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Santha Muller, SF J AIDS HIV Res, 2017, 1:1

SciFed Journal of AIDS & HIV Research

Research Article Open Access

Determination of Normal Reference Values for the CD Markers Amongst the Healthy Fijian Population

*[1]Santha Muller, [1]Atlesh Nand Sudhakar, [2]Mike Kama

*[1]Medical Research Laboratory,Fiji National University, Fiji

[2]Fiji Centre for Communicable Diseases Control, Fiji

Abstract

CD4+ T helper cells are part of lymphocytic cells that play an essential role in the human immune system. Their main function is to send signals to other types of immune cells, including CD8 killer cells, which then destroy the infectious cells. Immunophenotyping of these lymphocytes especially the CD4 T-cells has a significant clinical relevance for the diagnosis and management of many disease processes. A case control cohort study is carried out at Medical Research Laboratory (MRL) with the aim of enumerating the CD markers such as CD3+, CD4+, and CD8+ cells, expressed on the lymphocyte. The samples are processed using Accuri C6 Flow Cytometer based at MRL through the use of lyse-no wash procedure. From the total number of samples processed, it was found out that the HIV positive cases had lower mean CD4+ count and also lower count of other parameters in the lymphocyte populations such as CD45, and CD4:CD8 ratio when compared to the negative control samples. However, out of the 30 control samples analyzed, a wide range of CD4+ cell counts has been found (369-1309 cells/uL) with a mean and median of 752 and 742 cells/uL respectively. From this, 13 negative controls had the mean CD4+ count between 300-649 cell/uL, while only 5 negative control samples had the mean CD4+ count between 1000-1350 cells/uL. Comparison of mean CD4+ T-cell count between males and females in negative controls showed considerable lower CD4 T-cell count in females (742 ± 243 cells/uL) as compared to males (770 ± 222 cells/uL). The data also showed that the mean CD8+ count in negative controls was 450 ± 187 cells/uL. Although CD4+ T-cell count amongst the negative controls falls between the normal ranges, they are generally regarded as values within the lower normal limits. The findings of the current study also highlight the varied differences in CD4 T-cell count ranges amongst the negative control samples. Such differences could be due to ethnic and genetic differences in the lymphocyte subsets amongst the Fijian population. However, no such study has been carried out in the Fijian population to determine the reference range for the CD4+ and CD8+ T-cell counts. Therefore, a study needs to be conducted in different geographic locations, different ethnicities and gender in the Fijian population in order to determine the normal reference range for the lymphocyte subsets.

Key Words

CD4+; CD8+ Cells; Lymphocytes; Immunophenotyping

Introduction

Research information on the study of CD4 T-lymphocytes and its subset is generally lacking in the Fijian population. The enumeration of circulating CD4 lymphocyte subset is not only important for evaluating disease stage and progression in individuals with the Human Immunodeficiency Virus (HIV) but its measurement is also important for understanding the immune status amongst the healthy individuals in the Fijian Population. It is also of great interest to know how CD4 count differs in healthy population compared to other populations studied across the world, and whether such differences need to be taken into account while interpreting data with regard to the immune status of such individuals in the Fijian setting.

*Corresponding author: Santha Muller, Medical Research Laboratory, Fiji National University, Fiji. E-mail: Santha.Muller@fnu.ac.fj

Received Mar 23, 2017; Accepted June 2, 2017; Published June 15, 2017

Citation: Santha Muller (2017) Determination of normal reference values for the CD markers amongst the healthy Fijian population. SF J AIDS HIV Res 1:1.

Several studies have evaluated CD4 lymphocyte subset in Western nations[1-3]and other countries such as Singapore[4], Thailand[5], Malaysia[6-7], China[8], India[9], Thailand[10], Turkey[11] and Uganda[12]. Except for CD4 monitoring for HIV positive cases using Alere PIMA[13] machine based at Fiji Centre for Communicable Disease Control (Mataika House), data on normal ranges on CD4 T-cell subset amongst the healthy controls in Fijian population are generally not available. Hence, this study was conducted to establish and compare the mean absolute values of CD4 and CD8, CD45 T-lymphocyte subsets and the ratio of CD4 to CD8 cells amongst the healthy adults (controls) and HIV positive (Cases) in the Fijian population.

Materials and Methods

A prospective case control cohort study was conducted in thirty healthy adults (controls) and Forty Seven HIV positive (cases) attending to Reproductive Health Clinic (RHC-Suva). They participated in the study after giving voluntary informed consent. All the healthy participants (controls) were carefully screened for the absence of any HIV or any other Sexually Transmitted Infections (STI's) such as negative for hepatitis B surface antigen, syphilis and other venereal diseases.

