CD4 Cell Count Analysis Among HIV Patients at a Tertiary Hospital in South India

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ABSTRACT

Background: HIV (Human Immunodeficiency Virus) infection is characterized by a gradual deterioration of immune function. CD4 cell counts generally decrease as HIV progresses in untreated HIV-infected subjects .The present study was undertaken to know the effect of ART (Anti retroviral therapy) and the status of CD4 counts in study patients and among the genders in HIV patients.

Methods: This was a cross sectional study on secondary data maintained in the ART centre for the period of 6 months from March 2015 to October 2015. The patient reports (150 cases) in the ART centre during the period of the study (6 months) were selected. CD4 cell count was done using Flow Cytometry Absolute Cell Count System.

Result: Out of 150 patients 82 were male and 68 were female patients. The mean value for base line CD4 count for general group lies between those of female and male groups, with mean female (126.88cells/cmm) was lesser than that of male group(i.e. 141cells/cmm) indicating females were at higher risk of being Immunocompromised compare to males. Also that the standard deviation of female (5.4) was lesser to male (48.43) supporting the above statement. After 6 months of antiretroviral therapy, the mean CD4 counts was in the relation 352.74 cells/cmm(Male) < 378.82 cells/cmm(general) < 410.26cells/cmm (Female) with the standard deviation relation 162.68 (male) < 179.49(general) < 194.46 (Female).

Conclusion: The results of this study indicated that antiretroviral therapy plays an important role in maintaining the CD4cell counts and CD4 counts in female patients are much lower compared to the males and they respond well to therapy compared to the male population.

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Introduction

HIV (Human Immunodeficiency Virus) infection is characterized by a gradual deterioration of immune function. Most notably, crucial immune cells called CD4 cells are disabled and killed during the typical course of infection. [1] HIV is persistent and ultimately progressive in the vast majority of untreated hosts, there is increasing evidence that HIV have specific cellular immune responses playing a major role in determining the tempo of viral replication and thus the clinical outcome of infection. [2] At the end of 2010, an estimated 34 million people [31.6 million–35.2 million] were living with HIV worldwide, up 17% from 2001. This reflects the continued large number of new HIV infections and a significant expansion of access to antiretroviral therapy (ART), which has helped reduce Acquired Immunodeficiency Syndrome (AIDS) - related deaths, especially in more recent years. [3]

In humans, evidence supporting a protective effect of cellular immune responses in HIV infection is less direct. Long-term non progressive infection has been associated with both strong virus-specific CTL and with robust *gag* p24-specific CD4 cell proliferative responses. [4]Some studies have reported a direct inverse correlation between viral load and HIV-specific T-cell responses in untreated HIV-infected subjects. CD4 cell count is used to determine how well the immune system is working in people who have been diagnosed with HIV. The pattern of CD4 cell counts over time is more important than any single CD4 cell value because the values can change from day to day. The CD4 cell pattern over time shows the effect of the virus on the immune system. CD4 cell counts generally decrease as HIV progresses in untreated HIV-infected subjects. [5] Chamarajanagar is located in southernmost part of Karnataka. The population of Chamarajanagar is 10.20 Lakhs with a sex ratio of 989 females for every 1,000 males, and a female literacy rate of 54.32% with an overall literacy rate of 61.12% (Census 2011). According to 2012 PPTCT data, the level of HIV positivity was low (0.19%) among the PPTCT attendees, with a declining trend. [6]

We are on the verge of a significant breakthrough in the AIDS response living with and affected by HIV. The vision of a world with zero new HIV infections, zero discrimination, and zero AIDS-related deaths has captured the imagination of diverse partners, stake holders and people living with and affected by HIV. New HIV infections continue to fall and more people than ever are starting treatment. With research giving us solid evidence that antiretroviral therapy can prevent new HIV infections, it is encouraging that 6.6 million people are now receiving treatment in low- and middle-income countries: nearly half those eligible. [3]

Hence, the present study was undertaken to clearly understand about the effect of ART and the status of CD4 counts in study patients and among the genders. This gives the insight into the need and urgency of antiretroviral therapy to HIV/AIDS infected people.

Materials and Methods

This was a cross sectional study on secondary data maintained in the ART centre for the period of 6 months from March 2015 to October 2015. Permission was obtained from the district surgeon, district hospital Chamarajanagar, to collect the secondary data therefore there were no ethical issues. Subjects for the study were the reports of HIV patients registered in the centre during March 2015 to October 2015.

Methodology: Sample size: According to 2012 ICTC (Integrated Counseling and Testing Centre)data for Chamarajanagar district, the HIV prevalence was low among male (2.13%) and female (1.70%) attendees.

