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Original Research Article

Enumeration of CD4 + T lymphocytes Count among Healthy HIV Seronegative Individuals & People Living with HIV in Manipur

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Abstract

Introduction: The CD4+ T lymphocytes are the primary target cells for Human Immunodeficiency Virus (HIV). The enumeration of CD4+ T cells is the most important marker for HIV disease progression. The estimation of CD4+ T cell counts is used to decide the initiation of anti retroviral therapy (ART), to monitor the efficacy and to start treatment for opportunistic infections (OIs). Hence it is important to establish the reference ranges for the CD4+ T cell counts in the target population to understand the immune dysfunction.

Objectives: To determine the reference range of CD4 + T cell counts in healthy HIV seronegative individuals and its comparison with those people living with HIV in Manipur.

Materials and Methods: The study was carried out between August 2013 to June 2015 in the Dept. Of Microbiology, RIMS, Imphal, Manipur. Blood samples from 334 HIV seronegative volunteers comprising group I (188 males and 146 females) and 1058 HIV-positive patients comprising group II (525 males and 533 females) in the age group of 17-60 years, were analyzed for enumeration of CD4+ T cell counts by fluorescent activated cell sorter (FACS) counter (Becton Dickinson).

Results: In group I mean CD4 levels were 741 ± 180.349 cells/µl. In normal healthy males CD4 levels were 656.99 ± 122.73 cells/µl and 849.36 ± 185.190 cells/µl among healthy females. In group II mean CD4 levels were 341.43 ± 301.885 cells/µl. Among the HIV positive males CD4 levels were 300.35 ± 200.634 cells/µl while among the females CD4 levels were 381.89 ± 371.638 cells/µl.

Conclusion: As there is heterogeneity in baseline CD4 + T cell counts, our findings on T-cell subset reference ranges of normal healthy seronegative people in Manipur validate the utility of determination of CD4 cell count.

Keywords: CD4, HIV, Reference range, T lymphocytes

1. Introduction

CD4+ T helper lymphocytes play a central role in regulation of immune response.[1] They have capacity to help B cells for generating antibodies, to recruit and activate macrophages, to recruit neutrophils, eosinophils, and basophils to sites of infection and inflammation.[2] As the CD4+ T lymphocytes are the main targets of Human Immunodeficiency Virus (HIV), CD4+ T lymphocyte counts are recognized as the most important measurement of overall HIV-induced immune impairment.[3] The enumeration of CD4+ T-lymphocytes in HIV infected individuals is an essential tool for staging HIV disease, to make decisions for initiation of anti-retroviral therapy (ART), for monitoring response to ART and to initiate chemoprophylaxis against opportunistic infections.[4-6] Besides HIV disease, the clinical applications of CD4+ T lymphocytes include diagnosis of primary and secondary immunodeficiency disorders, evaluation of immune-mediated diseases and the assessment of immune reconstitution following stem cell transplantation.[7-9]

Globally 36.9 million (34.3 million–41.4 million) people are living with HIV, 2 million (1.9 million–2.2 million) people became newly infected and 1.2 million (9,80,000–1.6 million) people died from AIDS-related illness by the end of 2014.[10] In India the prevalence of HIV is 0.27%, total adult population living with HIV/AIDS (PLHA) is estimated to be 20,88,638 with 1,16459 new infections and 1,47,729 deaths due to AIDS related diseases[10]. Manipur a small north-eastern state of India with hardly 0.2% of India's population has prevalence of 1.22%. [11]

The information on the reference ranges of CD4+ T cell counts in a population is required for the application in the clinical settings such as various immune deficiencies. Reference values help in proper assessment of the degree of immunodeficiency in various conditions including HIV infection.[12,13]

Flow cytometry is an accepted standard method for determination of absolute count of CD4+, CD3+ and CD8+ T- lymphocytes.[14] Absolute T-lymphocyte subset counts are preferred over percentages by both clinicians and laboratory personnel [15] as the percentages are relative values and involve the use of multiplatform methods, which are prone to errors and analytical bias, while single platform methods have the potential to yield a less variable analysis.[16] The correct interpretation of the results of these tests depends on the precision and reproducibility of the method used and the availability of reference ranges of these counts in healthy individuals in a community. [17]