Blood specimens were collected by venepuncture and in Ethylenediammine tetra-acetic acid (EDTA) anticoagulated tube and transported to the Medical Research Laboratory (MRL) for processing and analysis. Anticoagulated blood samples were tested within 5-6 hours of collection.

For each sample, one test tube was labeled for the respective monoclonal antibody. One hundred microliters of anticoagulated blood was pipetted into the bottom of each properly labeled test tube, thus ensuring that the inside surface and top of the tube were free of blood. Next, 20uL PE CD3, FITC CD4, APC CD45 and 5 uL of PE-Cy7 CD8 were added to each set of the sample in the tube, mixed well and incubated for 30 minutes in dark at room temperature. Once the time had elapsed, 2mililitres of haemolysing reagent (red blood cell lysing buffer) was added to the respective tubes containing the blood sample and the antibodies. The tubes were again incubated in dark for 15 minutes to allow the red cell to hemolyse.

Flow Cytometry Immunophenotyping

CD3PE, CD4 FITC, CD8PECy7 and CD45APC counts were performed by no wash lysing procedure [14]. The Processed samples were acquired on the same day using 4 colored Accuri C6 flow cytometry method to determine the accurate absolute cell counts. Samples giving discrepant results were repeated in duplicate using the same method. All reagents, hardware and software were purchased from BD Biosciences, Australia and used according to manufacturer's instructions. Data was analyzed using CFlow Plus software which comes with the Accuri C6 system.

The protocol has been standardized based on the validation and optimization of the sample processing, setting up of the Accuri C6 and acquisition at the Medical Research Laboratory, Fiji National University.
The absolute values of CD4 and CD8 and CD45 cells were calculated based on the particular T-cell subset obtained by Flow Cytometry.

Statistical Analysis

The percentage of CD4 T-lymphocyte was determined automatically using the C Flow plus software. From this percentage value, the absolute count of CD4+T lymphocytes was calculated by using a factor 21.65 multiplied by the total count of lymphocytes. Microsoft Office Excel was used to compile the date to calculate the mean value of the absolute count of 30 control samples. The mean with the standard deviation was calculated for the normal distribution of the CD4 count. The line graph analysis was done for the individual data and the histogram was performed for the range of 100cell /uL versus the percentage of CD4+ T lymphocytes.

Result

From a total of seventy-seven adult's blood samples analyzed, 30 samples of healthy controls were included in this study. Among the 30 controls, 10 (33%) were male and 20 (67%) were female. The means, and the standard deviations, for the $CD3^{+/}CD4^{+}$ T helper cells between HIV positive cases and controls and gender differences (male and female) are shown in Table 1. The table shows that the mean absolute CD4+ T-lymphocyte count for the 30 healthy controls was 752± 233. The mean value for male and female controls depicts 770 ± 222 and 742 ± 237 cells/uL respectively (Table 1). No significant gender difference was observed in the mean CD4 T-lymphocyte

count amongst male and female control samples that were analyzed, showing a p value of 0.6.

Comparison of our data for the mean CD4 T-lymphocyte was performed with a review of the published data from different countries (Table 2). The observed difference in the CD4 T-Lymphocyte count from different countries can be taken as reference values and this might be due to the heterogeneity of the population as a result of ethno-racial variations**.**

Analysis of the CD4 T-lymphocyte for individual control sample amongst the healthy individuals showed that majority (21/30) had the absolute CD4 count of less than 1000 cells/uL when considering the universal reference range for the CD4 T-lymphocytes which is between 500-1500 cells/uL. 2/30 samples shows CD4 count less than 500cells/uL. (Figure 1). The red line (500cells/uL) shows the expected minimum cutoff and the green line shows the expected average (1000cells/uL) for the normal healthy controls. Percentage distribution of the individuals CD4 T-Lymphocyte count showed 71% of the individuals had the CD4+ count below 1000 cells/uL while some had the values below 500 cells/uL (Figure 2).

