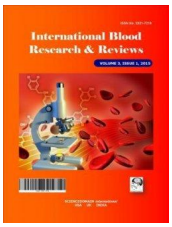

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Normal CD4 Count in Healthy Voluntary Blood Donors in the Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TT, TC, KW and T. Yangdon conceived and designed the study and wrote the study protocol. Authors TC, KW, T. Yangdon, TL, PK, JW, BR and T. Yangzom recruited the participants and performed laboratory analysis. Author TT performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed to estimate the normal CD4 counts in healthy Bhutanese blood donors.
Study Design: A prospective descriptive study.
Place and Duration of Study: This study was carried out in the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH), Thimphu, between July 2015 to April 2016.
Methodology: We recruited healthy blood donors in the JDWNRH collecting demographic characters and blood samples from consenting donors. Blood samples were analyzed using the BD FACS count system.
Results: A total of 413 healthy blood donors, 288 (69.7%) males with a mean age of 27.3 years

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(18-62 years) were enrolled. Ethnically, 351 (85.0%) were northern and 62 (15.0%) were southern Bhutanese. The mean CD4 count was 668.3 cells/µl (range 259 -1591 cells/µl) and the median was 663 cells/µl. Females had significantly higher counts (p=0.004) and CD4 counts also significantly increased with increasing age but these differences were numerically small. Ethnicity did not produce significant differences in the CD4 count. In about 21% of the participants, counts were below the reference ranges and published data for Indians and Caucasians but comparable to other Asian, Middle East and African population.

Conclusion: Upon validation with a larger study, a somewhat different CD4 cutoff may be required for the Bhutanese population. However, within the Bhutanese population, a single reference count may be advocated for adults disregarding the negligible numerical differences between age groups and gender.

Keywords: Bhutan; CD4 count; healthy blood donors; Thimphu.

1. INTRODUCTION

A cluster of differentiation 4 (CD4) is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages and dendritic cells. CD4+ T helper cells are white blood cells that circulate throughout the body to find and destroy germs invading the human body. In addition, CD4 T cells also have powerful antitumor effects even greater than CD8 T cells, in a mouse model [1]. In conditions such as untreated HIV infection or following immune suppression prior to an organ transplant, the CD4 cells become depleted and the body becomes vulnerable to a wide range of infections. The CD4 count measures how many CD4 cells a person has in his blood. The absolute CD4 count for adults for most laboratories was known to fall in a range of 800 to 1050 cells/µl and upon considering laboratory variations of two standard deviations, the normal CD4 count ranged within 500 to 1400 cells/µl [2]. This count is very much dependent on the total white blood cell count, the percentage of lymphocytes, and the percentage of lymphocytes that bear the CD4 receptors [3]. In HIV patients, CD4 count decreases over time and at counts below 200cells/µl, patients become susceptible to a wide variety of opportunistic infections. In addition to immunodeficiency, factors like medications, smoking, alcohol consumption, chronic medical conditions and pregnancy are known to affect CD4 count [3].

Normal CD4 count for Caucasian populations was reported to be 844+/-247 cells/µl [4]. Shanghai adults had a mean CD4 count of 727cells/µl which was approximately 100 fewer cells less than Caucasians with no significant differences between age and gender [5]. In healthy blood donors in India, the normal CD4 count was 865 cells/µl (95% CI, 430–1740 cells/µl), with significantly higher counts in females [6]. The CD4 counts among Indian adults were not different to the Caucasian population and differences between age, ethnicity, smoking, alcohol consumption, and the interval between drawing the blood sample was not significant. Another Asian study found lower CD4 counts in Asian males compared to Asian females and Caucasian males [7]. The importance of CD4 count in antiretroviral therapy (ART) has changed over the years as recommended by the WHO ART guidelines of 2002 [8] and 2013 [9]. Recently, with the understanding that all people living with HIV can benefit from HIV treatment and with its “treat-all” policy, the WHO 2016 guideline has now removed all limitations on eligibility for ART among people living with HIV making everyone with HIV eligible for ART [10]. However, CD4 count measurement can be useful in HIV patients to monitor response to treatment and progress of disease as well as in other non-HIV related clinical situations.

