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International Journal of Current Research
Vol. 4, Issue, 07, pp.086-091, July, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

DETERMINATION OF NORMAL CD^{4+} LYMPHOCYTE VALUES AND ITS ASSOCIATION WITH TOTAL LEUCOCYTE COUNT IN NORMAL ADULT NIGERIANS RESIDENT IN MAIDUGURI, NIGERIA

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ARTICLE INFO

Article History:
Received 18th April, 2012
Received in revised form
24th May, 2012
Accepted 12th June, 2012
Published online 30th July, 2012

Key words:
Antiretroviral therapy,
HIV,
TLC.

ABSTRACT

Background: Determination of normal CD4 count and understanding the total lymphocyte count (TLC)-CD4 count relationship could assist in making clinical decisions during antiretroviral therapy, especially in underserved resource-poor settings. The aim of this study is to establish the normal reference values of CD4 count in healthy HIV negative adults residing in our environment.

Methods: Three hundred and eighty four apparently healthy adult participants who presented at the HIV clinic for HIV counselling and testing (HCT) between March 2009 and January 2010 were equally recruited based on sex into the study. Those that tested negative had their CD4 count and TLC assessed using cy flow method and hematology analyzer.

Result: Their ages at presentation ranged from 18- 67 years (males, 18–49 years; females, 18–67 years) total mean age was 27.29 years (SD = 6.92 years). The male´s mean age of (27.43 years, SD = 5.95 years) was similar to (27.15 years, SD = 7.7 years) in females ($p > 0.05$). The participants' CD4 cell count at presentation ranged from 247-1840cells/µl with a mean of 766.66 (SD = 245.69 cells/µl). Mean CD4 cell count at presentation was significantly higher in females (828.41 cells/µl, SD = 268.68cells/µl) than in males (704.26cells/µl, SD =202.37 cells/µl) (f=22.82, $p = 0.000$). The mean ± SD (min-max) TLC of the studied participants was 5.26 ±1.97 (1.2-14.3). The mean TLC was similar in females ($4.94x10^9$/l, SD = 1.95 and males ($5.57x10^9$/l, SD =1.97) ($p = 0.9$). The mean CD4 counts of subjects were similar across the different groups. Similarly there was no statistically significant difference in the mean TLC across all the groups. A positive correlation was observed between CD4 count and TLC ($r=0.36$, $p=0.000$).

Conclusion: This study shows that females have higher CD4 count than males. There was no observed difference in mean CD4 count across all age groups. A positive correlation was observed between CD4 count and TLC ($r=0.36$, $p=0.000$); this association shows that TLC is a suitable surrogate marker for CD4 count in normal adults in our environment.

INTRODUCTION

Enumeration of CD4 + lymphocytes alongside other parameters is of central importance in monitoring the immune system. There is paucity of data on reference values of CD4+ lymphocytes from West African populations[1]. Rather values from textbooks and other populations based on studies in western countries are largely employed for clinical decision making. However, there is evidence in the literature of significant geographical and racial differences in these parameters. Work done to establish reference values for haematological parameters in Nigeria have been limited in the number of subjects studied and parameters established [2,3]. The information on haematological values from the Northern part of Nigeria is even scantier in spite of peculiar socio-economic and demographic characteristic**s**. The environment and mode of life in Nigeria are very different from those in Western countries. Since such factors can affect references ranges, differences in the references values for a Nigerian population can be expected. Estimation of CD4+ T lymphocyte is one of the measures of ascertaining the immune competence of the HIV infected individuals [4,5], throughout the broad spectrum of HIV disease [6,7]. The present

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knowledge concerning the staging of disease, monitoring of progression, initiation of therapeutic regimen and response to therapy depends heavily on determination of peripheral lymphocyte subpopulations [8]. With the reported high adult prevalence of human immunodeficiency virus (HIV) infection in Nigeria and with increased access to antiretroviral therapy, the need to determine local reference value for CD4+ lymphocytes count, for accurate monitoring of response to treatment and other treatment outcomes becomes more urgent [9]. Presently, there are no locally established reference standards for CD4+T lymphocyte parameters in Borno state, and evaluation of laboratory results is still based on reference to Caucasian values. This study proposes to determine essentially the reference value of CD4+T lymphocytes and its association with TLC in healthy HIV negative adult Nigerians in Maiduguri.

