International Journal of Community Research http://www.arpjournals.com
ISSN: 2315 – 6562 E-ISSN: 2384 - 6828

CD4 T LYMPHOCYTE COUNT OF APPARENTLY HEALTHY ADULT SUBJECTS IN EKPOMA, EDO STATE, NIGERIA

***[1]Babatope, I. O., [2]Eromon, P. E., [1]Eromon, A.**

[1]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria; [2] Department of Family Medicine, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria
*Corresponding author email: babatope_olaniyi@yahoo.com

ABSTRACT

CD4 cells are subgroups of lymphocytes and are a major determinant of the integrity of human immune system. The present cross-sectional study was undertaken to determine the CD4 T lymphocyte count of apparently healthy adult population in Ekpoma, Edo State, Nigeria. A total of 278 subjects (155 males, 123 females), aged 18-50years were recruited into the study. Five millilitres (5ml) of whole blood was collected from the antecubital vein and dispensed into E.D.T.A. bottles. Flow cytometry was used to determine the absolute counts (cells/µl) of CD4+ T lymphocytes. The mean absolute CD4 count of the population studied (n=278) was 924.07±298.00 cells/µl (Median 873.50; Range 422.00-1509.60). The mean absolute CD4 count of the male population (n=155) was 889.00±268.17 cells/µl (Median 874.50; Range 453.33-1481.05), while the mean absolute CD4 count of the female population (n=123) was 959.49±324.41 cells/µl (Median 867.50; Range 486.16-1572.90). Based on both sex and age-ranges, the female subjects had a higher mean absolute CD4 count compared to their male counterparts, but these variations were not statistically significant (P>0.05). In conclusion, our study has revealed that the mean absolute CD4+ T lymphocyte obtained in our study population is different from those of other populations.

Keywords: Ekpoma, CD4 count, Reference ranges, Apparently healthy adults

INTRODUCTION

Cluster of Differentiation 4 count (CD4 count), alongside other parameters, is of central importance in the monitoring of immune function (Aina *et al.*, 2005). CD4 refers to a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages and dendritic cells. In humans, the CD4 protein is encoded by the CD4 gene (Isobe *et al.*, 1986; Ansari-Lari *et al.*, 1996). T lymphocytes are divided into:

1. Helper cells which help in the function of the immune the system
2. Cytotoxic T cells also called kill infected cells
3. Suppressor T cells which are capable of suppressing the functions of both cytotoxic and helper T cells.

CD4+ T helper lymphocytes play a central role in the regulation of immune response (Zhu and Paul, 2008). They have the 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 (Mossman and Cofffman, 1989). The immunophenotyping of the lymphocytes, especially CD4+ T cells from peripheral blood is being used to assess the extent of immune dysfunction in the primary and secondary immunodeficiency, chronic infectious diseases and various cancers such as Hodgkin's disease, lymphoma, etc. The clinical

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
Indexed By: *African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

applications of immunophenotyping of CD4+ T cells including the monitoring of disease progression in HIV infection (Pattanapanyasat and Thakar, 2005), to determine the initiation of highly active antiretroviral therapy (HAART), and monitor the response to HAART (Hoffman, 1971; Tsegaye *et al.*, 1999; Uppal *et al.*, 2003), diagnosis of immunodeficiency disorders (Nicholson,1989; Jain *et al.*, 1999), evaluation of immune-mediated diseases (Bleesing *et al.*, 2000) and the assessment of immune reconstitution during stem cell transplantation (Storek and Witherspoon, 2000).

Full understanding of reference values in African populations will therefore provide useful information for HIV/AIDS management policy, clinical trials and other therapies that use T cell subsets as markers. However, only little data are available on CD4 T cells in Nigeria and most of the studies are on immunocompromised populations (Nwokedi *et al.*, 2007; Forbi *et al.*, 2010). Besides the few studies performed on apparently individuals in Nigeria did not exclude subjects that were hepatitis B and C viruses seropositive (Aina *et al.*, 2005; Oladepo *et al.*, 2009) and the possibility that this may affect the CD4 T cell outcomes cannot be underestimated. Therefore, it is imperative to determine the CD4 T lymphocyte count of apparently healthy adult subjects in Ekpoma, Edo State, Nigeria.

