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EFFECT OF ANTI-RETROVIRAL THERAPY ON CD4+ T LYMPHOCYTE COUNT IN HIV INFECTED PATIENTS ATTENDING REGIONAL INSTITUTE OF MEDICAL SCIENCES HOSPITAL, MANIPUR

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ABSTRACT: PURPOSE: The initiation of anti-retroviral therapy has reduced the HIV related mortality substantially .But recent reports of decreased efficacy have caused concerns even though it has helped to reduce the burden of opportunistic infections as well as disease progression. This study has been done to study the effect of anti-retroviral therapy (ART) on CD4 + T lymphocyte count in HIV infected and AIDS patients undergoing ART. **METHOD**: 624 HIV infected patients on ART during the period of February 2007 to February 2009 were included in the study. Effect of ART on CD4+ T lymphocyte count was done in these patients by using Fluorescent Activated Cell Sorter (FACS) count system. **RESULTS:** Of the 624 patients, 67.46% were males and 32.53% were females. Out of the 624 patients on ART, 95.35% (595/624) showed an increase in CD4 count while 4.64% (29/624) showed a decrease in CD4 count. Of this 89.10% (556/624) showed a continuous increase in their CD4 count without any fluctuation while 6.25% (39/24) showed increase in CD4 count although there was fluctuation during the period of 24 months and 2.88% (18/624) showed a continuous decrease in their CD4 count while 1.76% (11/624) showed decreasing trend although there was fluctuation during the period of 24months. **CONCLUSION:** The monitoring of CD4 count is useful to check the disease progression and effectiveness of ART.

KEY WORDS: Anti-retroviral therapy, CD4+ T lymphocyte, Fluorescent Activated cell Sorter, HIV, AIDS.

INTRODUCTION: Anti-retroviral drugs have established their role as agents which can delay the progression from HIV to AIDS and can give a reasonably good quality of life to AIDS patients [1,2].

Anti-retroviral therapy (ART) reduces the severity of the HIV infection and its progression causing drastic reduction in the occurrence of opportunistic infection (OI) when compared to pre era [3].

The introduction of ART has drastically changed the prognosis of HIV infected patients, in terms of decreasing mortality rate and decreasing incidences of opportunistic infections (OIs).The effect of ART is also well established for surrogate –markers such as the viral load and CD4 cell count[4]. Recommendations for initiation of ART are largely based on CD4+ T lymphocyte cell count [5].

Typically, the CD4 cell count should increase by at least 50 cells/µl at 4 to 8 weeks after starting therapy followed by an increase of 50–100 cells/µl per year until a threshold is reached[6, 7, 8]. Immunologic failure is indicated by a fall in CD4 counts higher than 30% from the peak or return to or below the pre therapy baseline[9].

The advent of ART brought hope to patients with HIV/AIDS but recent reports of decreased efficacy have caused concerns even though it has helped to reduce the burden of opportunistic

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infection (OIs) as well as disease progression. Manipur has a high prevalence of HIV/AIDS and OI constitutes the major cause of morbidity and mortality.

Many HIV infected persons are under ART but no proper study has been done to evaluate its effect on CD4 count in Manipur. As the incidence of HIV infection/AIDS is very high in Manipur, a proper study to document the effect of ART is highly essential for both physicians and patients at large.

Hence the present study was undertaken to know the effect of ART and the CD4 response.

MATERIALS AND METHODS: The study group comprises of 624 HIV infected patients on ART during the period of February 2007 to February 2009. The ART drugs used are: 1- Zidovudine 300 mgm b.d., 2- Lamivudine 150 mgm b.d., 3- Nevirapine 200 mgm b.d. If the patient is on antitubercular therapy, then the following regime is used: 1- Zidovudine 300 mgm b.d., 2- Lamivudine 150 mgm b.d., 3- Efavirenz 600 mgm o.d. There was no problem of compliance among the patients studied. These patients were followed up with CD4+ T lymphocyte count to see the effect of ART on this subset of T lymphocyte.

The inclusion criteria in this study were- diagnosed cases of HIV/AIDS, cases above 12years and cases on ART while the exclusion criteria was children below 12 years of age.