Studies have been carried out worldwide to establish reference ranges for CD4+ T cell counts. Variations in the reference ranges for CD4+ T cell counts have been observed in different populations. The CD4+ T cell counts are known to be influenced by race and environmental factors. Hence it is important to establish the reference ranges for the CD4+ T cell counts in the target population to understand the immune dysfunction.[18]

There is need to study the reference range of CD4+T cell counts and estimate the immune progression of disease in Manipur as it is a high HIV prevalent state in India. Therefore the present cross-sectional study was carried out to determine the reference ranges of CD4+ T cell counts in normal HIV seronegative healthy individuals and to compare these values with those in HIV positive, ART naïve individuals in Imphal, Manipur.

2. Materials & Methods

The present study was carried out in the Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Imphal, from August 2013 to June 2015.

Integrated Counselling and Testing centre (ICTC) for HIV and centre for the estimation of CD4 + T lymphocyte (FACS count centre) and National Reference Laboratory (NRL) are located in the Department of Microbiology, Imphal. HIV testing and CD4 estimation are carried out in these centres.

2.1 Selection of subjects:

Healthy volunteers (Group I) - 334 normal healthy individuals (188 males and 146 females) were included in this study. The exclusion criteria include a) Chronic illness, b) Vaccination in the past six months, c) Pregnant women, d) Seropositive for HIV and HBsAg. e) Consumed alcohol in last one week. f) Chronic smokers, g) Major surgery in past six months.

The selected individuals were resident doctors, nurses, laboratory staffs and volunteers in the age group of 17-60 yrs. HIV seropositive (ART naïve) (Group-II) - 1058 (525 males and 533 females) cases/clients attending STD Clinic/ICTC or those patients referred to this centre for HIV testing from different OPDs/Wards /ART centre of RIMS Hospital were included in this study.

2.2 Pre- and post- test counselling:

Necessary pre test counselling, informed consent was taken from the participants before HIV testing. Post test counselling was also given after knowing the result of the test.

2.3 Collection of blood:

Blood (5 ml) was collected from all the participants of study aseptically using sterile, disposable needle and syringe. 3 ml of the blood was transferred to a sterile vial for HIV serology and 2 ml to K-3 EDTA vacutainer (Becton Dickinson, Mountain View, CA) for CD4+ T cell count.

2.4 HIV testing:

Sera were separated on the same day and tested for HIV antibodies on the same day of collection. Strategies of HIV testing by Elisa /Rapid(ER) test were followed as recommended by NACO, Government of India. [19]

The different kits used for the tests were i) HIV 1+2 Immunodot test combaids-RS (Span Diagnostic, Surat, India), ii) Pareekshak HIV 1+2 Triline card test (Bhat Bio-Tech India Pvt. Ltd, Bangalore, Karnataka, India) and iii)SD Bioline HIV 1+2 Immunochromatography (Bio standard diagnostics Pvt. Ltd, Gurgaon, Haryana, India).

2.5 CD4 testing:

The blood samples were processed immediately within 2 hrs of collection, for determination the CD4+ T cell counts by fluorescence activated cell sorting (FACS) count system (Becton Dickinson Immunocytometry system, San Jose, CA 95131 - 1807).

The FACS count instrument is a compact cell counter with a built-in computer. When whole blood is added to the reagent, fluorochrome labelled antibodies in the reagent bind specifically to lymphocyte surface antigen. After a fixative solution is added to the reagent tubes, the sample is run in the instrument. The cell comes in contact with the laser beam, which causes the fluorochrome labelled cells to fluoresce. The fluorescent light provides the information necessary for the instrument to count the cells. The software identifies T lymphocyte subpopulations and correlates with the absolute count. Results provide absolute counts of CD4+, CD8+, CD3+ and CD4/CD8 ratio.