MATERIAL AND METHODS

Study area: This study was carried out in Ekpoma, the administrative headquarters of Esan West Local Government Area of Edo state. The geographical coordinates of Ekpoma are $6^0 45^{'} 0^{''}$ North and $6^0 6^{'} 0^{''}$ East. It is the fourth largest town in Edo State and has an area of 502km^2 and a population of 89,628 in 1991 and 127,718 in 2006, majority of which are civil servants, traders, business men/women, farmers, teachers, lecturers and students by occupation. Ekpoma since its designation as headquarters and as the host of the state-owned university (Ambrose Alli University) has grown into an urban center (Aziegbe, 2006).

Study population: A total of two hundred and seventy eight (278) apparently healthy adult subjects comprising of one hundred and fifty five (155) males and one hundred and twenty three (123) females were recruited into the study. The study subjects were HIV 1/2, Hepatitis (B and C) and VDRL seronegative and aged between 18 and 50 years.

Study design: Apparently healthy adults, HIV1/2, Hepatitis (B and C) and VDRL seronegative subjects belonging to different age-groups and social backgrounds were recruited into this study. The participants were selected by simple random sampling technique. Each consenting participant was asked to fill a questionnaire and administered a consent form A and form B to read and sign respectively. All participants were screened in or out based on both the inclusion and exclusion criteria captured in the questionnaire. HIV 1/2, Hepatitis (B and C) viruses and VDRL sero-reactivity were also determined and only those who were seronegative were recruited into the study.

.

Inclusion criteria: Only apparently healthy adult subjects who have not been on medication for the last one month and aged between 18 and 50 years as well as subjects seronegative for HIV 1/2, Hepatitis (B and C) viruses and VDRL were recruited into the study.

Exclusion criteria: The exclusion criteria for these individuals included any minor illness in the past one month; any major illness in the past six months; Individuals who took any vaccination in the past six months; Pregnancy; and subjects seropositive for HBV, HCV, HIV 1/2 and VDRL

Blood collection: Within the time frame of 9.00am-12.00noon, five (5) milliliters of blood was collected through venepuncture from the antecubital vein into ethylene diamine tetraacetic acid (E.D.T.A.) tubes in accordance with biosafety precautionary measures. All the samples were transported immediately at cold chain temperature ranges of 2^0C to 8^0C to the laboratory and were analyzed within six hours of sample collection.

Specimen Analysis: HIV Sero-reactivity was determined according the national algorithm II. Serial testing was carried out using Determine HIV- 1/2 test kit

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
Indexed By: *African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

in the first instance and Unigold HIV-1/2 kit was only used when Determine HIV1/2 test was sero-reactive and discordant results were resolved with the third kit (tie breaker). All the three test kits (Determine, Alere Medical Co. Ltd, Japan; Unigold, Trinity Biotech Plc, Ireland; and Stat Pak, Chembio Diagnostics systems, Inc, USA) were used according to the manufacturers' instructions. Participants were categorized as HIV non-reactive when they did not react with Determine HIV – 1/2 rapid test kit. Venereal Disease Research Laboratory (VDRL) test and Hepatitis (B and C) viruses were screened using three different test strips. These are quantitative membrane strips based immunoassay methods.

The appearance of a red bar both in the control and test line indicated a positive result, while the appearance of a red bar only on the control line with no red bar on the test line denoted a negative result.

The CD4 count was determined using Parteccyflow machine (Sysmex Partec GmbH, Görlitz, Germany) according to the manufacturer's instructions. The cyflow counter is based on the simultaneous measurement of multiple physical characteristics of CD4 T-cells in a single file as it flows through a light source, usually a laser beam. The counter separated the CD4 T cell from the monocytes – CD4 bearing cells and noise using a gating system.

Ethical approval: Approval for the study was obtained from the University Ethics Committee in accordance with the code of ethics for biomedical research involving human subjects. Written informed consent of each participant was obtained. However illiterate participants had their consent forms read and interpreted to them in their native languages by an interpreter.

