

Cluster of differentiation 4+ cell count mean value, reference range and its influencing factors in Human Immunodeficiency Virus-seronegative pregnant women in Lagos

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ABSTRACT

Background: Immunity in pregnancy is physiologically compromised and this may affect cluster of differentiation four (CD₄) count levels. It is well established that several factors affect CD₄ count level in pregnancy. This study aims to determine the effects of maternal age, gestational age, parity and level of education as they influence CD₄ count in pregnancy and also to determine the mean and reference range of CD₄ count in pregnancy in Lagos, Nigeria. **Materials and Methods:** A descriptive cross-sectional study was carried out at Ante-natal clinics in Lagos State, Nigeria. About 5 mls of blood was collected into Ethylene Diamine Tetracetic Acid (EDTA) bottles from HIV-negative pregnant women in various gestational ages of pregnancy. CD₄+ cell count and full blood count of all samples were done within 3 hours of collection. The descriptive data was given as means ± standard deviation (SD). Pearson's chi-squared test and correlation were used for analytical assessment. **Results:** A total of 74 pregnant women were recruited. The age range was 19–41 years and a mean age of 30.42 ± 5.34 years. The CD₄+ cell count was not statistically significant when compared with participants ages $P = 0.417$, neither with gestational ages $P = 0.323$, nor with parity $P = 0.247$ nor level of education $P = 0.96$. An overall mean CD₄+ cell count was 771.96 ± 250 cells/μl and the range was 193–1370 cells/μl. **Conclusion:** Maternal age, gestational age, parity and level of education had no significant effects on CD₄+ cell count levels in pregnancy. The mean CD₄+ cell count of HIV-negative pregnant women in Lagos is 771.96 ± 250 cells/μl.

Key words: CD₄+ cell count, mean value, pregnant women, reference range

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INTRODUCTION

Cluster of differentiation four (CD₄) T-lymphocytes and other lymphocytes synchronise the immune system's response to pathogens.¹ In human immunodeficiency virus (HIV)-un-infected individuals, CD₄+ cell count provides a picture of immune system health, with higher CD₄ counts typically signifying healthier immune systems. Children

have high CD₄+ cell counts, which decline slowly through adolescence and then plateau.²

Associated with variations in CD₄+ cell counts in HIV-negative populations are underlying demographic, gender, genetic factors, pregnancy, current exposures to infectious diseases and behavioural factors.³ It has been reported that healthy African and Asian populations typically have lower CD₄ lymphocyte counts than their Western European and Caucasian Counterparts.³⁻⁵ Ironically, cigarette smoking has been associated with higher CD₄ counts in several studies.⁶⁻¹¹ Underlying infectious diseases, such as pneumonia and tuberculosis (TB), have been associated with decreased CD₄ levels.^{12,13} Commercial sex workers (CSW), who are exposed typically to a wide variety of sexually transmitted infections, have somewhat lower lymphocyte counts than females who are not involved in the sex trade.^{14,15} In Western populations, black race, low

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Quick Response Code:	Website: www.nigeriamedj.com
	DOI: 10.4103/0300-1652.129640

body mass index (BMI) and injection drug use have also been associated with lower CD4 lymphocyte counts,^{13,16} and women tend to have CD4 levels 1–200 cells/ μ l higher than men with comparable demographic and behavioural patterns.⁹

In a pilot study by Audu *et al.*,¹⁸ CD4 T lymphocyte reference range was determined amongst apparently healthy male Nigerian blood donors to be 324 to 1,160 cells/ μ l. A mean CD4 count of 742 ± 209 cells/ μ l was also obtained. A reference value of CD4 T Lymphocytes in HIV-negative adult Nigerians was determined by Aina *et al.*,¹⁹ using 864 subjects in 2005. The 95% confidence interval for CD4+ cell counts in healthy adult Nigerians was determined as 547–1,327 cells/ μ l. In a more recent study, higher samples size of 2,570 and a multi-centred study in 2009, involving all the six-geopolitical regions of the country, Oladepo *et al.*,²⁰ established in healthy Nigerian adults a reference value for CD4 of 365–1,571 cells/ μ l, while the reference range for CD8 was 145–884 cells/ μ l. The mean CD4+ cell count of 847 cells/ μ l, in this study, is similar to the mean value of 828 cells/ μ l recorded by Aina *et al.*, in an earlier study in Nigeria. Contrary to reports from the earlier limited study by Aina *et al.*, in Nigeria, females were found to have significantly higher values of absolute CD4+ cell counts, although the results of this study agree with the reportedly higher CD4/CD8 ratios in females. This observation has also been reported in several other countries among Africans and Caucasians,²¹ like Uganda²² and Ethiopia.²³ The effect of sex hormone is one possible explanation for the reported difference in CD4 counts between genders that has been suggested.²³