Table 1: Mean CD4 Count Between Cases and Controls and Comparison of Mean CD4 Count Between Males and Females in Controls and Cases

TESTS	CASES (n=47)	CONTROLS (n=30)
CD3+/CD4+	292± 140	752± 233
CD4+ MALES	295 ± 117 (n=24)	770 ± 222 (n=10)
CD4+ FEMALES	317 ± 171 (n=23)	742 ± 237 (n=20)

Table 2: Mean CD4 T –Lymphocyte Immunophenotyping Data from Published Studies

Country	Mean CD4 Count(cells/uL)
Tanzania	746
Botswana	759
China	727
Iran	749
Ethiopia	753
United Kingdom	830
India	860
Thailand	910

Figure 1: Absolute CD3+/CD4+ Count for Individual Control Samples Amongst the Fijian Population Compared to HIV Positive Patients (Cases)

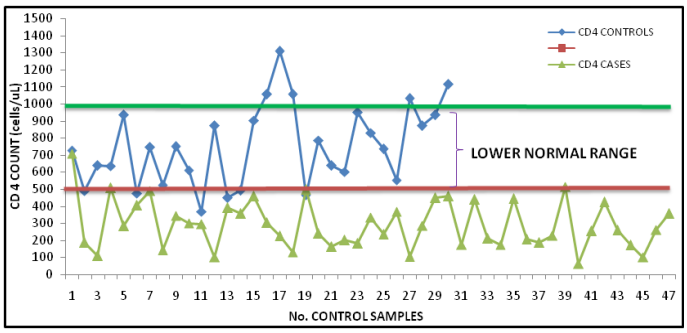
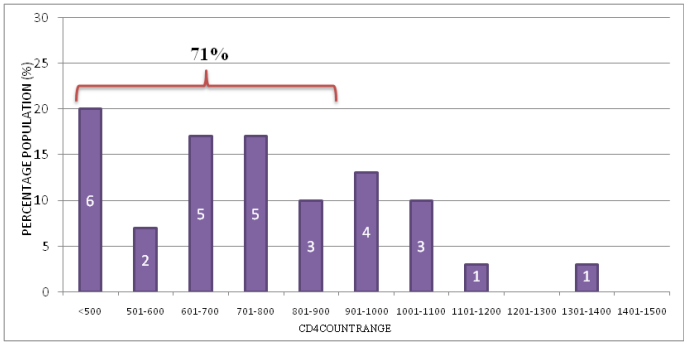


Figure 2: Percentage Distribution of CD3+/CD4+ Count Amongst the Healthy Control Samples from the Fijian Population



Discussion

This study provides the first estimates of CD4+ T-lymphocyte counts among healthy individuals in the Fijian population. A wide variation in mean CD4+T-lymphocyte count has been reported from various parts of the world. The absolute CD4+ count in 21 (71%) healthy controls were <1000 cells/uL. Whereas the absolute CD4 count in 6 (20%) of the healthy controls in the present study were even <500 cells/µL. This is higher than the value of 10.6% reported in a study in normal south Indian healthy individuals[15]. Although CD4+ T-cell count amongst the healthy controls falls between the normal ranges, they are generally regarded as values within the **lower normal limits**. However, it was noted that 5 controls had the absolute CD4+ count of above 1000 cells/uL (figure 3). These 5 individuals were found to be involved in regular physical activity and yoga exercises.

The mean CD4 count reported in the present study amongst the healthy controls were significantly lower than those documented in the Turkish[11] and Ugandan[12] population and paralleled those of the Chinese[8]

population. The observed difference in the CD4 reference values might be due to the heterogeneity of the population as a result of ethno-racial variations within populations also due to the differences in the methodologies where the variations were not controlled[16] .

Since Fiji is a heterogeneous country and the current study set up only involved the participants from the central division of the Fijian population, it is essential that a similar number of participants from all four geographical divisions be included in the future studies. This must also ensure that larger population size is taken in order to determine the national reference range for CD4 T-lymphocyte. Hence, this metacentric study covering the entire country will gave an opportunity to assess the differences in CD4+ T-cell counts in different populations amongst Fijians.

Acknowledgement

The authors thanks all the precious HIV positive patients and healthy volunteers as the study participants for this research, staff and Medical Officer at the reproductive Health clinic, the Ministry of Health and Medical services, Fiji. The authors also thank the Fiji National University, URPC for funding ACT 275 to conduct this study.

Abbreviations

AIDS- Acquired Immune Deficiency Syndrome

APC-AlloPhycoCynine

ART-Anti-Retroviral Therapy

PE- Phyho Erythrin

CD-Cluster of Differentiation

FITC- Fluorescein IsoThyoCynine

CD3/CD4/CD8+ - Positive cells

PECy7- Phyco Erythrin Cynine7

MRL-Medical Research Laboratory

EDTA- Ethylene Diamine Tetra-ace-tic Acid

CMNHS-College of Medicine, Nursing and Health Sciences

HIV-Human Immunodeficiency Virus

URPC- University Research and Publication Committee.

UNAIDS- United Nations programme on HIV/AIDS.

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