infections (OIs). The laboratory investigations include baseline and follow up CD4+ T- cell count, hematology, liver and kidney function tests to monitor drug toxicity and others to exclude OIs.

Prior to initiation of ART, all ART naïve patients were clinically evaluated and staged using revised WHO HIV clinical staging criteria. All patients with CD4+ T cell count $\leq$ 200 cells/μl regardless of the stage, stage III disease with CD4+ T cell count $\leq$ 350 cells/μl and those with stage IV disease regardless of the CD4+ T cell count were all considered eligible for ART initiation. No patient was started on ART unless ART adherence counseling was done and readiness for treatment was assessed to be possible. All ART naïve patients were started on generic fixed dose combination regimen stavudine + lamivudine + nevirapine (triomune) as required by the national guidelines for management of HIV and AIDS.[3] Patients intolerant to triomune, or those with concomitant anti-tuberculosis therapy were initiated on other generic drug combinations including zidovudine + lamivudine + nevirapine; stavudine + lamivudine + efavirenz; and zidovudine + lamivudine + nevirapine.

Follow up visits were made monthly at the clinic and involved sustained monthly ART education and adherence counseling, clinical evaluation, management of opportunistic diseases, drug refill, and regular laboratory investigations. All clinical and laboratory data were entered into the hospital ART clinic database access software.

We reviewed retrospectively the data of patients that were enrolled in the ART scaling up program from July 2004 to August 2005. We excluded for data analysis those patients: who were already on ARVs prior to initiation of the program, those whose age was not known, patients whose latest baseline CD4+ T cell counts were done more than a month prior to the program before starting ART, and patients with incomplete information about the dates for baseline and follow up CD4+ T cell counts. Data analysis was performed using SPSS version 13.0

Results

A total of 1435 patients were recruited for ART between July 2004 and August 2005, of these 1285 (89.6%) were aged 13+ years. A total of 361 patients were included in the data analysis. The baseline demographic and immunological characteristics of the study population are summarized in Table 1. The mean (range) age was 39 years (13 – 69 years). Two hundred and fourty seven (68.4%) were females. The overall mean baseline CD4+ T cell count was 113 cells/μl; 105 (29.1%) patients had baseline CD4+ count < 50 cells/μl.

Table 1. Baseline demographic and immunological characteristics of HIV-1-infected patients (N=361) at MNH, Dar es Salaam, Tanzania

Characteristics	Sex		Total (%)
	Male (%)	Female (%)	
Number	114 (31.6)	247 (68.4)	361 (100)
Mean age (years)	42	37	39
Mean baseline CD4+ count (cells/ul)	95	121	113
CD4+ T-cell count:			
1 – 49	44 (41.9)	61 (58.1)	105 (29.1)
50 – 99	21 (28.4)	53 (71.6)	74 (20.5)
100 – 200	42 (31.3)	92 (68.7)	134 (37.1)
201 – 350	5 (12.8)	4 (87.2)	39 (10.8)
351+	2 (22.2)	7 (77.8)	9 (2.5)

Table 2 summarizes the distribution of ART regimens and mean CD4+ T cell increase per month in HIV-1 infected individuals. Two hundred and ninety five (81.7%) received triomune. The overall mean CD4+ T cell count increase was 23 cells/μl. The mean CD4+ T-cell count monthly increase among patients on combined stavudine, lamivudine and nevirapine, and combined zidovudine, lamivudine and efavirenz regimens were 25 cells/μl and 15 cells/μl, respectively, and there was no significant difference in CD4+ T cell count increase between the two regimens (p=0.052). The mean CD4+ T-cell monthly increase for male and female patients were 22.7 cells/μl and 22.9 cells/μl, respectively, (p=0.963). The mean CD4+ T-cells increase per month for patients below 50 years (320) and those aged 50 and above (41) were 23.3 cells/μl and 19.5 cells/μl, respectively, (p=0.378).

Table 2: Distribution of mean CD4+ T cells increase per month according to ART regimens in HIV-infected patients (N=361) at MNH, Dar es Salaam, Tanzania.

