International Journal of Advanced Robotics and Intelligent Systems
Volume 1, Issue 03, 2025

Normal levels of CD4, CD8 T-lymphocytes, and leucocyte baseline in healthy pregnant HIV-seronegative women in Ekpoma, Edo State, Nigeria

FUNKE, CHINASA . Department of Laboratory Systems, USA

Research Article

Date of Submission: 03-04-2025 Date of Acceptance: 6-05-2025 Date of Publication: 09-06-2025

ABSTRACT

The purpose of this research was to determine the baseline absolute counts of CD4 and CD8 in healthy pregnant women in Ekpoma, Edo State, Nigeria. Thirty (30) non-pregnant women (control) and ninety (90) seemingly healthy pregnant women between the ages of 18 and 39 served as research participants. Depending on the trimester of pregnancy, the pregnant participants were split into three groups. Flow cytometry was used to assess the absolute numbers of CD4 and CD8. The leucocyte counts were calculated using an automated blood analyzer. The pregnant women's mean WBC (total) count (x103/µl) was 7.71±1.76 compared to the control individuals' 4.97±0.82. The test and control groups' mean CD4 counts differed statistically significantly, although there was no statistically significant difference between the two groups' total WBC and absolute CD8 levels (P>0.05). Comparing the CD4 absolute count to the control participants' mean levels, however, revealed a statistically significant difference. In conclusion, compared to non-pregnant controls, pregnancy considerably raised the mean WBC count but dramatically lowered the mean CD4+ cell count; however, there was no change in the mean CD8+ cell count among the participants examined.

Key words: Leucocyte counts, CD4 and CD8 counts, healthy pregnant women, and Ekpoma

INTRODUCTION

A protein known as the "CD," or Cluster of Differentiation, is expressed on the surface of hematopoietic system cells. Lymphocyte nomenclature is based on the expression of these proteins (Wingood, 2003). Thus far, more than three hundred (300) "CD" molecules have been identified.As of April 21, 2016, the human CD number was 371 (Zola et al., 2007; HCDM, 2016). These proteins are often linked to a particular cell function. It is a procedure for identifying and examining molecules on the cell surface that serve as targets for cell immunophenotyping (Chan et al., 1988). diverse CD molecules are expressed by cells with diverse functions. For instance, CD8+ cells are cytotoxic T-lymphocytes, CD4+ cells are T-helper cells, and CD3+ cells are total T-lymphocytes.

The CD4 T-lymphocyte plays a key role in immune function regulation. The body's immune response is coordinated by CD4 T-lymphocytes, commonly referred to as T-helper cells. For example, they assist B-cells in producing antibodies and enhance the cellular immunological response to antigens (Kapiga et al., 2009).

The transmembrane glycoprotein CD8 functions as a co-receptor for the T cell receptor (TCR). An important part of the adaptive immune system are CD8 T cells.According to Janeway et al. (2001), cytotoxic T cells, sometimes referred to as TC, killer T cells, or cytotoxic T-lymphocytes (CTL), are a subset of T cells that cause the death of cells that are damaged or malfunctioning or infected with viruses or other pathogens. They have strong cytolytic action against host cells infected with pathogens, but they also play a role during pregnancy by supporting fetal implantation and preventing fetal abortion. In order to regulate T-cell activity, the host regulatory response expresses activated CD8 T cells (Scaife et al., 2006).

A woman's physiology is drastically changed during pregnancy to make room for the growing "organ," the fetus (Loh et al., 2004). The body undergoes several structural (anatomical) and functional (physiological) changes during pregnancy. They are brought on by the demands of the growing fetus, placenta, and uterus as well as the rising levels of pregnant hormones, particularly progesterone and estrogen (Murray and Mcjibbey, 2010). Although physiological changes cause a condition of relative immune-suppression in cellular immune response,

pregnant women are not immune-suppressed in the traditional sense. The mother's immune system has a special difficulty during pregnancy: it must withstand the presence of a semiallogeneic fetus while still mounting a robust defense against invasive infections (Ogawa, 2003).

CD4 and CD8 cell counts are routinely measured in people with HIV infections and are used as indicators to evaluate immunological state and make decisions about starting antiretroviral medication (ART) (Gange et al., 2003). Pregnant women and newborns are the target of several health initiatives, including prevention of mother-to-child transmission (PMTCT), since the introduction of antiretroviral medication (ART) and other measures to enhance maternal and child health. Accurate laboratory reference intervals are necessary for proper interpretation and decision-making when recruiting pregnant women for clinical studies and for general clinical treatment (Miri-Dashe et al., 2014).

In general, there is a dearth of information on the typical ranges of CD4, CD8, and leucocyte counts in Ekpoma, particularly in pregnant women. Establishing suitable normal reference values for T cell subsets among the obstetric population of Ekpoma, Nigeria, would thus be necessary.