Several studies have been published on CD4 +cell counts during normal pregnancy. In a study in Maiduguri, Nigeria,²⁴ the normal CD4 T-lymphocyte baseline in healthy HIV-negative pregnant women was determined; the mean CD4 count of the pregnant women was 751.41 cells/ μ l, which was significantly lower than the mean CD4 count of 869 cells/ μ l for the non-pregnant women. Primigravidas had a lower mean CD4 count than both multiparas and grandmultiparas. Similarly, the mean CD4 count was higher in the first trimester than in the later parts of pregnancy. There was no significant difference in the mean CD4 count across all age groups. There was a slight fall in the mean CD4 count in pregnancy, which was more in the first trimester of pregnancy and in primigravidas.²⁴

Aina *et al.*,¹⁹ also reported a lower mean CD4 count of 771 cells/ μ l in pregnancy compared with 828 cells/ μ l for men and non-pregnant females. A similar study in the U.S.²⁵ examined the changes in CD4+ and CD8+ cell levels during pregnancy and post partum in women seropositive and seronegative for HIV-1. The study concluded that the percent CD4+ cell levels declined steadily during pregnancy and post partum among HIV-seropositive women indicating that HIV disease continues to progress during this period.

The percent CD8+ cell levels increased at or near delivery and declined to baseline post partum in both seronegative and seropositive women.

In 1989, a study was published²⁶ of normal pregnancy which found reduced CD4 percentages in the 1st and 2nd trimester, as well as reduced CD4/CD8 ratios in the 2nd trimester. Sridama *et al.*, (1982)²⁷ found reduced absolute CD4 counts, as well as reduced percentages of CD4+ T-cells in 76 women with normal pregnancies.

There is the need to determine the CD4 count mean value and reference range in pregnancy in Lagos which will likely differ from values obtained in other part of the country giving the differences in demographic composition and genetic factor. This study may bring to the fore variability in CD4+ cell counts and offer explanation for this differences.

MATERIALS AND METHODS

A descriptive cross-sectional study was carried out at the ante-natal clinics of the General hospital Isolo and Ifako Ijaiye General hospitals both in Lagos State, Nigeria between January 2012 and February 2012. All consenting patients that screened HIV negative using determine²⁸ rapid kit were recruited into the study. Determine rapid HIV kit is used routinely for screening in the two ante-natal clinics. Approval was obtained from the institutions' research and ethics committees. Participants were asked and aided to fill a structured questionnaire including demographic information, parity, last menstrual period, gestational age and details of drugs history.

Inclusion criteria

All consenting HIV-negative pregnant women. Irrespective of parity, maternal and gestational ages.

Exclusion criteria

All HIV-positive pregnant women.

Collection of samples

About 5 ml of blood was collected into Ethylene Diamine Tetraacetic Acid (EDTA) bottles from HIV-negative pregnant women in various gestational ages of pregnancy on each clinic day at the same time because of the effect of diurnal rhythm on the CD4 count assay. CD4+ cell count and full blood count of all samples were done within 3 hours of collection. Full blood count was done with Sysmex KN-21N, (manufactured by Sysmex corporation Kobe, Japan) a three-part auto analyser able to run 19 parameters.

Cluster of differentiation 4 count assay procedure

Partec Cyflow counter for CD4+ cell count was used for the assay. Into partec test tube (Rohren tube) containing 20 μ l CD4 antibody, 20 μ l of well mixed whole blood was

added. The solution was mixed gently and incubated in the dark for 15 minutes at room temperature. A total of 800 μl of CD4+ buffer was added and mixed gently. Tube was plugged on to the counter and allowed to run ensuring CD4 cells, monocytes and noise are well separated and gated. Results were automatically displayed in the screen in counts/ μl .