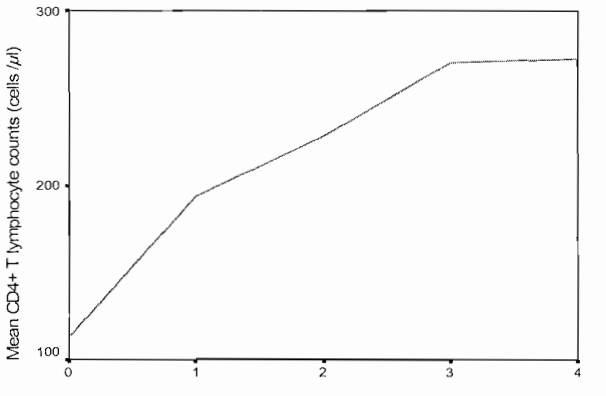
Regimen	Patients No. (%)	Mean monthly CD4+ T-cell increase (cells/ul)
D4T, 3TC and NVP	295 (81.7)	25
AZT, 3TC and EFZ	32 (8.9)	15
D4T, 3TC and EFZ	11 (3.3)	23
AZT, 3TC and NVP	4 (1.1)	25
Changed regimen	18 (5.0)	27
Total	**361 (100)**	**23**

D4T, stavudine; 3TC, lamivudine; NVP, nevirapine; AZT, zidovudine; EFZ, efavirenz

The curve of the overall immunological responses overtime showed that the CD4+ T cell count increased rapidly in the first nine months of treatment with more rapid increase in the first 3 months (Figure 1). The trends of the overall means were 113, 194, 228, 270 and 272 cells /μl at baseline, 1-3, 4-6, 7-9 and 10-12 months intervals, respectively. Of all the patients, only 12 (3%) patients had CD4+ T-cell counts that kept on declining overtime, 8 (2%) patients had CD4+ T-cell counts that remained static even after having been started on ART.

It was also noted that patients starting treatment with baseline CD4+ T cell counts <50, 50 – 99, 100 – 200, 201 – 350 and 351+ cells/μl had the mean CD4+ T cell count increase per month of 27, 23, 24, 8, and 9 cells/μl, respectively, (F=4.46, p=0.001 using ANOVA analysis).

Figure 1: The overall trends of immunological responses to ART over 12 months



Discussion

CD4+ T cell count is one of the essential laboratory parameters that is used as eligibility criterion for initiation of ART and remains as the gold standard for evaluating the immunological response to ART in resource-limited countries. Other parameters that are used to monitor and evaluate the progress of the patients are viral load and clinical progression over time. Although viral load is a good prognostic marker of HIV disease progression and is routinely used in clinical practice in developed countries, the assay is expensive and may not be affordable for every facility in resource limited countries. It is thus recommended in Tanzania that, except where viral load assay is available, monitoring of the patients on ART should be done using $CD4^+$ T lymphocytes and clinical progression. In the current study, we described the immunological response to ART in HIV-1 infected ART naïve patients in relation to age, sex, baseline CD4+ T cell counts and generic ART regimens.

More than two thirds (68.4%) of all patients that were included in the analysis were females. This reflected the proportion of patients attending ART clinic at Muhimbili National Hospital. However, the proportion is relatively higher compared to the proportion (53.3%) of women with HIV and AIDS in the general population.[1] The study finding suggests that women have a higher tendency to seek treatment than their male counterparts. It could also be due to the positive impact of screening for HIV infection during antenatal clinic (ANC) visits where pregnant mothers are counseled and screened. In antenatal clinics, which are entry points, HIV seropositive women are also informed about the available prevention of mother to child transmission (PMTCT) and comprehensive treatment services for HIV and AIDS and they are therefore more likely choose to attend the ART clinic for care, treatment and support compared to males who do not have equivalent ANC services.

Study findings showed that about one third of the patients started treatment (ART) with CD4+ T cell count below 50 cells/μl. This could be due to the fact that MNH was the first health facility in the country to offer comprehensive management of HIV and AIDS free of charge. Prior to the National ART scaling up program the drugs were sold at very high prices mainly in private health facilities and many HIV and AIDS patients could not afford them. The provision of free services for ART could have motivated the very sick patients with severe immunosuppression to seek health care in this facility. It is also a common observation that HIV-infected patients tend to come late to the clinic to seek treatment because of less awareness about the ART program.