Bhutan has an estimated population of 770,000 in 2016 [11]. Ethnicity in Bhutan is comprised mainly of three groups; the Ngalops of western and northern Bhutan, the Sharchops of eastern Bhutan and the Lhotshampas of southern Bhutan [12]. While it is practically difficult to distinctly separate the Ngalops and Sharchops, the Lhotsampas are relatively unique owing to their mostly Hindu culture although ethnicity is becoming increasingly diluted due to much inter-marriages. Therefore, practically, the two most prominent ethnic groups, relevant to this study, are the largely Buddhist northern Bhutanese and the largely Hindu southern Bhutanese. No work has been done on assessing the normal CD4 count in the Bhutanese population. This study, therefore, assessed the normal CD4 counts amongst healthy voluntary blood donors in the national referral hospital in Bhutan’s capital city.

2. MATERIALS AND METHODS

2.1 Research Design and Study Site

A prospective descriptive study was carried out in the microbiology and blood bank units of the Department of Laboratory Medicine, Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) in Bhutan's capital city, Thimphu. The JDWNRH is the apex hospital in Bhutan providing health services to the population in the capital city and serves as the referral centre for all the district hospitals in Bhutan.

2.2 Participants and Sample Size

Participants included healthy voluntary blood donors visiting the blood bank unit of the JDWNRH. All blood donors visiting the unit were offered the option to participate in the study and willing people were enrolled. Considering a 95% confidence interval, a standard normal variate of 1.96, an expected proportion of the population with normal CD4 count at 0.5 (no previous data), an absolute error of 0.05 and an expected participation rate of 95%, the sample size for the study was calculated at 413 donors and these were recruited over 10 months from July 2015 to April 2016. Blood samples were tested in the microbiology unit.

2.3 Test Principles

When monoclonal antibodies are added to human whole blood, the fluorochrome-labelled antibodies bind specifically to the antigens on the surface of nucleated blood cells (such as leucocytes). These antibodies may be used to identify lymphocyte subpopulations. When an aliquot of the stained human blood sample is introduced into the flow cytometer and passed through a narrow stream along the path of a laser beam, they fluoresce on being excited by the laser beam and the emitted light is collected and processed by the flow cytometer. In addition, the reagent tubes contain a known number of fluorescent reference beads to which a precise volume of whole blood is added. The software automatically identifies the lymphocyte populations of interest and calculates the CD4 counts (cells/µl) by comparing cellular events to bead events.

2.4 Test Procedure

Whole blood samples were collected in EDTA tubes during the blood donation process. Samples were stored no longer than 48 hours at room temperature (20-25°C) before testing in batches. Absolute CD4 count values were generated with the BD FACS count system, BD FACS reagent kit and BD FACS control kit following the manufacturer's instructions [13]. A single test required one ready-to-use reagent tube to which a 50µl of blood sample was pipetted. After incubating at room temperature (20 to 25°C) for 60 minutes, 50ul of fixative was added to each tube and the controls beads to the control sample prepared. These preparations of the samples and control were run in the BD FACS count system. Every test was preceded by a run of the quality control tests followed by sample run under the same conditions upon the satisfactory result of the quality run.

2.5 Statistical Analysis

Data were analyzed using STATA software version 14. Frequency, means, standard deviations and ranges of CD4 counts were calculated. Linear regression analysis was done to find differences in CD4 count between gender, age groups and ethnicity (northern and southern Bhutanese) considering a p-value of <0.05 as significant. For this study, the Ngalops and Sharchops were taken together as northern Bhutanese and the Lhotsampas as southern Bhutanese for assessing ethnic differences in the CD4 count.