Statistical analysis: The data obtained were expressed as means ± standard errors of means (S.E.M). The medians were calculated and the reference values were determined at 2.5^{th} and 95^{th} percentiles. Statistical significance was determined using the analysis of variance (ANOVA) or the Student's t-test as appropriate. $P < 0.05$ was considered significant. All statistical analyses were done using SPSS version 21.0

RESULTS

Table 1 reveals the socio-demographic profile of the study subjects. A total of two hundred and seventy eight (278) apparently healthy adult subjects residing in Ekpoma were recruited for this study. Distribution according to sex revealed that more men were recruited compared to women (55.76% vs 44.24%). Female subjects in the age range of 24-29 years were the most predominant participants (17.62%), closely followed by male subjects in the age ranges of the both 24-29 years and 30-35 years (16.91%) while the least predominant age group of 30-35 years were females who recorded a frequency distribution of 7.91%. Most of the study subjects were Christians (97.84%) while Muslims accounted for 2.16%. University undergraduates (students) constituted half of the study subjects while farmers were the least (1.80%). Undergraduates accounted for 50% on the basis of educational qualification, followed by university graduates 36.69% and subjects with no formal education recording 1.80%.

Table 2 depicts the CD4 counts of apparently healthy adult subjects in Ekpoma. The absolute mean of CD4 count for the male population (n=155) was 889±268.17 cells/µl (Median 874.50, Range 453.33-1481.05 cells/µl). For the female population (n=123), the mean absolute of CD4 count was 959.49±324.41 cells/µl (Median 867.50, Range 486.16-1572.90) while the mean absolute of CD4 count for the total population (n = 278) was 924.07±298 cells/ µl (Median 873.50, Range 422.00-1509.60).

Table 3 shows the CD4 counts of the study subjects according to age. The CD4 reference ranges for males and females were determined at 2.5^{th} and 95^{th} percentiles. The means and medians of CD4+ T lymphocytes were also determined according to sex and age groups. In all the age ranges except one, the female subjects had a higher CD4 mean absolute count compared to their male counterparts but these variations were not statistically significant ($P>0.05$).

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
Indexed By: *African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

International Journal of Community Research http://www.arpjournals.com
ISSN: 2315 – 6562 E-ISSN: 2384 - 6828

TABLE 1: SOCIO-DEMOGRAPHIC PROFILE OF APPARENTLY HEALTHY SUBJECTS STUDIED

VARIABLE	CATEGORIES	NUMBER OBSERVED	FREQUENCY (%)
Sex	Male	155	55.76
	Female	123	44.24
Age (Male)	18 – 23	26	9.35
	24 – 29	47	16.91
	30 – 35	47	16.91
	36 and above	35	12.59
Age (Female)	18 – 23	27	9.71
	24 – 29	49	17.62
	30 – 35	22	7.91
	36 and above	25	8.99
Religion	Christian	272	97.84
	Muslim	6	2.16
	Traditional	0	0
Marital Status	Single	103	37.05
	Married	161	57.91
	Divorced/Separated	14	5.04
Occupation	Student	139	50.0
	Employed	99	35.61
	Farmer	5	1.80
	Business	11	3.96
	Artisan	11	3.96
	Unemployed	13	4.68
Education	Undergraduates	139	50.00
	University	102	36.69
	Polytechnic	10	3.60
	Secondary	11	3.96
	Primary	11	3.96
	No Formal Education	5	1.80

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
Indexed By: *African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

TABLE 2: CD4 COUNT OF THE STUDY SUBJECTS IN EKPOMA

	MALES N=155			FEMALES N=123				TOTAL N=278		
Parameter	Mean±SD	Median	2.5-95TH Percentile	Mean±SD	Median	2.5-95TH Percentile	P-Value	Mean±SD	Median	2.5-95TH Percentile
CD4	889.80 ± 268.17	874.50	453.33 - 1481.05	959.49 ± 324.41	867.50	486.16 - 1572.90	0.071	924.07 ± 298.58	873.50	482.00 - 1509.60

TABLE 3: CD4 COUNTS OF THE STUDY SUBJECTS ACCORDING TO AGE

	FEMALES				MALES				TOTAL			
Age Yrs	N	Mean±SD	Median	2.5TH-95TH Percentile	N	Mean±SD	Median	2.5TH-95TH Percentile	P-value	N	Mean±SD	Median	2.5TH-95TH Percentile
18-23	27	1043.78 ± 368.90	906.00	717.00 - 1513.00	26	1062.31 ± 201.66	1042.00	526.00 - 1823.60	0.822	53	1052.87 ± 296.11	1021.00	550.85 ± 1717.30
24-29	49	879.90 ± 291.54	800.00	457.20 - 1534.00	47	866.64 ± 280.32	880.00	405.25 - 1456.50	0.821	96	873.41 - 284.67	856.00	452.28 - 1468.55
30-35	22	1011.68 ± 330.99	954.00	428.00 - 1426.65	47	879.00 ± 243.19	804.00	630.00 - 1592.65	0.113	69	938.57 ± 290.54	881.00	422.00 - 1501.00
>36	25	983.30 ± 309.58	931.50	444.00 - 1527.00	35	865.17 ± 276.79	804.00	583.00 - 1567.95	0.151	60	931.17 ± 295.79	1460.00	468.80 - 1522.20