Patients and methods

Study Population: Apparently healthy HIV sero-negative subject 18 years and above Nigerians resident in Maiduguri were randomly selected into the study. The term apparently healthy is used because of the possibility of undisclosed or undiagnosed diseases, but negative HIV sero-status was confirmed. In this study, diurnal variation was avoided by collecting samples during a specified time of the day.

Inclusion Criteria

1. Adult Nigerians 18 years and above who have resided in Maiduguri for at least one year.
2. HIV negative status confirmed by western blot.
3. Informed (written) consent and willingness to participate in the study.
4. Absence of any evidence of severe systemic illness on general physical examination.

Exclusion Criteria

1. Less than 18 years of age
2. Evidence of HIV infection
3. Inability to give consent or unwillingness to participate in the study.
4. Evidence of severe systemic illness on physical examination
5. Subject on immunosuppressive agent/drugs.
6. Recent immunization.
7. Visitors or those that have not resided in Maiduguri for up to one year.

Study procedure: Participants underwent clinical assessment that includes standardized history and physical examination. Using a structured, pre evaluated questionnaire, information was obtained on demographic, Medical history, medication usage, blood transfusion, sexual and drug use behaviour, alcohol ingestion, family history of medical conditions such as diabetes, hypertension, Asthma etc. Participant were excluded from the study based on findings from history, physical examination and HIV seropositivity. No other investigation was conducted to unravel the possibility of undiagnosed or undisclosed illness.

Blood samples Collection: Blood samples from the participants that fulfilled the inclusion criteria was collected by venipuncture after scrubbing the area with sterile cotton soaked in methyleted spirit from the antecubital vein into ten millilitres of plain, EDTA, and fluoride vacutainer tubes bottles.

Blood samples Analysis Sample for Total lymphocytes count (TLC) and CD4+ T cell count was collected between 9:00-10:00am and assayed within 6 hours of collection of whole blood using standardized flow cytometric Cyflow machine (manufactured by Cytec, Partec, Germany) and haematology analyzer (manufactured by sysmex corporation, kobe Japan).

Ethical consideration: Written Informed consent (signed or thumb print) was obtained from patients, Permission was obtained from the University of Maiduguri Teaching Hospital (UMTH) Ethical Committee.

Data of Analysis

Data obtained were analysed using Statistical Package for social sciences version 15.0(SSPS Chicago III.USA) for windows. Results were presented as mean ± standard deviations (SD). Paired student t –test will be used to test the significances between age groups. Two-way analysis of variance was used in comparing the means of males and female participants where necessary, with statistical significance set at p (probability) value less than 0.05. Spearman's correlation analysis was used in determining correlation between CD4 counts and TLC. Tables was be used to present data, Microsoft excel was used to plot graphs where applicable.

RESULTS

Socio-Demographic Characteristics

Three hundred and eighty four apparently healthy adult participants who presented at the HIV clinic for HIV counselling and testing (HCT) between March 2009 and January 2010 were equally recruited based on sex into the study. Their ages at presentation ranged from 18- 67 years (males, 18–49 years; females, 18–67 years). The overall mean age was 27.29 years (SD = 6.92 years). The male´s mean age of (27.43 years, SD = 5.95 years) was similar to (27.15 years, SD = 7.7 years) in females ($p > 0.05$). Two hundred and nineteen (107 males and 112 females) were married, 156 (82 males, 76 females) were singles (never married), 7 (3 males, 4 females) were divorced, 2 (2 males, 0 female) were separated, at the time of presentation. There was no significant difference between males and females in the proportion of participants based on social status.

A total of one hundred and thirty six (64 males and 72 females) were illiterate, 62 (28 males, 34 females) had primary education, 121 (64 males, 57 females) were secondary school leaver while 65 (36 males, 29 female) had tertiary education, at the time of presentation. There was no significant difference between males and females in the proportion of participants based on literacy level. The age profile of the study population were categorized into seven groups as:- 18-25, 26-35, 36-45, 46-55, 56-65, 66-75, 76-85. The proportion of participants in different groups were 24.7%, 14.1%, 29.4%, 18%, 12%, 1.3% and 0.5% respectively as depicted in Table 1.