The descriptive data was given as means \pm standard deviation (SD). Pearson's chi-squared test and correlation were used for analytical assessment. The differences were considered statistically significant when P value obtained was <0.05 .

RESULTS

A total of 74 pregnant women were recruited, 71 (96%) were married while three (4%) single. Majority of them 55 (74.2%) had tertiary education, 17 (23%) had secondary and two (2.8%) had primary education. More than a quarter of them 25 (33%) were primigravida, 13 (17.6%) gravid two, 14 (19%) gravida three, 10 (13.9%) gravida four and five (6.9%) gravida five. About three-quarters of the patients, 50 of 74 (67.56%) were in third trimester, 18 of 74 (24.32%) in second trimester and only six (8.1%) in first trimester [Table 1].

The age range was 19-41 years and a mean age of 30.42 ± 5.34 years. The gestational age of participants ranged from 10 weeks to 40 weeks and a mean of 31.71 ± 6.58 weeks.

The Kilmogorov-Smirnov and the Shapiro-Wilk tests of normality for CD4+ count and age, gestational age and parity were not significant, which indicates that the data meets the normality assumptions. The relationships between these variables were also linear as determined by scatter diagrams of the variables. The CD4+ count was neither statistically significant when compared with participants ages P value = 0.417, gestational ages P = 0.323, parity P = 0.247 nor level of education P = 0.96. There were insignificant correlation between CD4 count and age r = 0.021 with a significance (two-tailed) of 0.857, gestational age r = -0.013 a significance of 0.927, parity r = 0.059 with a significance of 0.636 and level of education r = -0.018 with a significance of 0.88 [Table 2].

The CD4+ cell count ranged from 193–1,370 cells/ μl , mean CD4+ count in the first, second and third trimesters were 883.50 ± 311.78 cells/ μl , 733.53 ± 267.86 cells/ μl and 777.64 ± 239.99 cells/ μl , respectively. An overall mean was 771.96 ± 250 cells/ μl [Table 3]. Median CD4 + count was 756 cells/ μl while the mode was 500 cells/ μl .

The CD4+cell count had a significant correlation with lymphocyte percentage and number, r = 0.25 with a significance of 0.035 and r = 0.62 with a significance of 0.001, respectively.

Table 1: Socio-demographic parameters

Parameter	Frequency	Percent
Marital status		
Married	71	96%
Single	3	4%
Total	74	100%
Level of Education		
Primary	2	2.8%
Secondary	17	23%
Tertiary	55	74.2%
Total	74	100%
Gravidarity		
Gravida 1	25	33%
Gravida 2	13	17.6%
Gravida 3	14	19%
Gravida 4	10	13.9%
Gravida 5	5	6.9%
Total	74	100%
Gestational age		
First trimester	6	8.1%
Second trimester	18	24.32%
Third trimester	50	67.56%
Total	74	100%

Table 2: CD4+ cells mean values/Semester

CD4+ cells/Semester	Mean value
First trimester	883.50 ± 311.78 cells/ μl
Second trimester	733.53 ± 267.86 cells/ μl
Third trimester	777.64 ± 239.99 cells/ μl
Overall mean	771.96 ± 250 cells/ μl
Reference range	193-1370 cells/ μl

Table 3: Bivariate analysis of CD4+ cells and its influencing factors

Factors	P value	r	significance
CD4+ cell count vs. Participant ages	0.417	0.021	0.857
CD4+ cell count vs. Gestational ages	0.323	-0.013	0.927
CD4+ cell count vs. Parity	0.247	0.059	0.636
CD4+ cell count vs. Level of education	0.96	-0.018	0.88

DISCUSSIONS

Immunity in pregnancy is physiologically compromised and may affect the CD4+ cell count, as lower CD4+ cell count was reported in pregnancy compared with non pregnant females.¹⁹ Reference range of CD4+ cell count amongst Nigerians has been reported earlier by Aina¹⁹ and Oladepo *et al.*,²⁰ and the effects of various variables as they affect CD4+ cell count levels in pregnancy were highlighted. This study considered and established that age, gestational age, parity and level of education did not influence CD4+ cell count in pregnancy.