Estimation of CD4+ T lymphocyte was done by using Fluorescent Activated Cell Sorter (FACS) count system [Becton Dickinson Immunocytometry System, San Jose, CA 95131-1807]
Interval of CD4+ T lymphocyte estimation was done at every 6 months interval.

RESULTS: Of the 624 patients, 421 (67.46%) were males and 203(32.53%) were females. The ratio of male to female was approximately 2:1.

The age of the patients ranged from 20-60 years. Majority of the patients were in the age group of 30-39 as shown in Table 1.

Age	Male	Female
20-29	32	33
30-39	230	104
40-49	130	54
50-59	24	11
60 & above	5	1
Total	421 (67.46%)	203 (32.53%)
Table 1: Age and Sex distribution of cases		

Patient on ART	Effect of ART on CD4 count	No. of patient Increased in CD4 count	CD4 Count	Baseline (cells/μl)	6months (cells/μl)[mean ↑sed in CD4 count]	12months (cells/μl)[mean ↑sed in CD4 count]	18months (cells/μl)[mean ↑sed in CD4 count]	24months (cells/μl)[mean ↑sed in CD4 count]
CD4 count	Increased	556 (89.10%)	Mean CD4	138.27	256.26 *[117.99]	341.15 *[97.79]	410.50 *[84.18]	475.30 *[78.64]
Table 2: Effect of ART on CD4 count								

(*figure in parenthesis indicate mean increase in CD4 count)

Of the 624 patients on ART, 556 (89.10%) showed a continuous increase in their CD4 count (i,e their CD4 count doesn't fluctuate). The mean baseline CD4 count is 138.27 cells/μl and the CD4 counts at 6,12,18,24 months are 256.26cells/μl,341.15 cells/μl,410.50cells/μl and 475.50cells/μl

respectively. While the mean increases in CD4 count at 6, 12, 18 and 24 months are as follows - 117.99cells/µl,97.79cells/µl,84.18cells/µl and 78.64cells/µl respectively.

No. of Patients whose CD4 is increased but fluctuates			Increased in CD4 range with respect to 24months	
39 (6.25%)	0-49 (cells/µl)	50-99 (cells/µl)	100-199 (cells/µl)	200-299 (cells/µl)	>300 (cells/µl)
	10 patients	9 patients	13 patients	4 patients	3 patients
Table 3: Effect of ART on CD4 count for those where CD4 count showing increase with fluctuation				

Of the 624 patients on ART,CD4 count fluctuates in 39(6.25%) cases (i,e at one interval of 6 months CD4 count increase and at another interval of 6 month CD4 count decrease). But when the baseline CD4 count was compared with 24months, 10 patients showed an increase in CD4 count in the range of 0-49 cells/µl, 9 patients showed an increase in the range of 50-100cells/µl, 13 of them in the range of 100-199 cells/µl, 4 patients in the range of 200-299cells/µl and 3 patients in the range of > 300cells/µl.

Thus all together 95(95.35%) showed an increase in their CD4 count.

Patient on ART (total)	Effect of ART on CD4 Count	CD4 count	Baseline (cells/µl) [mean ↓sed in CD4]	6 months (cells/µl) [mean ↓sed in CD4]	12 months (cells/µl) [mean ↓sed in CD4]	18 months (cells/µl) [mean ↓sed in CD4]
624	Decreased	18 (2.88%)	222	177.22 *[44.78]	135.58 *[49.75]	100.33 *[44.00]
Table 4: Cases showing decreased CD4 count on ART						

(* figure in parenthesis indicates mean decreased in CD4 count)

Out of the 624 patients on ART, 18(2.88%) patients showed a continuous decrease in CD4 count. The mean baseline CD4 count is 222cells/µl and the CD4 counts at months of 6,12 and 18 are 177.22 cells/µl, 135.58 cells/µl respectively.

Only 3 times reading at 6 months interval i,e from baseline to 18months was taken for 18 patients who showed decrease in CD4 count after ART as these patients did not turn up at 24months.