Table 1. Socio-demographic characteristics of the study participants

	No (%) of or parameter for participants			
	M (n=192)	F (n=192)	p-value	M and F (n=384)
Characteristics				
Sex				
Male	192			
Female		192		
Marital status				
Married	107(55.7)	112(58.4)	> 0.05	219 (57.0)
Single	82(42.7)	76(39.6)	> 0.05	158(40.6)
Divorced	3(1.6)	4(2.0)	>0.05	7(1.8)
Separated	2(1.0)	0(0.0)		2(0.6)
Educational status				
Illiterate	64(33.3)	72(37.5)	>0.05	136(35.4)
Primary Education	28(14.6)	34(17.7)	>0.05	62(16.2)
Secondary Education	64(33.3)	57(29.7)	>0.05	121(31.5)
Tertiary Education	36(18.8)	29(15.1)	>0.05	65(16.9)
Age (years)				
18-25	43(22.4)	52(27.1)	>0.05	95(24.7)
26-35	23(12.0)	31(16.1)	>0.05	54(14.1)
36-45	51(26.6)	62(32.3)	>0.05	113(29.4)
46-55	47(24.5)	22(11.5)	>0.05	69(18.0)
56-65	22(11.5)	24(12.5)	>0.05	46(12.0)
66-75	4(2.0)	1(0.5)		5(1.3)
76-85	2(1.0)	0 (0.0)		2(0.5)

Table 2. Mean CD4+T cell count and Total leucocyte count

	Male (Mean ±SD (min max)	Female (Mean ±SD(min-max)	P - value	Male and Female (Mean ±SD(min -max)
CD4 (Cells/µl)	704.26±202.37 (247-1352)	828.41±268.68 (400-1840)	<0.05	766.66±245.69(247-1840)
TLC x 10^9/l	4.94±1.95 (1.20-14.3)	5.57±1.97 (1.30-13.1)	>0.05	5.26±1.97(1.20-14.3)

Table 3(a). Mean CD4 count of the various age groups

Age group (years)	Mean CD4 ± SD	Min - Max
18-25	766.58 ± 233.59	402.0 - 1598.0
26-35	801.53 ± 260.35	400.0 - 1840.0
36-45	779.00 ± 254.58	553.0 - 1411
46-55	786.00 ± 241.7	479.0 - 1519
56-65	794.32 ± 255.9	403.0 - 1672

CD4 count (cells/µl)

Table 3(b). Mean Total leucocytes count (TLC) count of the various age groups

Age group (years)	Mean CD4 ±SD	Min - Max
18-25	5.11±1.79	1.30-10.6
26-35	5.31±2.0	1.20-13.1
36-45	5.60±2.1	1.4-12.6
46-55	5.13±1.8	1.36-12.9
56-65	4.96±2.1	1.32-11.7

TLC (x10^9/l)

Table 4. Relationship of CD4 ranges and corresponding mean Total leucocyte count (TLC)

