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RESEARCH ARTICLE

PROSPECTIVE STUDY OF IMMUNOLOGICAL RECOVERY IN HIV PATIENTS BY CD4 COUNT AFTER SIX MONTH ON HAART

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ABSTRACT

Introduction: In chronic HIV infection disease progression and response to Highly Active Anti Retroviral Therapy is monitored by CD4 + T lymphocyte count measurement and plasma viral load quantification. In resource limited countries CD4 + T lymphocyte count measurement is commonly used.

Methodology: Newly diagnosed HIV seropositive subjects (n= 68) in clinical stage 1&2 with CD4 count less than 500 cells/ µl was started on therapy and CD4 count response was evaluated after 06 months with adherence more than 95%.

Results: There was overall increase in mean CD4 cell count of 129 cells/ µl after 06 month follow up on therapy from the baseline in the study cohort but actually in 15 patients there was statistically significant fall in mean CD4 cell count of 92 cells/ µl. Therapy dependent increase in CD4 cell count at 06 month follow up is significantly seen in subject with pre treatment baseline CD4 count less than 350 cells/ µl and there is no correlation between age and immunological recovery with therapy.

Conclusion: Therapy has favourable outcome in immune recovery and CD4 count monitoring is a powerful tool to determine the response in most of the individuals but paradoxical response cases will need powerful indices like viral load quantification. Therapy response has favourable outcome in pre treatment CD4 count of less than 350 cells/ µl.

INTRODUCTION

Chronic HIV infection leads to progressive decline in counts of CD4 T-helper lymphocytes in the peripheral blood along with its functional impairment[1,2].Currently flow cytometry based CD4+ T lymphocyte count(CD4 count) measurement and molecular assay to quantify plasma viral load (PVL) are the standard methods in use to monitor HIV infection. These laboratory measures are used in conjunction with clinical assessment to determine stage, progression and therapy response[3,4].Several issues concerning use of CD4 count as marker of immunological recovery has arisen nowadays like difference in long and short term prediction, impact of use of antiretroviral therapy(ART) but it continues to be pivotal in monitoring both treated and untreated patients in resource limited settings[5,6].HAART almost invariably causes reduction in plasma viremia and rapid increase in peripheral blood CD4 count in majority of individuals[7,8].During first few weeks of starting HAART ,the increase in CD4 count is rapid due to initial redistribution of cells to the peripheral circulation from stores in the lymphoreticular system[9].Thereafter on prolonged therapy rapidity of rise of CD4 count decreases because it is dependent on generation of the naive CD4 cells from the thymus which is not sufficient to sustain the rapid rise seen initially[10].However immunological recovery in treatment naive patients when started on HAART have been shown in studies to be dependent on different factors like sex related difference in immune response due to estrogen related effect on immune system[11],age of the patient with rise in CD4 count seen more in younger individuals[12],baseline CD4 count level[13,14].In the present study we sought to evaluate therapy response to immunological recovery as characterised by increase in CD4 count in newly diagnosed naive HIV seropositive patients starting HAART regimen in a ART centre of North Eastern India. We also evaluate effect of age and initial baseline CD4 count at the start of HAART on the rate of immunologic reconstitution.

METHODOLOGY

The study was performed at an ART centre from January 2012 to December 2014. Eligibility Criteria for inclusion were Age ≥ 22 years, WHO clinical stage 1 & 2, CD 4 count ≤ 500 cells/ µl and treatment naive. Individuals in stage 3 & 4, CD 4 count > 500 cells/µl, already on HAART, females and children were excluded from the study. Demographic data of age, sex and written consent were obtained from all study participants. A total of 68 newly diagnosed adult male HIV seropositive

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subjects were enrolled. The subjects were staged according to WHO clinical staging [15] and individual in stage 1 & 2 were included in the study. At the time of recruitment whole blood samples were collected by venepuncture using 10 millilitres hypodermic syringe into EDTA anti- coagulated tubes (5 millilitres) and non anti- coagulated tubes. Sera derived from the non anti-coagulated tube were screened and confirmed for HIV 1 & 2 infection using WHO strategy involving rapid test with Tridot and p24 antigen (4th generation), Immunochromatography assay (Immunocheck) and HIV 1 & 2 indirect ELISA by ELISA reader in each sample. CD4 T-Helper lymphocyte count was measured by FACS count system (Becto, Dickinson and company, USA) from sample collected in EDTA tubes. Routine Hematological and biochemical investigations along with screening of HBsAg, anti HCV, VDRL was done. All the subjects were started on HAART according to recommendation of WHO guidelines and were followed up after 6 months. HAART regimen given was AZT+ 3TC+ NVP with Cotrimaxazole prophylaxis when CD4 count was < 350 cells/µl and AZT was replaced by TDF when Hb was < 8gm/ dl. All study subjects were categorised into three study groups based on CD4 count at baseline as ≤ 200 cells/ µl, 201 to 350 cells/ µl and 351 to 500 cell/ µl. The subjects were also divided into Age groups of ≤ 35 years and > 35 years. These subjects were followed up after a period of 06 months to monitor immune recovery and disease progression on > 95% adherence to HAART.