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
Indexed By: *African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

DISCUSSION

Studies have been carried out worldwide to establish reference ranges for CD4+ T cell counts in normal healthy individuals. Variation in the reference ranges for CD4+ T cell counts have been observed in different populations. The CD4 T-lymphocyte absolute count is one of the best surrogates for assessing the risk of progression to AIDS among HIV-infected individuals. This marker is also of diagnostic tool in determining certain AIDS related opportunistic infections or immune compromised conditions and the time of initiating antiretroviral and prophylactic antimicrobial therapies (Idoko *et al.*, 2001; Oladepo *et al.*, 2009).

Our study revealed that the mean CD4+ T lymphocytes count in Ekpoma was 924 cells/µl. This finding is consistent with the previous reports of Malone *et al.* (1990), Dapper *et al.* (2008), Vithayasai *et al.* (1997) and Santagostino *et al.* (1999) who found the mean CD4+ T lymphocyte counts of 910 cells/µl in Thailand, 920 cells/µl in South South Nigeria, 933 cells/µl in Central Africa Republic and 940 cells/µl in Italy respectively. However, higher mean CD4+ absolute counts of 980 cells/µl (Cameroon), 980 cells/µl (Tanzania), 993 cells/µl (Netherlands) and 1095 cells/µl (Turkey) were reported by Janossy *et al.* (2000), Schizlein-Bick *et al.* (2000), Janossy *et al.* (2000) and Yaman *et al.* (2005) respectively. Afolabi *et al.* (2014) suggested that the observed differences in CD4 reference values might be due to the heterogeneity of population as a result ethnoracial variations, inter-laboratory variability and the differences in laboratory methodologies of measuring CD4 count especially where variations were not controlled. In comparison, lower CD4+ mean absolute values of 759 cells/µl (Botswana), 765 cells/µl (Western Nigeria), 775 cells/µl (Ethiopia) and 808 cells/µl (North Central Nigeria) were reported by Bussmann *et al.* (2004), Akinbo *et al.* (2015), Janossy *et al.* (2000) and Afolabi *et al.* (2014) respectively.

The CD4 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 lymphocyte auto-antibodies and procedures like splenectomy (Miyawaki *et al.*, 1984; Maini *et al.*, 1996). Other factors that can cause variations in the CD4 count were type of equipment and techniques used such as multiplatform conventional flow techniques (Singh *et al.*, 2000; Uppal *et al*, 2003; Saxena *et al.*, 2004), processing and analyzing the whole blood samples, staining reagents and fluorochromes, equipment calibration, preference and gating strategies used for the analysis of the results (Gelma *et al.*, 1993; Gratama *et al.*, 1997). ELISA – based immunocapture kits (Kannangai *et al.*, 2000) were used in different studies and could have given rise to procedural and instrumental errors (CDC, 1997).

In this study, females had higher CD4 counts compared to males. The higher values seen in the women in our study as earlier described by other investigators (Oladepo *et al.*, 2009; Miri-Dashe *et al.*, 2014) is also consistent with findings elsewhere (Prins *et al.*, 1999; Lugada *et al.*, 2004). Sex hormone effect could be the possible explanation for the observed gender difference in CD4 counts as the circulating lymphocytes have receptors for androgens and estrogen (Grossman, 1985). Furthermore, Akinbo *et al* (2015) suggested that this might be as a result of haemopoietic feedback stimulus following monthly blood loss due to regular menstruation. It has also been speculated that gender and age related diseases such as auto-immune disorders in female patients (Santagostino *et al.*, 1999; Mair *et al.*, 2007; Ngowi *et al.*, 2009).

Analysis of the CD4 count in the different age groups revealed variations, though it was not statistically significant. Our finding is similar to early studies carried out in Nigeria by Aina *et al.* (2005) who reported that there was no significant difference

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***Indexed By**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate*)

between the CD4 counts when compared by age groups. This is also consistent with the findings of Lugada *et al.* (2004) who found a decrease with age that was not statistically significant. A more comprehensive design is suggested to give a clear picture of how CD4 lymphocyte count changes with age. It is however well known or established that the pattern of T lymphocyte generation with age originate from dynamic changes in thymic as well as extrathymic functions, along with sequential developmental steps from stem cell to ultimately mature cells (Globerson, 1997).