2.6 Quality control:

All tests were performed according to the manufacturer's recommendations. Manufacturer's guidelines were strictly adhered to with regard to biosafety practices, troubleshooting, and maintenance of equipment. The laboratory also participates in the external quality assurance

scheme (EQAS) for HIV testing and CD4 testing conducted by National AIDS Research Institute (NARI), Pune.

2.7 Statistical analysis:

The values of mean, median and standard deviation of CD4+ T-lymphocytes were calculated using SPSS 16 and Epi-Info22.

2.8 Ethical issues

Ethical approval was obtained from the Institutional Ethics Committee, RIMS, Imphal.

3. Results

The predominant age groups in Groups I and II were 31-40 years. In Group I, males consisted of 188(56.2%) while females consisted of 146(43.7%). In group II, males were 525(49.6%) while females were 533(50.3%) (Table 1).

In healthy males CD4 counts were 656.99±122.73 cells/µl and in healthy females it was 849.36 ± 185.190. Among the healthy males, maximum mean CD4 count i.e. 668.90±82.458 was in the age group 41-50 years. Among the healthy females, maximum mean CD4 count i.e. 910.28±214.776 was in the age group 41-50 yr (Table 2)

In HIV infected males, the maximum CD4 count i.e. 354.9±153.419 was seen in the age group of cases with less than 20 yrs. The CD4 count was seen to be decreasing with increasing age. In HIV infected females the maximum CD4 count was seen in the age group of 31-40 yrs i.e. 410.32±518.319. The females are found to have a higher CD4 count compared to the males. (Table 3).

On comparing the CD4 count in normal healthy individuals and in individuals infected with HIV, the mean CD4 count of normal healthy individuals was 741±180.349 while the mean CD4 count for the HIV infected individuals was 341.43±301.885. Hence, the mean CD4 count of the normal healthy individuals was significantly higher than the mean CD4 count of the HIV infected cases with p<0.001.(Table 4)

Table 1: Age wise distribution of study groups

Age group (yr)	Study groups					
	Group I (Normal healthy individuals)			Group II (HIV infected individuals)		
	Male	Female	Total	Male	Female	Total
<20	2	4	6(1.7%)	29	38	67(6.3%)
21-30	46	48	94(28.1%)	86	152	238(22.4%)
31-40	103	72	175(52.3%)	212	209	421(39.7%)
41-50	29	18	47(14%)	151	104	255(24.1%)
>50	8	4	12(3.6%)	47	30	77(7.2%)
Grand total	188(56.2%)	146(43.7%)	334	525(49.6%)	533(50.3%)	1058

Table 2: Absolute CD4 count of normal healthy individuals (Group I)

	Male		Female	
Age group (yr)	Number (n)	Mean±SD (cells/µl)	Number (n)	Mean±SD (cells/µl)
<20	2	661.50±17.678	4	767.75±142.320
21-30	46	642.04±95.782	48	792.02±157.216
31-40	103	662.50±140.371	72	877.85±190.970
41-50	29	668.90±82.458	18	910.28±214.776
>50	8	627.75±160.076	4	832.25±148.291
Total	188	656.99±122.739	146	849.36±185.190

SD=Standard deviation.

Table 3: Absolute CD4 count of HIV infected individuals (Group II)

	Male		Female	
Age group (yr)	Number (n)	Mean±SD (cells/µl)	Number (n)	Mean±SD (cells/µl)
<20	29	354.9±153.419	38	342.92±141.518
21-30	86	328.60±199.193	152	384.50±213.958
31-40	212	326.85±213.432	209	410.32±518.319
41-50	151	256.98±189.097	104	346.46±280.175
>50	47	234.77±167.859	30	342.90±223.820
Total	525	300.35±200.634	533	381.89±371.638

Table 4: Comparison of CD4 count between normal healthy individuals and HIV infected cases