The study findings also showed that more than half of the patients had the first follow up CD4+ cell count tests done between 4 – 6 months and these were statistically significant different (p=0.010) from the CD4+ cell counts in the other intervals for the 1st follow up. These findings are in line with the Tanzania National Guidelines for Management of HIV and AIDS that recommends follow up CD4+ T cell count at every 4 - 6 months for patients on ART. [3]

The overall increase of CD4+ T cell count per month was found to be 23 cells/μl regardless of regimen, sex, age and baseline CD4+ count. Similar CD4+ T cell count increase (21 cells/μl/month) has been reported among HIV-1 infected ART naïve patients starting treatment containing a protease inhibitor.[11] CD4+ T cell count increase per month in relation to the two most used ARV drug combinations (combined stavudine, lamivudine and nevirapine and combined zidovudine, lamivudine and efavirenz) were 25 and 15 CD4+ T-cells/μl per month, respectively. Although the findings appeared to be in favour of combined stavudine,

lamivudine and nevirapine, the difference between the two regimens was not statistically significant (p=0.052). Our findings do not provide adequate evidence for the choice of one particular combination over the other. These findings are in line with the findings from other studies that reported equal effectiveness of ART drug combinations in HIV-1 infected patients starting ART.[10, 12]

There was no significant difference in the mean CD4+ T-cells monthly increase between sex (p = 0.963) as well as between the treatment age groups (p = 0.378). Our findings are in contrast with recent study findings from 68 hospitals in France which showed that patients over 50 years of age had their CD4+ T-cell reconstitution significantly slower than in younger patients, despite a better virologic response.[13] The difference between our study and theirs is that they had 401 patients over 50 years of age compared to very few patients (41) in our study.

The mean CD4+ T cell count increase per month was significantly higher among patients starting treatment with baseline CD4+ T cell counts ≤ 200 cells/μl than those who started treatment with baseline CD4+ T cell count >200 cells/μl (F=4.46, p=0.001). The mean CD4+ T cell count increase per month was as high as 27 cells/μl among patients started treatment with severely depleted immunity (CD4+ T cell counts ≤ 50 cells/μl) and as low as 8 cells/μl per month among those started treatment with baseline CD4+ T cell counts above 200 cells/μl. Despite such good immunological response among patients who started ART with severe immunosuppression, it is still under debate whether patients with more advanced HIV-1 infection as shown by CD4+ T cell count <50 cells/μl at baseline could have the capacity in the immune system to fully replenish the depleted CD4 T-cells or whether a plateau is possibly reached after three to four years, beyond which there is no further improvement.[14,15] It has been reported that patients starting ART at low CD4+ T cell levels rarely attain complete immune reconstitution.[16,17] Since our evaluation was only done one year after the program started, it is important to note that further follow up studies are needed to ascertain whether these patients who started treatment with low baseline CD+ T cell levels will ever attain complete immune reconstitution or not. The trends of the overall immunological responses showed that CD4+ T cell counts increased more rapidly in the first three months of treatment (27 cells/μl per month) and much slower thereafter and almost plateauing at 10 - 12 months. However, our findings that showed slight increase in the mean CD4+ T cell count at 10 - 12 months are inconclusive because there were very few patients that had already reached this time interval on treatment. Similar trend of CD4+ T cell count increase overtime has been reported following initiation of ART with a rapid increase of CD4+T cells within the first three to four months (21 cells/μl per month) and much slower rise (0.5 cells/μl per month) thereafter.[18] It has been suggested that the initial rapid increase in CD4+ cells could be due to redistribution which is followed by production of new CD4+ T-cells. Other study findings have attributed this to diminished programmed cell death (apoptosis), one of the mechanisms of CD4+ T cell depletion that occur to a greater extent in HIV-infected individuals than in non-infected persons, both in the peripheral blood and lymph nodes.[19]

In conclusion, initiation of ART resulted in overall good immunological responses even among patients who had CD4+ count of < 50 cells/μl. It is recommended to enhance the scale up of the ART program country wide in order to provide access to care and treatment to all HIV-infected individuals.