In conclusion, our study has established reference ranges among the adult population of Ekpoma and revealed that the mean CD4+ T lymphocyte count obtained in our study population are different from those of other populations.

ACKNOWLEDGEMENT

The authors wish to acknowledge the management of Ambrose Alli University, Ekpoma, Nigeria, for providing the enablement for this work. We also thank the participants whose consent made this work possible.

REFERENCES

Afolabi, J. K., Fadeyi, A., Desalu, O. O., Durotoye, I. A., Fawibe, A. E., Adeboye, M. A. N., Olawumi, H. O., Babatunde, A. S., Ernest, S. K., Aderibigbe, S. A., Saadu, R., Salami, A. K., Aboyeji, A. P. (2014). Normal CD4 count range among healthy Nigeria population in Ilorin. *J Int Ass Prov Aids Care*; 10:1-8.

Aina, O., Dadik, J., Charurat, M., Amangaman, S., Gurundi, E., Mang, R., Guyit, N., Lar, P., Datong, C., Daniyan, P., Kanki, A., Abimiku, A. (2005). References of CD4 T-lymphocytes in Human Immunodeficiency Virus-negative adult Nigerians.*Clin Diagn Lab Immunol*; 12(4): 525-530

Akinbo, B. D., Atere, A. D., Fatunade, H. B., Iyabor, N. O. (2015). Haematological indices and absolute CD4 counts of apparently healthy population in Ondo State, Nigeria.*Brit J Med Med Res*; 8(8):717-723.

Ansari-Lari, M. A., Muzny, D. M., Lu, F., Lilley, C. E., Spanos, S., Malley, T., Gibbs, R. A. (1996). "A gene-rich cluster between the CD4 and trisephosphateisomerase genes at human chromosome 12p 13". *Genome Res*; 6(4): 314-326.

Aziegbe, F.I. (2006). Sediment sources, Redistribution, and Management in Ekpoma,Nigeria. *Journal of Human Ecology*; 20(4): 259-268.

Bleesing, J. J., Straus, S. E., Fleisher, T. A. (2000).Auto-immune lymphoproliferative syndrome: a human disorder of abnormal lymphocyte survival. *Paediatr Clin North Am J*; 47:1291-1310.

Bussmann, H., Wester, C. W., Masupu, K. V., Peter, T., Gaolekwe, S. M. ,Kim, S., Reich, A. M., Ahn, S., Wu, Y., Thior, I., Essex, M., Marlink, R . (2004). Low CD4+ T lymphocyte values in human immunodeficiency virus-negative adults in Botswana. *Clin Diagn Lab Immunol*; 11(5): 930-935.

Centres for Disease Control and Prevention (CDC)(1997). 1997 revised guidelines for performing CD4+ T-cell determinations in persons infected with human immunodeficiency virus (HIV). *MMWR Recomm Rep*; 46:1-29.

Dapper, V., Emem-Chioma, P., Didia, B. (2008). Some haematological parameters and the prognostic value of CD4, CD8 and total lymphocyte counts and CD4/CD8 cell count ratio in healthy HIV sero-negative, HIV sero-positive and AIDS subjects in Port Harcourt, Nigeria. *Turk J Haematol*; 25:181-186.

Forbi, J. C., Forbi, T. D., Agwale, S. M. (2010). Estimating the time period between infection and diagnosis based on CD4+ counts at first diagnosis among HIV-1 antiretroviral naïve patients in Nigeria. *J Infect Dev Ctries*; 4:662-667.

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***Indexed By**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate*)

Gelman, R., Cheng, S. C., Kidd, P., Waxdal, M., Kagan, J. (1993). Assessment of the effects of instrumentation, monoclonal antibody, and fluorochrome on flow cytometric immunophenotyping: a report based on 2 years of the NIAID DAIDs flow cytometry quality assessment program. *Clin Immunol Immunopathol*; 66:150-162.

Globerson, A. (1997). Thymocytopoiesis in aging: the bone marrow thymus axis. *Arch Gerontol Geriatr*; 24:141-155.