3. RESULTS

A total of 413 healthy blood donors, 288 (69.7%) males, were enrolled in the study. The mean age of the participants was 27.3 years (range 18-62 years, SD 7.3), the median age was 26 years and age group of 18-25 years were the maximum participants comprising almost half of the total. Ethnically, 351 (85.0%) were northern Bhutanese and 62 (15.0%) were southern Bhutanese. The mean CD4 count of the participants was 668.3cells/µl (95% CI 648.8, 687.8) ranging between 259 -1591cells/µl (SD±201.2) and the median count was 663cells/µl. The mean count was higher in females who recorded lower minimum count but a higher maximum count resulting in a wider range. In addition, southern Bhutanese appeared to have higher CD4 counts numerically than northern Bhutanese (Table 1).

In linear regression analysis, females showed significantly higher CD4 counts than males (p=0.004). Age group of 26-30, 31-35 and more than 40 years were likely to have higher CD4

counts compared to the younger age group of 18-25 years, except for the age group of 36-40 years. Ethnicity did not affect CD4 count since the numerically higher counts amongst southern Bhutanese compared to northern Bhutanese was not statistically significant (p=0.177) (Table 2).

Although there was a significant difference between age groups, the numerical differences in mean CD4 counts were small. However, younger people showed wider gaps between the maximum and minimum counts (higher range) which gradually reduced as a person aged (Fig. 1).

When the differences in counts in age groups were analyzed further by gender, it was observed that the mean count gradually increased with age in males but in the females, the mean count reached a peak at 31-35 years and then gradually decreased, as shown in Table 3 and Fig. 2.

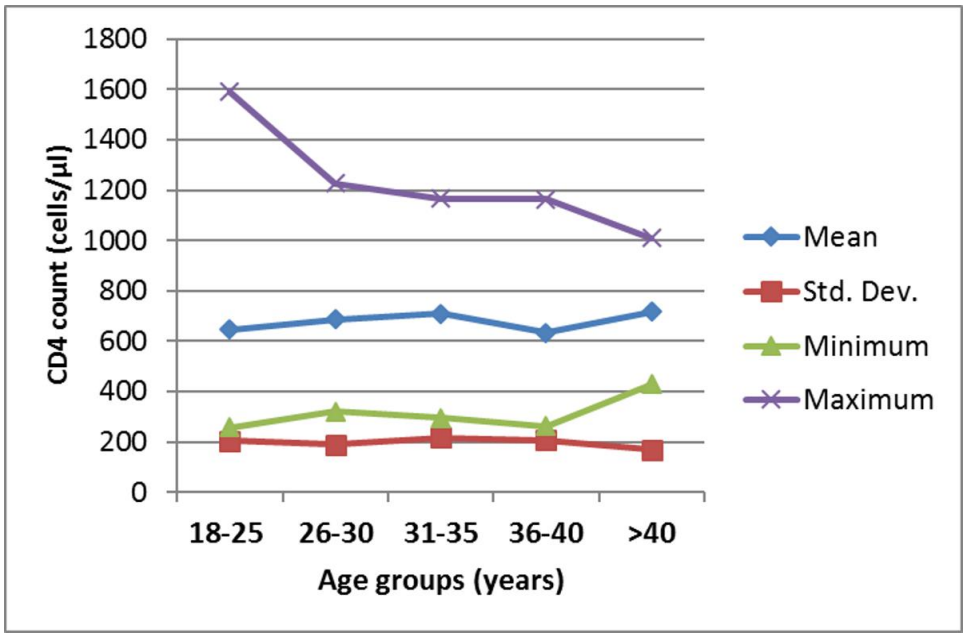

Fig. 1. Overall variation of CD4 count by age groups

Table 1. Participants' demography and CD4 count parameters by gender, age group and ethnicity