With level of significance 5% and absolute allowable error 5%using "estimation set up technique" the inflated sample size for male and female were 34 and 27 respectively and the total sample size was 61. However all the available patient reports (i.e.150) in the ART centre during the period of the study (6 months) were selected. Regimen of ART used was Zidovudine Lamivudine and nevirapine and was the same to all subjects in this study.

CD4 cell count was done using Flow Cytometry Absolute Cell Count System in the hospital ART centre laboratory and values were maintained in the hospital records. The results of the test were recorded in excel-sheet and analyzed using standard statistical tool called R software.

Result

CD4 count base line value and after 6 months on ART value follow normality assumption. (Figure1&2) The base line CD4 values for the general population group were minimum 11cells/cmm, maximum 297cells/cmm and the mean of 135cells/cmm. The minimum value corresponds to the female and maximum value corresponds to the male. The minimum and maximum values for female were lesser than those of male group. The mean value for base line CD4 count for general group lies between those of female and male groups, with mean female (126.88cells/cmm) was lesser than that of male group(i.e. 141 cells/cmm) indicating females were at higher risk of being Immunocompromised compare to males. Also that the standard deviation of female (5.4) was lesser to male (48.43) supporting the above statement. The standard deviation of female group indicates that the entire group behaves in the similar manner indicating ART was crucial for the group, in other

wards the female group should be prioritized to male in giving therapy. The standard deviation of female indicates that the sharper 95% confidence interval to female than that of male (Table 1&2)

The mean CD4 counts was in the relation 352.74cells/cmm (Male) < 378.82cells/cmm (general) < 410.26cells/cmm (Female) with the standard deviation relation 162.68 (male) < 179.49(general) < 194.46 (Female). The 6 months post ART therapy indicates that the risky female group was behaving well with wider variability compared to the male

group. On the whole, all the groups after ART had shown better CD4 counts. (Table 3) Thus there was a significant improvement in the CD4 counts in the patients after ART was initiated. (Figure 4& 5)

However when we compare the SD for base line and after 6 months groups , it was more in the after 6 months group than baseline group indicating the wide variation among the 150 patients and was supported by coefficient of variation also.

Table 1: Descriptive values for CD4 counts

CD4 cell count	Baseline Male	Baseline Female	Baseline Combined	After 6 months Male	After 6 months Female	After 6 months Combined
No of cases	82	68	150	82	68	150
Minimum	17.00	11.00	11.000	116.00	99.00	99.000
Maximum	297.00	200.00	297.000	876.00	1029.00	1029.000
Median			136.500			340.000
Mean	141.80	126.88	135.040	352.74	410.26	378.820
95% CI Upper	152.45	137.66	142.646	388.49	457.33	407.779
95% CI Lower	131.16	116.10	127.434	317.00	363.19	349.861
Std. Error	5.35	5.40	3.849	17.96	23.58	14.655
Standard Dev	48.43	44.53	47.140	162.68	194.46	179.489
C.V.			0.349			0.474
SW Statistic	0.97	0.97	0.971	0.94	0.93	0.937
SW P-Value	0.03	0.07	0.003	0.00	0.00	0.000

Table 2:Paired t test table for equality of CD4 counts

	Male	Female	Combined
Mean difference	-210.94	-283.38	-243.780
95.00% CI	-244.30 to -177.57	-328.83 to -237.93	-271.583 to -215.977
SD of difference	151.85	187.76	172.327
t	-12.58	-12.45	-17.326
df	81	67	149
p-value	0.00	0.00	0.000 (indicating the significance)
Bonferroni adj p-value	0.00	0.00	0.000

Table 3: Two-sample t-test on CD4 after 6 Months grouped by SEX against Alternative = Female 'greater than 'Male Pooled variance:

Difference in means	57.5208
95.00% confidence bound	9.2591
t	1.9728
df	148
p-value	0.0252
Bonferroni adj p-value	0.0252

The absolute value of mean difference CD4 counts satisfying the relation 210.94cells/cmm (male) < 243.78 cells /cmm < 283.38 cells/cmm (Female) indicating better increase after treatment.(Table 2) The p value of paired t test and Bonferron adjusted p value show the significance mean difference for all individual groups. (Table 2& Figure3)

Most of the patient values for CD4 counts are <200 cells/cmm after treatment ,it shows that more number of percentage of patients have better CD4 counts with minimum variability indicating patients are responding in a better way to treatment. (Figure 4 and 5)