Gratama, J. W., Krawan, J., van der Beemed, R., Hooibrink, B., van Bockstaele, D. R., Hooijkaas, H. (1997).Analysis of variation in results of flow cytometric lymphocyte immunophenotyping in a multicenter study.*Cytometry*. 30:166-177.

Grossman, C. J. (1985). Interactions between gonadal steroids and the immune system. *Science*. 227:257-260.

Hoffman, R. G. (1971). Establishing quality control and normal ranges in the clinical laboratory. Jericho, NY; Exposition Press Inc.

Idoko, J. A., Njoku, M. O., Sirisena, M. D., Jelpe, D. (2001). CD4 T-lymphocyte counts in Human Immunodeficiency Virus (HIV) infected healthy Nigeria population. *Niger Med Pract*. 39: 53-56.

Isobe, M. Huebner, K., Maddon, P. J., Littman, D. R., Axel, R., Croce, C. M. (1986). "The gene encoding the T-cell surface protein T4 is located on human chromosome 12". *Proc of the Natl Acad Sc U. S. A.* 83(12): 4399-4402.

Jain, A., Atkinson, T. P., Lipsky, P. E., Slater, J. E., Nelson, D. L., Strober, W. (1999). Defects of T cell effector function and post-thymic maturation in C-linked hyper-IgM syndrome. *J.Clin Invest.* 103:1151-1158.

Janossy, G., Jani, I., Godhe, W. (2000). Affordable CD4(+) T-cell counts on single platform flow cytometers/primary CD4 gating. *Br J Haematol*; 111: 1198-1208.

Kannangai, R., Prakash, K. J., Ramalingam, S., Abraham, O. C., Matthew, K. P., Jesudason ,M. V.,Sridharan,G. (2000). Peripheral CD4+/CD8+ T-lymphocyte counts estimated by immunocapture method in the normal healthy south Indian adults and HIV seropositive individuals.*J Clin Virol*; 17:101-108.

Lugada, E. S., Mennin, J., Kaharuza, F., Ulvestad, E., Were, W., Langeland, N., Asjo, B., Malamba, S., Downing, R. (2004). Population-based haematologic and immunologic reference values for a healthy Ugandan population. *Clin Diagn Lab Immunol*; 11(1): 29-34.

Maini, M. K., Gilson, R. J. C., Chavda, N., Gill, S., Fakoya, A., Ross, E. J., Phillips, A. N., Weller, I. V. (1996). Reference ranges and sources of variability of CD4 counts in HIV-seronegative women and men. *Genitourin Med*; 72:27-31.

Mair, C., Hawes, S. E., Agne, H. D., Sow, P. S., N'doye, I., Manhart, L. E., Fu, P. L., Gottlieb, G. S., Kiviat, N. B. (2007). Factors associated with CD4 lymphocyte counts in HIV-negative Senegalese individuals. *Clin Exp Immunol*; 151 (3): 432-440.

Malone, J. L., Simms, T. E., Gray, G. C., Wagner, K. F., Burge, J. R., Burke, D. S. (1990). Sources of variability in repeated T-helper lymphocyte counts from human immunodeficiency virus type 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. *J AIDS*; 3(2): 144-151.

Miri-Dashe, T., Osawe, S., Tokdung, M., Daniel, N., Choji, R. P., Mamman, I., Deme, K., Damulak, D., Abimiku,A. (2014). Comprehensive reference ranges for haematology and clinical chemistry laboratory parameters derived from normal Nigerian adults. *PLOS ONE*; 9(5): e93919.

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***Indexed By**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate*)

Miyawaki, T., Taga, K., Nagaoki,T., Seki, H., Suzuki,Y., Taniguchi, N.(1984). Circadian changes of T lymphocyte subsets in human peripheral blood. *Clin Exp Immunol*; 55:618-622.

Mossman, T. R., Coffman, R. L. (1989). TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol;* 7:145-17.

Ngowi B. J., Mfinanga, S. G., Bruun, J. N., Morkve, O. (2009). Immunohaematological reference values in human immunodeficiency virus-negative adolescent and adults in rural northern Tanzania.*BMC Infect Dis*; 9:1.

Nicholson, J. K. (1989). Use of flow cytometry in the evaluation and diagnosis of primary and secondary immunodeficiency diseases. *Arch Pathol Lab Med*; 113:598-605.