Variables	Number (%)	CD4 count parameters			
		Mean	Std. dev	Range	Median
Overall	413 (100)	668.3	201.2	259 -1591	663
By Gender					
Male	288 (69.7)	654.2	193.5	263 -1226	641
Female	125 (30.3)	700.8	215.0	259 - 1591	700
By age group (Years)					
18-25	199 (48.1)	646.6	205.5	259 -1591	625
26-30	118 (28.6)	686.9	189.4	322 -1226	681
31-35	42 (10.2)	710.5	218.0	297 - 1166	743
36-40	28 (6.8)	635.1	206.7	263 - 1165	638
>40	26 (6.3)	717.2	168.9	430 - 1011	727
By ethnicity					
Northern Bhutanese	351 (85.0)	663.0	200.8	259 - 1591	658
Southern Bhutanese	62 (15.0)	698.2	202.2	356 - 1206	683

Table 2. Differences in the absolute CD4 count by age, gender and ethnicity

CD4 count	Coefficient	95% CI		p-value
Gender				
Male	Ref.			
Female	63.76	20.53	106.99	0.004*
Age group (years)				
18-25	Ref.			
26-30	49.45	3.13	95.76	0.036*
31-35	76.26	9.45	143.07	0.025*
36-40	-3.07	-83.00	76.86	0.940
>40	89.03	6.29	171.78	0.035*
Ethnicity				
Northern Bhutanese	Ref.			
Southern Bhutanese	37.44	-17.03	91.90	0.177

Table 3. Comparison of absolute CD4 values between males and females of same age group

Age groups (Yrs.)	Number (%)	Mean count		Minimum count		Maximum count	
		Males	Females	Males	Females	Males	Females
18-25	199 (48.1)	625.8	675.7	295	259	1136	1591
26-30	118 (28.6)	671.7	740.8	322	394	1226	1146
31-35	42 (10.2)	679.5	824.1	364	297	1166	979
36-40	28 (6.8)	620.5	702.4	263	521	1165	827
>40	26 (6.3)	721.6	664.5	430	533	1011	796

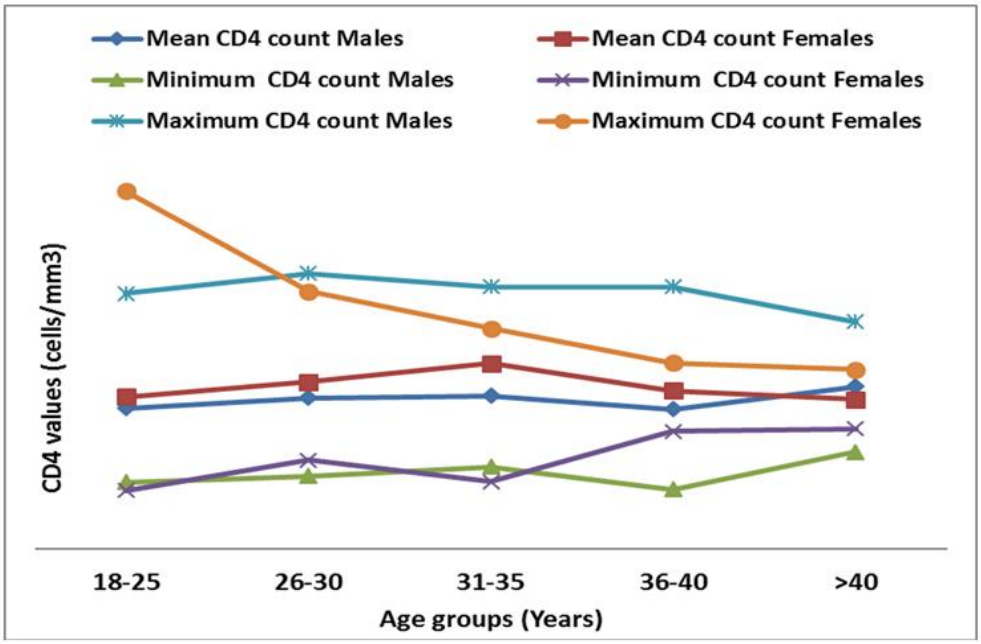

Fig. 2. Variation in CD4 count by age groups for each gender

4. DISCUSSION

In this study, the mean CD4 count in the healthy Bhutanese population was 668.3cells/µl ranging between 259-1591cells/µl. Although this mean count was within the normally recommended laboratory reference ranges of 500 to 1400 cells/µl, about 21% (88/413) of the participants had a CD4 count of less than 500cells/µl (the lower limit of the recommended reference range).