The Equality of Variance test was passed. The t test for independent sample means was adopted with the alternative "Female is greater than the male CD4 counts" to know whether females responding well to the treatment. (F=1.43, DF=67, 81, p-value =0.12) The p-value indicates that the data do not provide sufficient information to reject the insignificance difference and hence the alternative hypothesis was accepted. (Table 3) That is Females were responding well to ART than males. (Figure 6)

Discussion

As per NACO guidelines, currently in India, absolute CD4 cell count is being used as the basis for initiation of ART.
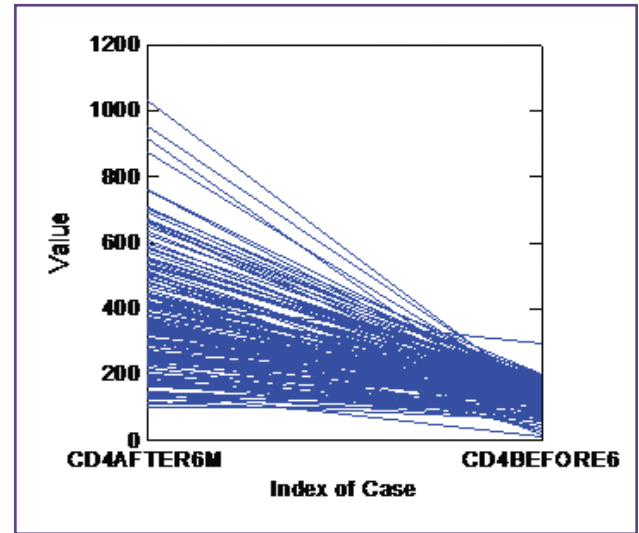

Fig. 1 : Probability plot showing normality for CD4 before treatment
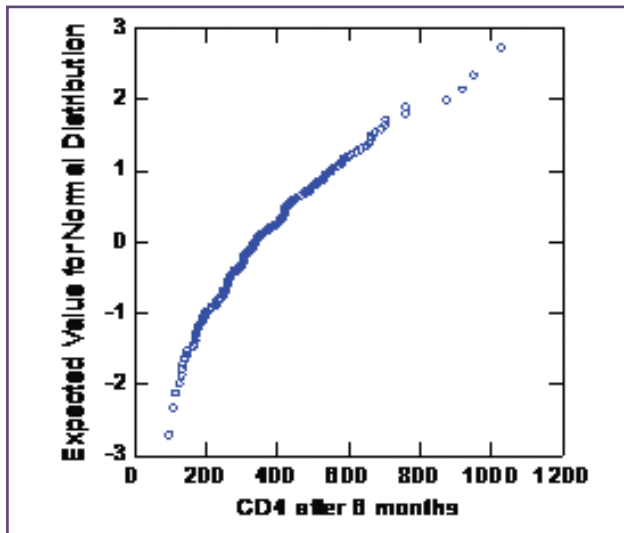
Fig. 2: Probability plot showing normality for CD4 after treatment

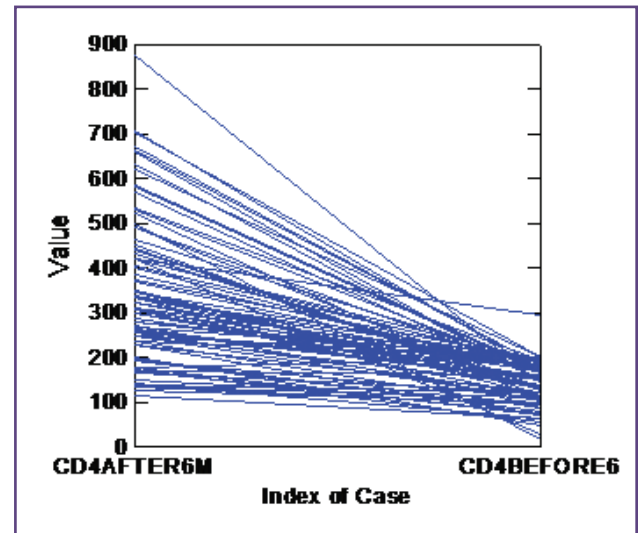**Fig. 3: indicates that there is an increase in the CD4 count after ART supporting the p-value**