No. of patients where CD4 is decreased but fluctuates	Decrease in CD4 range with respect to 24 months					
	0-10 cells/µl	11-20 (cells/µl)	21-30 (cells/µl)	31-40 (cells/µl)	41-50 (cells/µl)	>50 (cells/µl)
11 (1.76%)	2 Patients	2 Patients	1 Patients	2 Patients	2 Patients	2 Patients
Table 5: Effect of ART on CD4 count for those where CD4 count showing decrease with fluctuation						

Out of 624 patients, CD4 count of 11 (1.76%) patients fluctuates and when baseline CD4 count was compared with CD4 count of 24 months, there was a decrease in the CD4 count as shown in Table 5.

DISCUSSION: The initiation of anti-retroviral therapy has drastically changed the prognosis of HIV infected patients in term of decreasing mortality rate and decreasing incidence of opportunistic infections. In this study we have observed a preponderance of male patients (67.46%) in comparison to female (32.53%). Our finding is consistent with other published report whereby male subject are more often the victims of this disease than their female counterparts probably due to their high risk behavior [9,10].

In the present study 95.35% (595/624) showed an increase in CD4 count while 4.64% (29/624) showed a decrease in CD4 count. Of this 89.10% (556/624) patients showed continuous increase of CD4 count without any fluctuation (as shown in Table 2). 6.25% (39/624) of the patients showed increase in CD4 count although there was fluctuation during the period of 25 months (as shown in Table 3) While 2.88% (18/624) of the patients showed decrease in the CD4 count (as shown in Table 4) and 1.76% (11/624) showed a decreasing trend although there was fluctuation during the period of 24months (as showed in Table 5). Similarly, several studies from India have reported an increase in CD4 count after starting HAART (highly active anti-retroviral therapy). Good treatment response was seen in 87.5% and deterioration in 12.5% in HIV infected patients on HAART in south India [11]. Similar good treatment response of > 80% was seen in HIV patients on HAART in a study conducted at Lok Nayak Hospital, New Delhi [12]. In a similar study at Karnataka, 87.5% HIV seropositive patients on ART showed an increase response in their CD4 T lymphocyte count after 6months [11].

Several other studies from around the globe found good treatment response after starting HAART [13, 14]. Study conducted in Nigeria found out that 86.48% showed a marked increase in their CD4 count level in the initial 6 months of therapy indicating that HAART is effective at suppressing HIV viral load and increasing CD4 cells count [15]. This is consistent with our findings in which 89.10% showed a continuous increase in their CD4 count while 2.88% showed a continuous decrease in 24 months.

In our study the mean baseline CD4 count was 138.27 cells/μl and the mean increases at 6,12,18 and 24 months was as follows 117.99 cells/μl, 97.79 cells/μl, 84.18cells/μl and 78.64cells/μl respectively. Another Indian study from Chennai found good treatment response with a mean CD4 gain 188cells/μl in a mean duration of 4.3 months after HAART [16].

CONCLUSION: The initiation of anti-retroviral therapy has drastically changed the prognosis for HIV infected patients in terms of decreasing mortality rate and decreasing incidence of opportunistic infections. The benefits of HAART in HIV infection have been attributed primarily to its suppression of viral replication. [17]

Further the monitoring of CD4 count is a reliable indicator of the immune status of the individual. CD4 count differentiates the staging of HIV infection [18]. It can predict the type of opportunistic infection that is likely to occur thereby enabling decisions on the need of prophylaxis. Also monitoring of CD4 count helps in deciding on the initiation and change of combination of ART. The fluctuations in the study are minor fluctuations as fluctuations in the CD4 count is a natural phenomenon. The fluctuation in the CD4 count might be due to movement of the CD4 cells between

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blood and tissue. The fluctuations are due to factors like diurnal variations, strenuous exercise, stress, corticosteroid therapy, some medications, smoking etc.[19]