Subset	Groups	Number	Mean	Standard deviation	*p value*
CD4	Normal healthy individuals (Group I)	334	741.08	±180.349	<0.001
	HIV infected cases (Group II)	1058	341.43	±301.885	

p<0.001

4. Discussion

The baseline CD4 counts/µl in healthy Indians has been reported from various parts of the country. The CD4+ T lymphocyte count has been shown to be influenced by sex, age, race, time of specimen collection (diurnal rhythms), physical and psychological stress, pregnancy, drug administration (zidovudine, cephalosporin, cancer chemotherapy, nicotine and steroids), tuberculosis, viral infections, presence of antilymphocyte auto antibodies and procedures like spleenectomy.[20,21] Other factors that cause variations in the CD4 counts were type of instrument used, processing and analyzing the whole-blood samples, integrity of the blood samples, staining reagents and fluorochromes, equipment calibration, preference and gating strategies used for the analysis of the results.[22,23]

Studies carried out by various workers in India reported the occurrence of mean CD4 count as follows: Das BR *et al*[24] reported mean CD4 absolute count of 771 cells/µL in the study from western India; Ray K *et al*[25] reported mean CD4 count of 703 cells/µL in the study from north India; Ramalingam S *et al*[26] reported mean CD4 count of 799 cells/µL in the study from south India; Singh YGK *et al*[27] reported mean CD4 count of 848±395 cells/µL in a previous study carried out in Manipur; Kannagai R *et al*[28] reported mean CD4 absolute count of 1048 cells/µL in south India, Murugravel KG *et al*[29] reported the CD4 count of 926 cells/µL in south India; Uppal SS *et al*[30] reported mean CD4 count of 865 cells/µL in west India.

Hence some of the studies carried out in India showed figures of CD4 count almost similar to the present study while the figures of CD4 count by other workers are higher than the present study. Hence, it is evident that a wide variation in mean CD4 count has been found after assessing the figures of the various workers.

Studies carried out among healthy individuals in various parts of the world reported the occurrence of mean CD4 count as follows: Bofill M *et al*[31] reported mean CD4 of 830±290 cells/µL for Bristons; Vithayasai V *et al*[32] reported mean CD4 count 910±310 cells/µL for Thailand; Kalinkerich A *et al*[33] reported 863.9±234.8 cells/µL for the Israelis; Jiang W *et al*[34] reported 727 cells/µL in China ; Bussmann H *et al*[35] reported 759 cells/ µL in Botswana; Janwossy *et al*[36] reported 775 cells/ µL in Ethiopia. Hence, the findings of these workers show huge variation as the figures of some of the workers are almost similar with the present study while the findings of some of the workers give value higher than that of the present study. Hence there is huge variation in the mean absolute CD4 count as documented from various parts of the world.

Though, the base line CD4 cell count in healthy females in the present study was higher than that of males, the difference was statistically insignificant. In our study the CD4 range in normal healthy individuals was from 252cells/µl to 1449 cells/µl. The data therefore, showed that the lower cut-off is much below the clinically significant level of 500 cells/µl. Various published studies reported CD4 reference range from 250-1510 cells/µl. Our results are in agreement with these findings.

The levels of CD4+ cells were significantly low and ratio inverted in HIV positives in comparison to healthy volunteers. Taylor JMG *et al* [37] have reported earlier that each person has a unique level of CD4 cell number that is reflective of his/her immunocompetence to protect himself/herself against the development of clinical symptoms.

The distribution of mean absolute CD4 count among different age groups in our study revealed that both males and females in the age group of 41 to 50 years had significantly higher CD4 count, 668.90±82.458 cells/µl and 910.28±214.776 cells/µl respectively.

5. Conclusion

Our study emphasize that there is heterogeneity in the data of CD4 count in this region. Similar findings are also reported by various workers. However, it is important to know the base line data in normal healthy individuals in regions with high HIV prevalence. It will facilitate the interpretation of results in HIV infected and other immunodeficient patients. These reference ranges can be used to guide clinical decision.

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