CD4 count Range (cells/µl)	Overall mean TLC (10^9/l)	Mean TLC(10^9/l) (males)	Mean TLC(10^9/l) (females)
200-299	2.1 ± 0.6	2.0 ± 0.7	2.3 ± 0.4
300-399	2.4 ± 0.5	2.3 ± 0.9	2.5 ± 0.7
400-499	3.2 ± 0.8	2.7 ± 1.1	2.8 ± 1.2
500-599	3.6 ± 1.1	3.1 ± 1.3	3.1 ± 1.1
600-699	4.3 ± 0.9	3.6 ± 1.6	3.8 ± 1.5
700-799	5.0 ± 0.7	3.6 ± 1.0	3.7 ± 1.9
800-899	4.8 ± 1.2	4.7 ± 1.1	4.0 ± 1.7
900-999	5.2 ± 1.5	4.8 ± 1.5	4.4 ± 1.6
1000-1099	5.1 ± 1.3	5.6 ± 1.3	4.9 ± 2.2
1100-1199	5.2 ± 1.2	5.4 ± 1.8	5.5 ± 1.9
1200-1299	5.3 ± 1.0	5.6 ± 2.0	6.0 ± 1.4
1300-1399	5.4 ± 1.4	6.5 ± 2.1	6.4 ± 2.2
1400-1499	5.6 ± 2.0	5.8 ± 1.9	6.7 ± 2.3
1500-1599	6.1 ± 1.8	7.7 ± 2.3	6.6 ± 1.9
1600-1699	6.8 ± 2.3	7.3 ± 2.1	7.3 ± 2.1
1700-1799	7.2 ± 1.7	7.7 ± 1.8	7.4 ± 2.3
1800-1899	8.0 ± 2.2	8.0 ± 2.2	7.9 ± 1.8

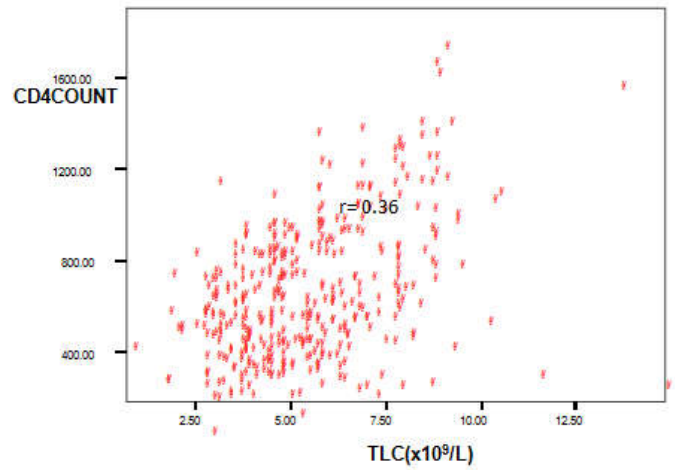

Figure 1. The correlation of Total leucocytes count (TLC) with CD4 count level, it shows that there is a positive correlation between Total leucocytes count (TLC) with CD4 count level (r =0.36, p =0.00).

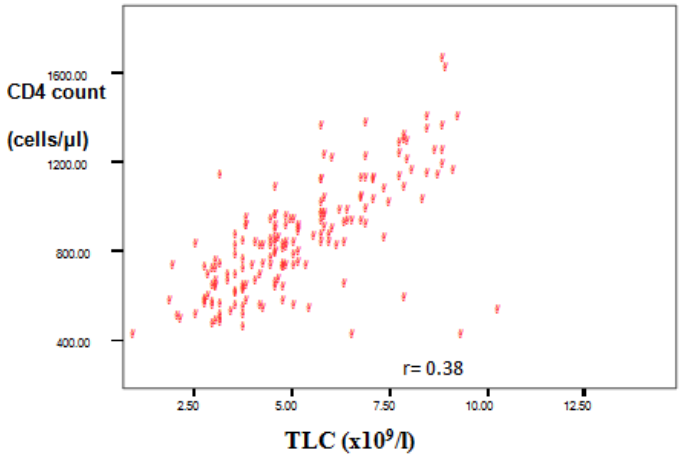

Figure 2. Correlation between CD4 Count and TLC in males

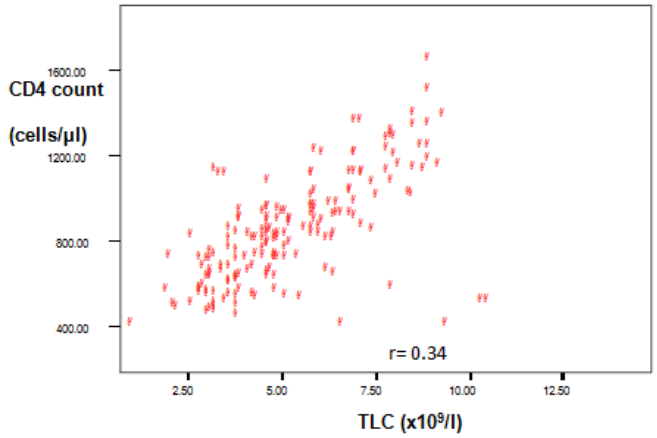

Figure 3. Correlation between CD4 Count and TLC in Females

CD4T+ cell Count and Total Leucocyte Count (TLC)