Fig. 4: Indicates that there is an increase in the CD4 count after ART supporting the p-value in Males
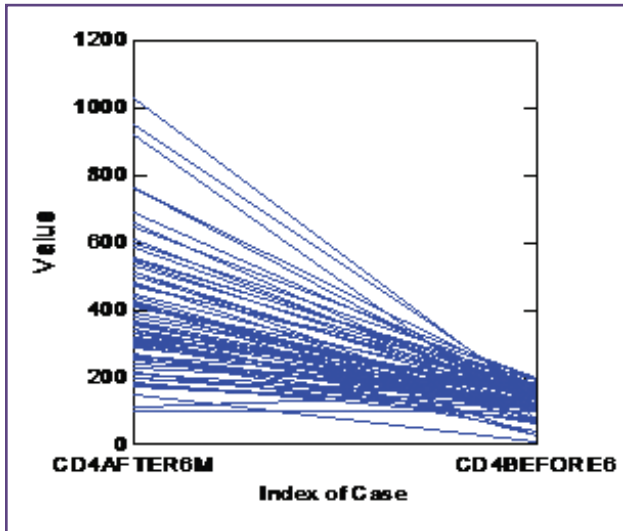
Fig. 5: Indicates that there is an increase in the CD4 count after ART supporting the p-value in Females

Fig. 6: Graph comparing Female and Male groups after ART

[7] In the present study, baseline mean CD4 cell count was 135 cells/cmm, with mean of 126.88cells/cmm in females and 141 cells/cmm in males, indicating females were at higher risk of being Immunocompromised compare to males. After 6 months of ART , the mean CD4 cell count increased to 378.82 cells /cmm, with 352.74cells/cmm in Males and 410.26 cells/cmm in Females. After 6 months of ART therapy the female patients showed an increase in the CD4 counts compared to the male patients with wider variability. These findings were similar to the findings in the previous studies with base line mean CD4 count being 155cells/cmm and154.2+/-47.3 cells/cmm respectively and the 6 months follow up counts of 297cells/cmm and 325.4 +/- 96.1 cells/cmm. [7&8] These findings suggest that the ART therapy was effective. Earlier studies reported that the gradual CD4 cell count rise are likely to reflect the generation of new cells by peripheral expansion of pre existing T-cell clones or generation of thymically derived naïve cells among ART patients . [9&10] There is a continuous increase of CD4 cell count among the HIV/ AIDS subjects who receive highly active antiretroviral therapy. [11] According to a previous study, lower percentages of CD4 T-lymphocytes are associated with adverse clinical outcomes among children and adolescents infected with human immunodeficiency virus (HIV). CD4 lymphocytes percentage generally increases with receipt of highly active antiretroviral therapy, but long term follow-up is required to assess whether these increases in CD4 cell percentages are maintained and whether they lead to normal CD4 cell percentages in children and adolescents with severe immunosuppression.[12] This finding indicated that the treatment was effective. It was reported that, the initial
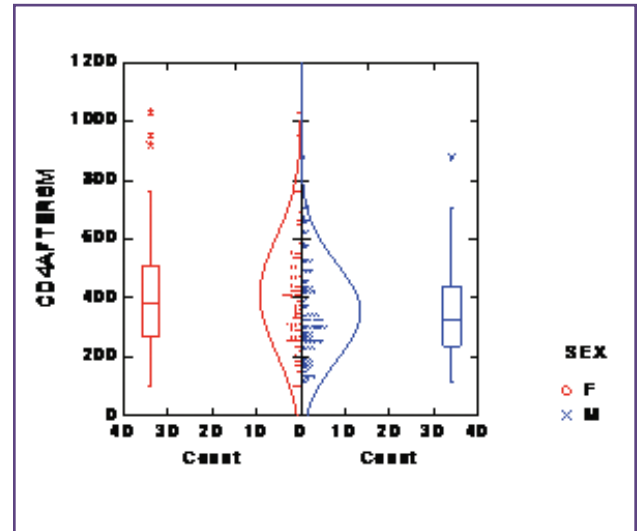
increases in CD4 cell percentage observed in the first year after ART initiation are sustained for at least 5 years after ART initiation among children and adolescents infected with HIV and that greater increases occur among those with the greatest degree of immunosuppression. [12]

The CD4 cell count decreased due to the disruption of the cell membrane as HIV buds from the surface or the intracellular accumulation of heterodisperse RNAs and unintegrated DNA.[7] Intracellular complexing of CD4 cell and viral envelope products can result in cell killing. [8]Similarly, other investigators proposed that the untimely induction of a programmed cell death as an additional mechanism for CD4 cell loss in HIV infection. [13]

Conclusion

HIV patients should be educated about the importance of continous intake of antiretroviral therapy which will prolong their survival and decrease the viral load and transmission of the disease hence, it can be concluded that ART is effective enough in slowing the progression of HIV infection to AIDS and increasing the survival rate of patients with good performance. Special reference is to be given to female patients since their CD4 counts are much lower compared to the males and they respond well compared to the male population

Competing Interests

None declared

Reference

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