To detect significant difference between CD4 count level at baseline and at 06 month follow up paired t test was used and statistical inference was made significant if p value < 0.05. To analyse whether there is significant difference between CD4 count at baseline and 06 months follow up for the age groups below 35 years, 35 years and above and for the entire sample as a whole, paired t test is used. If calculated p value is less than 0.05 then the null hypothesis can be rejected. Karl Pearson's correlation coefficient was used to find correlation with age and immune recovery on HAART.

RESULTS

The mean age of HIV sero-positive study subjects was 34.35 years and the median age was 32years. The highest HIV prevalence was seen in Age group ≤ 35 years (69.1%) in the study. Most common mode of acquiring infection in the study group was hetero sexual (96.38%) and intravenous drug user (3.62%). None of the individual screened was found to have HBV, HCV and VDRL reactivity.

Mean CD 4 count at baseline (pre HAART) was 305.191 cells/ µl with ± 106.0438 standard deviation of mean value. At 06 month follow up (post HAART) mean CD4 count increased to 434.956 cells/ µl ± 193.6892 standard deviation of mean value. In 53 subjects CD 4 count increase was seen at post HAART 06 monthly follow up with CD4 count increase from 288.47 cells/ µl to 481.15 cells/ µl. The mean difference of CD4 count increase was 192.67 cell/ µl in these patients and was statistically significant by paired t test ($p < 0.05$).

In 15 patients CD 4 count decreased during six month post HAART follow up from mean CD4 count of 364.27 cells/ µl to 271.13 cells/ µl. The mean difference in CD4 count fall in these patients was 92.53 cells/ µl which was statistically significant ($p < 0.05$). The fall in CD 4 count between 49.58 cells/ µl and 135.48 cells/ µl gives the 95% confidence limit by the study.

The relative number of subjects with CD4 count ≤200 cells/ µl, 201 to 350 cell/ µl and 351 to 500 cells/ µl at base line was 8 (11.7%), 40 (58.8%) and 20 (29.4%) respectively. After 06 months of HAART the numbers became 4 (5.8%), 23 (33.8%), 19 (27.9%) respectively and 22 (32.3%) achieving CD4 count of > 500cells/ µl. There was a statistically significant variation ($p < 0.05$) in therapy dependent increase in CD4 count based on pre therapeutic baseline CD4 count in groups with CD4 count ≤ 200 cells/ µl and 201 to 350 cells/ µl whereas in the group with CD4 count of 351 to 500 cells/ µl variation in therapy dependent increase was not statistically significant ($p = 0.117$).(Table 1.1 and 1.2)

Table 1.1 Mean CD4 count along with their standard deviation and mean increase in CD4 count after six months follow up for different groups classified according to baseline CD 4 count.

CD4 Count	Baseline		After 6 months		Mean increase	Percentage increase in mean
	Mean	Standard deviation	mean	Standard deviation		
Less than 200(n=8)	117.875	66.6707	250.75	102.42	132.875	109.16%
Between 200 and 350(n=40)	280.85	46.4297	432.5	171.9517	151.65	54%
Above 350 but below 500(n=20)	428.8	38.57	513.55	216.6427	84.75	19.76%
Total(n=68)	305.191	106.0438	434.956	193.6892	129.7647	42.52%

In the study mean CD 4 Count at baseline in age group < 35 years was 304.413 cells/ µl with ± 97.6847 standard deviation of mean value whereas in age group ≥ 35 years mean CD4 count at baseline was found to be 306.818 cells/ µl with ± 124.2013 standard deviation from mean value.

After 06 month on HAART mean CD4 count in age group < 35 years was 430.761 cells/ µl with ± 206.6170 standard deviation from mean value which showed a 41.51% increase in CD4 count from the baseline whereas in age group ≥ 35 years it was found to be 443.727 cells/ µl with ± 167.6107 standard deviation from mean value and it showed a 44.62% increase in CD4 count from the baseline. (Table2.1 and 2.2)

In order to test whether this change in mean increase is significant or not paired t test is performed and the result is tabulated in table 2.2 and it is seen that the result of paired t test was significant in both the age groups.

The calculated Karl Parson's coefficient of correlation between age of the patients and their improvement in CD4 count after 06 months treatment from the baseline CD4 count is 0.021 and it is not significant. This infers that there is no significant relationship between age of patients and their response to treatment with respect to increase in CD4 count.

Table1. 2 Result of paired t test for mean difference of CD4 at baseline and CD4 after 6 months for different groups classified according to baseline CD4 count.