CD4 count ranges also varied widely with the minimum (259cells/µl) and the maximum (1591cells/µl), much outside the recommended laboratory references. However, the mean counts in the healthy Bhutanese population was consistent with other Asians [5,14], Middle East [15] and Africans [16] population but lower than those in the Indian [6] and Caucasian [4] population. The finding of a significantly higher absolute CD4 count in older age groups and in female gender was similar with other studies [7, 17]. Since there were no significant differences in absolute CD4 counts between the two major ethnic groups in Bhutan, a single reference range would be suitable for both the ethnic groups. However, CD4 counts in females were higher by a coefficient of 63.8 meaning that by being a female, the person is likely to have a CD4 count higher by at least 64cells/µl. In addition, CD4 counts were significantly higher by coefficients of 49 to 89 cells/µl of blood in older age groups compared to age groups of 18-25 years. However, even though the differences between gender and age groups were statistically significant, the numerical differences seem negligible and may be disregarded in day to day clinical practice for convenient generalization of normal CD4 values in a small population like Bhutan.

CD4 lymphocyte counts are widely used for clinical classification, to determine prognosis, and to decide whether to prescribe prophylaxis for opportunistic infections in HIV patients. Low CD4 and CD8 counts were seen in HIV-negative TB patients compared to healthy control groups, with disseminated diseases occurring in patients with profound CD4 lymphopenia and the counts subsequently returning to normalcy after recovery [15]. Low levels of CD3 and CD4 also occurred commonly in Hodgkin's disease [18]. In a low resource setting like Bhutan, where HIV viral load testing is not yet available, CD4 count is still the key to important decisions in HIV patient care. At the observed low CD4 count in about 21% of healthy Bhutanese adults compared to the commonly used reference ranges that are derived from the Caucasian population, the classification of diseases such as HIV and other clinical situations where critical decisions are made based on CD4 counts may be unreliable for the Bhutanese as with other Asian population. However, this needs to be confirmed with more nationally representative samples of adequate gender and age distribution. Past studies also recommended aggressive investigation of persons with a CD4 count of less than 300 to 400/µl for HIV infection and other causes of immune deficiency [2]. Such generalized recommendations may not be valid for settings in Bhutan and other Asian countries with comparatively low CD4 counts in a significant proportion of the normal healthy people.

This study has several limitations. HIV status and other immunological conditions that may impair CD4 count were not assessed although the participants were presumably healthy as regular voluntary blood donors. In addition, socioeconomic status, dietary or other habits such as smoking and alcohol consumption which could potentially affect CD4 counts due to their effect on lymphocyte proliferation were not accounted for. No other T lymphocyte subsets like CD8, CD3 and total white cell counts were measured. The findings of the study were also limited by inclusion of participants of blood donation eligibility age group only.

5. CONCLUSION

The findings of this study need to be validated with a larger study before we conclude that a somewhat different CD4 cut-off may be required for the Bhutanese population. However, within the small Bhutanese population, a single reference count may be advocated for adults disregarding the negligible numerical differences between age groups and gender. Future studies should include children and the older age groups for a more comprehensive national reference value.

CONSENT

All authors declare that informed consent was obtained from all participants/blood donors for this study.

ETHICAL APPROVAL

Ethical approval was granted by the Research Ethics Board of Health (REBH), Ministry of Health, Bhutan through approval no. REBH/Approval/2014/011. All the participants provided informed consent before participation and all the samples were anonymized.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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