REFERENCES:
1. MMWR. (1998) Guidelines for the use of anti-retroviral agents in HIV infected adults and adolescents. Department of Health and Human services and Henry J Kaiser Family Foundation, MMWR Recmm Rep; 47: 43-82.
2. WHO. Treating 3 million by 2005, making it happen, The WHO Strategy. WHO 2003: http://www.who.int/3 by 5/publications/documents/en/3 by 5 strategy making it happen.pdf. Accessed on March 18, 2005.
3. Kovacs JA, Masur H (2003). Prophylaxis against opportunistic infections in patients with human immunodeficiency virus infection. New England Journal of Medicine; 342:1416-1429.
4. Kirk O, Lundgren JD, Pedersen C, Nielsen H, Gerstoft J. Can chemoprophylaxis against opportunistic infections be discontinued after an increase in CD4 cells induced by highly active antiretroviral therapy? AIDS 1999; 13: 1647-1651.
5. Hogg RS, Yip B, Chan KJ ,Wood E, Craib KJP et al(1999). Rates of Disease Progressive by Baseline Cd4 Cell count and viral load after Initiating Triple Drug Therapy, JAMA; 286(20): 2568-2577.
6. Yamashita TE, Phai JP, Munoz A, Munoz A, Margolick JB et al(2001). Immunologic and virologic response to highly active anti-retroviral therapy in the Multicenter AIDS Cohort Study, AIDS; 15(6):735-746.
7. Le Moing V, Thiebant R, Chere G, Leport C, Cailleton V et al(2002). Predictors of long term term increase in CD4+ cell counts in human immunodeficiency virus infected patients receiving a protease inhibitors containing anti-retroviral regime, Journal of Infectious Disease ; 185: 417-480.
8. Yeni PG, Hammur SM, Carpenter CC, Cooper, Fischl MA et al(2002). Anti-retroviral treatment for adult HIV infection in 2002: Updated recommendation of the International AIDS Society-USA panel, JAMA ; 288: 222
9. Ayyagari A, Sharma AK, Prasad KN, Dhole TN, Kishore J et al(1999) .Spectrum of OIs Human Immunodeficiency virus infected cases in tertiary care hospital Indian Journal of Medical Microbiology; 17:78-80.
10. Ebrahim RA, Farid EMA, Yensif A, Jamsheer AE (2002). Microbiological Infections in HIV positive Bahraini patients with low CD4+ T lymphocyte count, Journal of Communicable Disease; 34(3):160-170.
11. Shahapur PR, Bairy I,Shivananda PG (2005): Effect of HAART on CD4+ T lymphocyte count in HIV seropositive south Indian individuals – A follow up study. Indian Journal Pathology Microbiology; 48(2): 270-272.
12. Gautam H, Bhalla P, Saini S, Dewan R(2008). Corelation between baseline CD4+ T lymphocyte and Plasma viral load in AIDS patients and their early clinical and immunological response to HAART: A preliminary study. Indian Journal of Medical Microbiology; 26(3): 256-258.
13. Hughes MD, Danieles MJ, Fischl MA, Kim S, Schooley RT (1998). CD4 cell count as a surrogate end point in HIV clinical trial, a meta analysis of studies of the AIDS clinical trials group. AIDS; 12: 1823-1832.

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14. HIV Surrogate marker collaboration group. Human Immunodeficiency virus type 1 RNA level and CD4 count as a prognostic marker and surrogate end point, a meta analysis (2000). AIDS Res Human retrovirus ; 16: 1123 – 1133.

15. Mirabeau TY, Obinna NG, Marcellinus O, Edo AD, Ikennah AM et al(2005) .Effect of Lamivudine (Epivir), Nevirapine (Vivumine) and Stavudine (Stavir) on CD4+ count of HIV patients attending University of Benin teaching hospital (UBTH), Benin city, Edo State, Nigeria. Kuwait Medical Journal; 37(2): 86-90.

16. Kumarasamy N, Solomon S, Peter E,Amalraj RE, Purnima M et al(2000). Anti-retroviral drugs in the treatment of people living with human immunodeficiency virus: Experience in South Indian tertiary referral center, Journal Association Physicians India; 48: 390-393.

17. Lucas GM (2005). Anti-retroviral adherence, drug resistance, viral fitness and HIV disease progression: a tangled web is woven, Journal of Antimicrobial Chemotherapy ; 55: 413-416.

18. HIV Surrogate Marker Collaborative Group. Human Immunodeficiency virus type 1 RNA level and CD4 count as prognostic marker and surrogate end point: a meta-analysis(2000), AIDS Res. Human retrovirus ; 1123 -1133.

19. Monthly mortality weekly report(1997) Revised guidelines for performing CD 4 T lymphocytes determinations in persons infected with HIV: published by US Department of Health and Human Science, CDC, Atlanta, Georgia 30333, Jan.10. 1997;56:no.RR-2:29.

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