Nwokedi, E. E., Ochicha, O., Mohammed, A. Z., Saddiq, N. M. (2007). Baseline CD4 lymphocyte count among HIV patients in Kano, Northern Nigeria. *Afr J Health Sci.* 14:212-215.

Oladepo, D. K., Idigbe, E. O., Audu, R. A., Inyang, U. S., Imade, G. E., Philip, A. O., Okafor, G. O., Olaleye, D., Mohammed, S. B., Odunukwe, N. N., Harry, T. O., Edyong-Ekpa, M., Idoko, J., Musa, A. Z., Adedeji, A., Nasidi, A., Ya' aba, Y., Ibrahim, K.(2009). Establishment of reference values of CD4 and CD8 lymphocyte subsets in healthy Nigerian adults. *Clin & Vaccine Immunol*. 16 (9):1374-1377.

Pattanapanyasat, K., Thakar, M. R. (2005). CD4+ T cell count as a tool to monitor HIV progression and anti-retroviral therapy.*Indian J Med Res.* 121:539-549.

Prins, M. J., Robertson, J. R., Brettle, R. P., Aguada, I. H., Broers, B., Boufassa, F., Foldberg, D. J., Zangerle, R., Coutinho, R. A., Vandean Hoek, A. (1999). Do gender differences in CD4 counts matter. *AIDS.* 13:12361-2364.

Santagostino, A., Garbaccio, G., Pistorio, A., Bolis, V., Camisasca, G., Pagliaro, P., Girotto, M. (1999). An Italian national multicenter study for the definition of reference ranges for normal values of peripheral blood lymphocyte subsets in healthy adults. *Haematologica*; 84:499-504.

Saxena R. K., Choudhry V., Nath I., Das, S. N., Paranjape R. S., Baba G., Ramlingam S., Mohanty, D., Vohra H., Thomas, M., Saxena, O. B., Gangully, N. K. (2004). Normal ranges of some select lymphocyte sub-populations in peripheral blood of normal healthy Indians. *Curr Sci*; 86:969-975.

Schizlein-Bick, C. T., Spritzler, J., Wickening, C. L., Nicholson, J. K., O'Gorman, M. R. (2000). Elevation of Tri Count absolute count tubes for determining CD4 and CD8 cell numbers in human immunodeficiency virus-positive adults. Site investigators and the NIAID DAID New Technologies Evaluation Group. *Clin Diagn Lab Immunol*; 7:336-343.

Singh, Y. G. K., Dar, L., Singh, N. G. B. (2000). Levels of CD4 and CD8 among the inhabitants of Manipur, India. *J Commun Dis*; 32:201-206.

Storek, J., Witherspoon, R. P. (2000). Immunologic reconstitution after haematopoeitic stem cell transplantation. In: Atkinson, K., editor. Clinical bone marrow and blood stem cell transplantation. 2nd ed. Cambridge United Kingdom: Cambridge University Press. Pp 111-146.

Tsegaye, A., Messele, T., Tilahun, T., Hailu, E., Sawu, T., Doorly, R., Fontanet, A. L., Rinke De Wit, T. F. (1999). Immunohaematological reference ranges for adult Ethiopians. *Clin Diagn Lab Immunol*; 6:410-414.

Uppal, S. S., Verma, S., Dhot, P. S. (2003).Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effect of sex, age, ethnicity and smoking. *Cytometry*; 52:32-36.

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***Indexed By**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate*)

Vithayasai, V., Sirisanthana, T., Sakonwasun, C., Savanpiyasiri, C. (1997). Flow cytometric analysis of T-lymphocytes subsets in adult Thais. *Asian Pac J Allergy Immunol*; 15:141-146.

Yaman, A., Cetiner, S., Kibar, F., Tasova, Y., Scydaoghu, G., Dundar I. H. (2005). Reference ranges of lymphocyte subsets of healthy adults in Turkey. *Med Prin Pract*; 14(3): 189-193.

Zhu, J., Paul, W. E. (2008). CD4 T cells: fates, functions, and faults. *Blood*; 112:1557-1569.

AUTHORS CONTRIBUTIONS:

Research idea and design, drafted the work, and data analysis –B. I. O. Medically certified the subjects, contributed reagents/materials and reviewed the write-up-E.P.E. Field work/sample collection and also contributed reagents/materials-E.A.

Endorsed By: *Innovative Science Research Foundation (**ISREF**)* and *International Society of Science Researchers (**ISSCIR**).*
***Indexed By**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*