CD4 count	Mean difference	Std dev	Std error of mean	T	df	P value
Less than 200(n=8)	132.8750*	73.5322	25.9976	5.111	7	0.001
Between 200 and 350(n=40)	151.6500*	166.8785	26.3858	5.747	39	0.000
Above 350 but below 500(n=20)	84.7500	230.8832	51.6270	1.642	19	0.117
Total(n=68)	129.7647*	181.0638	21.9572	5.910	67	0.000

*Implies significant at 5% level of significance

Table 2.1 Mean CD4 count along with their standard deviation and mean increase in CD4 count after six months follow up for different age groups.

Age group	CD4 at baseline		CD4 after 6 months		Mean increase	Standard deviation of mean increase	Percentage increase in mean
	mean	Std dev	Mean	Std dev			
Below 35(n=46)	304.413	97.6847	430.761	206.6170	126.3478	201.5785	41.51%
35 and above(n=22)	306.818	124.2013	443.727	167.6107	136.9091	132.0804	44.62%
Total(n=68)	305.191	106.0438	434.956	193.6892	129.7647	181.0638	42.52%

Table 2.2 Result of paired t test for mean difference of CD4 at baseline and CD4 after 6 months for different age groups

Age group	Mean difference	Std dev	Std error of mean	t	df	P value
Below 35(n=46)	126.3478*	2012.5785	29.7211	4.251	45	0.000
35 and above(n=22)	136.9091*	132.0804	28.1596	4.862	21	0.000
Total (n=68)	129.7647*	181.0638	21.9572	5.910	67	0.000

*Implies significant at 5% level of significance.

DISCUSSION

In this study we observed the highest HIV infection rate in ≤ 35 years age group (69.1%) and 96.38% of them have contracted HIV through heterosexual contacts.

These findings are in agreement with that of the study by O.Erhabor *et al*[16].

In this study the mean CD4 count in 68 patients before ART was 305 cells/µl with standard deviation± 106 of mean value. After six months of ART the mean value rose to 434 cells/µl with standard deviation± 193 of mean value thus showing that all of them are benefitted from the ART. But on further analysis we found in the study that actual increase in the CD4 count from baseline after six months of ART was seen in 53 patients only with mean increase of 192 cells/µl which was statistically highly significant($p<0.01$).

Study by Mocroft *et al* have also shown mean increase in CD4 count of 100 cells/µl after 12 months of ART[17].CASCADE collaboration have reported the mean CD4 count rise of 119 cells/µl after six months on ART[18].

In 15 patients after six months of ART mean CD4 count reduced from 364 cells/µl to 271 cells/µl. The mean difference in fall was 92 cells/µl which was also statistically significant when compared using paired t test($p<0.05$). Similar findings was seen in a Indian study where 12 patients of total 57 patients studied showed mean difference in fall of CD4 count of 109 cells/µl after six months of ART[19]. Highley man[19] have also reported poor CD4 cell recovery in their study in few patients on HAART.

This paradoxical response can be due to the influence of regression of mean as baseline measure was used to calculate the change from baseline and we may have underestimated the strength between probability in increase in CD4 count and low CD4 count at baseline[20].

These subjects even though confirm their adherence to HAART > 95% might have recall bias in claiming adherence to therapy for 06 months. There is also a possibility of immunological failure and hence should have been investigated for viral load response and drug resistance testing if adherence to HAART > 95% with no evidence of opportunistic infection found but due to our resource limited setting we were unable to quantify viral load and comment on this issue.

In our study we found that subjects with pre- HAART CD4 cell count of ≤ 350 cells/ µl have shown more improvement in their CD4 counts on therapy. Absolute mean CD 4 count rise of 132 cells / µl in subjects with pre HAART mean CD4 cell count ≤ 200 cells/ µl group and 151 cells/ µl rise in mean CD4 count in subjects with baseline mean CD4 count of 201 to 350 cells/ µl was observed in the study which was statistically significant. There is conflicting evidence on this point with some [21,22] but not all [23,24] studies finding of higher response to HAART in subjects with higher baseline CD4 cell count. In our study we did not find any association between age of the subject and immunologic response on HAART as in both ≤ 35 years and > 35 years age group mean rise in CD4 cell count on therapy was not statistically significant.

Although some studies have found relationship between younger age group and immunological response [25, 26].

CONCLUSION

In our study if we consider all 68 patients together then CD 4 count of the cohort on HAART have significantly increased in 06 month follow up but actually this favourable outcome was

seen in 53 patients only. In 15 patients statistically significant fall in CD4 count post HAART at 06 month follow up was seen which can be influenced by regression to mean or immunological failure. Adherance in our country needs redressal during continuum of care in HIV even though in our study adherence to HAART was > 95%. The variation in therapy dependent increase in CD4 count based on pre therapeutic baseline CD4 count was seen in subjects with ≤ 350 cells/ µl which is being followed by National Aids Control Organisation in their recommendation of starting CD4 count based therapy. There was no correlation between Age and Immunological recovery on HAART in this study.

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