This study population falls in a bracket adult age (19-41 years), age and CD4+ cell counts were found to be insignificantly related. CD4+ cell count is highest during the early years of life; it declines steadily to a stable adult values and is lowest in the elderly.²⁹ However, Aina *et al.*,¹⁹ reported in pregnant women unlike men and non-pregnant females that the odds of having a low CD4+ cell count were significantly related to both age by decades and age of marriage by decades. They suggested that the effect of marriage age may be due to prolonged exposure to sexually transmitted diseases and other infections, with the development of chronic lymphocytosis in women who got married earlier.

This study also reported an insignificant association between CD4 count and gestational age. There was a slight variation in CD4+ cell count by trimester the highest in first trimester, then the third and lastly the second. Gestational age may or may not affect CD4+ cell count, while some authors reported an increased in CD4 count as gestational age increases.³⁰ Other reported no relationship exist between gestational age and CD4+ cell count in both HIV positive and HIV-negative women.³¹ However, Gomo *et al.*,³² reported a decline in CD4+ cell count by 25 for each week's increase in gestation, among women with low serum retinol.

Parity (<4 versus >4) was found to be insignificantly related to the odds of having a low CD4+ cell count.¹⁹ This study also reported no association exists between the two variables. Similar to our findings, educational level was not significantly associated with CD4+ cell count in Cameroun³³ and Uganda.³⁴

The mean CD4+ cell count of 771 ± 250 cells/μl reported in this study was the exact value reported by Aina *et al.*,¹⁹ among pregnant women in Jos, Nigeria despite use of a much higher sample size of 681. The mean is also similar to the works of Chama *et al.*, (751.41 cells/μl) in Maiduguri, Nigeria,²⁴ Dayama *et al.*, (764 ± 249 cells/μl) in India,³⁵ Eno-Tanjoung *et al.*, in Cameroun³³ (851 ± 254 cells/μl).

The reference range CD4+ cell count obtained in this study (193–1,370 cells/μl) has a much lower limit compared with the study of Aina *et al.*,¹⁹ showing 321 cells/μl. Some of our study participants with low CD+ cell count could have a rare disorder namely idiopathic CD4+ T-lymphocytopenia (ICL). ICL is defined as CD4+ T-cell count of less than 300 cells/μl in the absence of HIV infection or other known causes of immunodeficiency.³⁶ “Determine” –a rapid HIV kit was used to screen participants in this study. The sensitivity and specificity of rapid kit is lower²⁸ than the enzyme linked immunosorbent assay (ELISA) and Western blot used in the Aina study for screening. The implication of this is that some of the recruited patients in our study could have been screened negative by the kit despite been

positive to HIV antibodies. This could account for the low CD4 +cell count reported in some of them. Underlying infectious diseases have been associated with decreased CD4 levels.^{12,13} The pregnant women used in this study were not screened for any underlying infectious diseases, e.g. pulmonary tuberculosis, which could impact on the result. However, the upper limit of 1,370 cells/μl obtained in our study is similar to 1,314 cells/μl reported by Aina *et al.*, amongst pregnant women.

CD4+cell count significantly correlates with lymphocyte percentage and number in this study; this may be due to the fact that CD4 T-cell is a subset of T-lymphocyte and generally function as helper (induce) T-cells. It constitutes 65% of the blood T-cells. They produce lymphokines upon activation by foreign antigens presented by major histocompatibility complex molecules expressed on the surface of antigen-presenting cells.

CONCLUSION

Maternal age, gestational age, parity and level of education had no significant effects on CD4+ cell count levels in pregnancy. The mean CD4+ cell count of HIV-negative pregnant women in Lagos is 771.96 ± 250 cells/μl.

ACKNOWLEDGEMENT

We acknowledge Mr. Oluwamuhuru Phillip O., who carried out the CD4+ cell count and full blood count assays for the authors.

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How to cite this article: Akinbami AA, Dosunmu AO, Adediran A, Adewunmi AA, Rabi KA, Osunkalu V, *et al.* Cluster of differentiation 4+ cell count mean value, reference range and its influencing factors in Human Immunodeficiency Virus-seronegative pregnant women in Lagos. *Niger Med J* 2014;55:116-20.

Source of Support: Nil, **Conflict of Interest:** None declared.