The participants' CD4 cell count at presentation ranged from 247-1840cells/µl with a mean of 766.66 (SD = 245.69 cells/µl). Mean CD4 cell count at presentation was significantly higher in females (828.41 cells/µl, SD = 268.68cells/µl) than in males (704.26cells/µl, SD =202.37 cells/µl) (f = 22.28, p <0.05). The CD4 counts distributions are presented in Table 2. The mean ± SD (min-max) TLC of the studied participants was 5.26 ±1.97 (1.2-14.3). The mean TLC was similar in females (4.94x10^9/l, SD = 1.95 and males (5.57x10^9/l, SD =1.97) (f=0.78, p >0.05). The CD4 counts and TLC distribution are summarized in Table 2.

Mean CD4 Count and TLC Based on Age Group

The mean CD4 counts of subjects were similar across the different groups as depicted in Table 3(a). Similarly there was no statistically significant difference in the mean TLC across all the groups, the mean TLC in different age group is as shown in Table (3b). There was a linear relationship between CD4 count ranges and mean CD4 count, the mean CD4 count increased progressively with increase in CD4 count in both male and female cohort, as shown in Table 4. Figure 3. Shows the correlation of Total leucocytes count (TLC) with CD4 count level in females, it also shows that there is a positive correlation between Total leucocytes count (TLC) with CD4 count level (r= 0.34, p = 0.00). Figure 2. shows the correlation of Total leucocytes count (TLC) with CD4 count level in males, it shows that there is a positive correlation between Total leucocytes count (TLC) with CD4 count level (r = 0.38, p =0.00).

DISCUSSION

This study looked at the values of CD4 + T cell count in 384 healthy HIV negative adults. The CD4 + T cell count, a frequently used surrogate marker for immune suppression associated with human immunodeficiency virus (HIV) infection. It is an important parameter used to guide the initiation and monitoring of antiretroviral (ARV) prophylaxis and treatment in HIV infection [10]. The mean CD4 count of the participants was 766.66cells/µl. This is similar to the values for healthy adult reported previously in the same region, adult Ethopians and adult Chinese [11-13], lower than the values reported from Jos, Ghana, Tanzania and Uganda [14-17] and higher than values obtained from a Swiss study [18]. In the present study, females were found to have significantly higher mean absolute CD4 count than males. Several studies have reported similar observations of higher CD4 T-cell count in females compared to males in both Africans and Caucasians populations [13,17,19-23]. It has been suggested that a sex hormone effect could be one possible explanation for the reported gender difference in CD4 counts [21]. However, the clinical relevance of these difference of patient management remains to be elucidated; since there is no strong evidence to suggest that gender affects HIV disease progression, the biological relevance of difference in CD4 count between men and women remains unknown. No difference in mean CD4 count was observed across age group in study similar to previous report [24-26] in which no significant changes in CD4 counts with regard to age were found in adults.

We found that the total lymphocyte count (TLC) performed well in predicting CD4 count. Our findings suggest TLC, which is relatively inexpensive and available, is a reasonably accurate tool that can be used for monitoring the patients' immune status during therapy in addition to determining when patients should start antiretroviral therapy. These findings also imply that the possibilities for modifying the models to suit specific needs exist such as employing simple scores, algorithms, or risk calculators for use in clinics in underserved areas. Studies have described the relationship between TLC and CD4 count previously that support our study findings