References

1. Ministry of Health, Tanzania Mainland, National AIDS Control Programme (NACP). HIV/AIDS surveillance report no. 18, January – Dec. 2003, March 2004.
2. Tanzania Commission for AIDS (TACAIDS), National Bureau of Statistics (NBS) and ORC Macro. Tanzania HIV/AIDS indicator survey 2003-04, Mar. 2005, pp. 69.
3. Ministry of Health, Tanzania Mainland, National AIDS Control Programme (NACP). National guidelines for the clinical management of HIV and AIDS, 2nd edition, April 2005,
4. Giorgi JV, Lyles RH, Matud JL et al. Multicentre AIDS Cohort Study. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. J Acquir Immune Defic Syndr 2002; 29(4):346-355.
5. Jacobson LP, Phair JP, Yamashita TE. Update on the virologic and immunologic response to highly active antiretroviral therapy. Curr Infect Dis Rep 2004; 6(4):325-332.
6. Jacobson LP, Phair JP, Yamashita TE. Virologic and immunologic response to highly active antiretroviral therapy. Curr HIV/AIDS Rep 2004; 1(2):74-81.
7. Viard JP, Mocroft A, Chiesi A et al. Influence of age on CD4 cell recovery in HIV-infected patients receiving HAART: evidence from UroSIDA study. J Infect Dis 2001; 183:1290-1294.
8. Kolte L, Dreves AM, Ersboll AK et al. Association between larger thymic size and higher thymic output in HIV-infected patients receiving HAART. J Infect Dis 2002; 185:1578-1585.
9. Kaufmann GR, Furrer H, Ledergerber B et al.; Swiss HIV Cohort Study: Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/μl in HIV type 1-infected individuals receiving potent antiretroviral therapy. Clin Infect Dis 2005; 41(3):361-372.
10. French M, Amin J, Roth N et al. Randomized, open label, comparative trial to evaluate the efficacy and safety of three antiretroviral drug combinations including two nucleoside analogues and nevirapine for previously untreated HIV-1 infection: the OzCombo 2 study. HIV Clin Trials 2002; 3(3):177-185.
11. Le Moing V, Thiebaut R, Chene G et al. Predictors of long-term increase in CD4(+) cell counts in HIV-infected patients receiving a protease inhibitor-containing antiretroviral regimen. J Infect Dis 2002; 185: 471-480.
12. Foudraine NA, de Jong JJ, Jan Weverling G et al. An open randomised controlled trial of zidovudine plus lamivudine versus stavudine plus lamivudine. AIDS 2003; 12(12):1513-1519.
13. Grabar S, Kousignian I, Sobel A et al. Immunologic and clinical responses to highly active antiretroviral therapy over 50 years of age. Results from the French Hospital Database on HIV. AIDS 2004; 18(15):2029-2038.
14. Smith CJ, Sabin CA, Youle MS et al. Factors influencing increases in CD4 cell counts of HIV-positive persons receiving long-term highly active antiretroviral therapy. J Infect Dis 2004; 190:1860-1868.
15. Viard JP, Burgard M, Hubert JB et al. Impact of 5 years of maximally successful highly active antiretroviral therapy on CD4 cell count and HIV-1 DNA level. AIDS 2004; 18:45-49.
16. Valdez H, Connick E, Smith KY et al. Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. AIDS 2002; 16:1859-1866.
17. Kaufmann GR, Perrin L, Pantaleo G et al. CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV Cohort Study. Arch Intern Med 2003; 163:2187-2195.
18. Pakker NG, Notermans DW, de Boer RJ et al. Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. Nat Med 1998; 4:208-214
19. Roger PM, Breittmayer JP, Durant J et al. Early CD4(+) T cell recovery in HIV-infected patients receiving effective therapy is related to a down-regulation of apoptosis and not to proliferation. J Infect Dis 2002; 185:463-470.