[4,27]. A relatively high positive correlation has been established between absolute values of TLC and CD4 count [27] or between changes in TLC and CD4 cell count [28]. A range of TLC cutoffs have been used and reported as predictors of CD4 < 200 cells/µl. These cutoffs range from 1000 cells/µl with a specificity of 98% and a sensitivity of 53% to 1,400 cells/µl with a sensitivity of 73% and a specificity of 88%[4,29]. In another study, a TLC cutoff of 1,200 cells/µl was modeled with hemoglobin to improve sensitivity[30]. Our study explored the use of TLC in predicting CD4 count that would be readily available even in underserved resource-poor settings. Our study demonstrated that TLC could be used to predict CD4 count in healthy HIV negative adults, its application in various formats, both before and during HAART use in HIV patients need to be explored. The laboratory parameter most studied as a potential alternative to CD4 count is the total lymphocyte count (TLC). This is calculated by multiplying the total white blood cell count (wbc) by the lymphocyte percentage. The relatively low cost of this method, and the wider availability of necessary laboratory equipment make it an attractive parameter for resource-limited settings. The lymphocyte percentage of total wbc, which is necessary to calculate the TLC, is most accurate if determined within a few hours of phlebotomy. Many hematology workstations in resource-limited settings are unable to meet this stringent criterion, and there are unavoidable excursions in ambient temperature that accelerate the degradation of laboratory samples. As such, TLC calculations are prone to error. Another factor that can unravel potential correlation between TLC and CD4 count is that TLC captures both B and T cell subsets. Accordingly, a person with low CD4 count could have relatively high TLC if high amounts of B cells are expressed due to immune hyperactivation from exposure to the wide variety of circulating antigens in sub- Saharan Africa.

The World Health Organization [31] recommends initiation of antiretroviral therapy (ART) in all WHO stage 4 patients, irrespective of the CD4 count; WHO stage 3 patients with a CD4 count of 200-350cells/µl ; and in all patients with a CD4 count of less than 200cell/µl. In places where CD4 T cell testing in not available, the WHO recommends considering treatment for WHO Stage 2 disease if TLC is <1,200 cells/mm3. The use of TLC is not recommended in asymptomatic patients [31]. Evidence frequently cited to support the use of a TLC cut off of 1,200 cells/mm3 (for Stage 2 disease) includes a South African cohort study in which TLC <1,250 cells/ mm3 was found to be an equivalent predictor of disease progression compared to CD4T cell count <200 cells/ µl [32]. Other studies question this cut off, however, and an acceptable, reproducible cut off has been elusive. For example, in a study from Nigeria, one-third of patients with CD4 T cell count <200 cells/µl, had TLC >1,200 cells/mm3 [33]. Further, in an analysis of WHO Stage 2 patients in an ART program (n 5 1281), CD4 T cell and TLC counts were significantly positively correlated. Using TLC <1,200 cells/mm3 as a predictor of CD4 T cell <200 cells/µl resulted in 31.5% sensitivity, 96.0% specificity, 95.9% positive predictive value, and 31.6% negative predictive value. Increasing the cutoff value to 1,900 cells/mm3 resulted in 67.0% sensitivity, 67.9% specificity, 86.3% positive predictive value, and 40.4% negative predictive value. Taken together, TLC <1,200 cells/mm3 was a poor predictor of CD4 T cell count <200 cells/µl, and over half of patients with CD4T cell count <200 cells/µl would have been inappropriately excluded by TLC-guided treatment with a cut off of 1,200 cells/mm3[34]. Thus, TLC is of limited usefulness for guiding initiation of ART: some patients with CD4 count <200 cells/µl will not be started on ART because their TLC will be >1,200cells/mm3. A higher cut off, on the other hand, is likely to lead to cases of unnecessary ART, drug toxicity and probably higher costs. Some investigators have suggested that incorporating the hemoglobin level, and perhaps body mass index or platelet count, will improve the accuracy of TLC [35.36], these additional parameters such as these need to be evaluated in different population.

Conclusions

This was a cross sectional study that determined the normal CD4 count and its association with Total leucocyte count (TLC) in healthy HIV negative adult residing in Maiduguri. The following conclusions were arrived at: The mean CD4 count of 766.66 was obtained for the participants, females had higher CD4 count than males. There is no difference in mean CD4 count across age groups. A positive correlation was observed between CD4 count and TLC (r=0.36, p=0.00); this association shows that TLC is a suitable surrogate marker for CD4 count in our cohort.

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