MATERIALS AND METHODS

Study Area: The administrative center of the Esan West Local Government Area in Edo State, Nigeria, Ekpoma, is where this research was conducted. The region is located between latitudes 6043N and 6045N of the Equator and longitudes 606'E and 608'E of the Greenwich Meridian. With an area of 502 km2 and a population of 89,628 in 1991 and 127,718 in 2006, it is the fourth biggest town in Edo State. The majority of its residents work as public workers, dealers, businesspeople, farmers, teachers, lecturers, and students. Since being designated as headquarters and the location of Ambrose Alli institution, a state-owned institution, Ekpoma has developed into an urban hub (Aziegbe, 2006).

Study Population: Ninety (90) pregnant women who seemed to be in good health participated in this descriptive cross-sectional research, which was carried out in Ekpoma between January and June 2017 at the maternity clinics of Osemudiamen Specialist Hospital and Esan West Local Government Area. Thirty women who were not pregnant acted as control subjects, and the individuals ranged in age from 18 to 39.

Study Design: Pregnant participants in this research seemed to be in good health. This research only included participants who were seronegative for VDRL, HIV 1/2, and hepatitis B and C viruses. Simple random sampling was used to choose the participants. A questionnaire and permission forms A and B were given to each willing participant to complete and sign, accordingly. Based on the inclusion and exclusion criteria recorded in the questionnaire, each participant was screened in or out.

Inclusion Criteria: The research only included seemingly healthy pregnant women between the ages of 18 and 39 who were seronegative for VDRL, HIV 1/2, and hepatitis B and C viruses.

Exclusion Criteria: Excluded from the research were women who were nursing, had HIV, were menstruation, or were using any kind of oral contraceptive or antiretroviral medication.

Blood Collection: In compliance with biosafety precautionary procedures, four milliliters of blood were drawn by venepuncture from the antecubital vein between 9:00 am and 12:00 pm and placed in ethylene diamine tetraacetic acid (EDTA) tubes. Within six hours of sample collection, all of the samples were tested after being promptly transferred to the laboratory at cold chain temperatures ranging from 20 to 80 degrees Celsius.

Specimen Analysis (A): The national algorithm II was used to assess HIV sero-reactivity. The Determine HIV-1/2 test Kit was used for serial testing in the first instance, and the Unigold HIV-1/2 test was only utilized when

the Determine HIV-1/2 test was sero-reactive and the third kit, Stat Pak (tie-breaker), resolved inconsistent findings. The manufacturers' instructions were followed while using each of the three test kits (Determine, Alere Medical Co. Ltd., Japan; Unigold, Trinity Biotech Plc, Ireland; and Stat Pak, Chembio Diagnostics systems, Inc., USA). When a participant did not respond with the Determine HIV-1/2 quick test kit, they were classified as HIV non-reactive.

Specimen Analysis (B): Following the manufacturer's instructions, the Partec cyflow machine (SysmexPartec GmbH, Görlitz, Germany) was used to calculate the CD4 and CD8 counts. The foundation of the cyflow counter is the simultaneous monitoring of many physical traits of CD4 and CD8 T cells (at various periods) in a single file as it passes past a light source, often a laser beam. Using a gating method, the counter distinguished the CD4+ or CD8+ T cells from the monocytes that carried CD4 or CD8 cells and noise. The Sysmex KX-21-N Haematology auto analyzer (Sysmex Corporation, Japan) was used to determine the total and differential leucocyte counts.

Ethical Approval: In compliance with the code of ethics for biomedical research involving human beings, the University Ethics Committee granted approval for the study. Each subject provided written informed permission. Nonetheless, an interpreter read and explained the consent documents to individuals who were illiterate in their native tongues.

Statistical Analysis: Means ± standard errors of means (SEM) were used to represent the data. Reference values were established at the 2.5th and 95th percentiles, and medians were computed. The analysis of variance (ANOVA) or the Student's t-test, if applicable, were used to establish statistical significance. $P<0.05$ was regarded as significant. SPSS version 21.0 was used for all statistical analyses.

RESULTS

The sociodemographic details of both pregnant and non-pregnant women in the research region are compiled in Table 1. The pregnant women's mean age was 31.70 ± 5.55 years, whereas the control group's mean age was 28.70 ± 6.60 years. The first, second, and third trimesters had mean ages in weeks of 10.30 ± 0.72, 20.10 ± 1.10, and 30.70 ± 0.70, respectively. Civil servants made up the majority of the pregnant individuals recruited for the research, while those with no particular employment (i.e., others) made up the smallest percentage.

The WBC (total), CD4, and CD8 counts of the pregnant and control participants were shown in Table 2. The pregnant women's mean WBC (total) count (x103/µl) was 7.71 ± 1.76 compared to the control individuals' 4.97 ± 0.82. Pregnant women and control patients had a statistically significant increase ($P<0.05$) in this comparison. Pregnant women had mean CD4 absolute counts of 614.49 ± 266.87 cells/µl and 935.40 ± 306.56 cells/µl, respectively, compared to control participants. Pregnant women's CD4 counts were statistically significantly lower ($P < 0.05$) than those of control participants. Pregnant women and control participants had mean CD8 absolute counts of 411.31 ± 161.76 cells/µl and 380.87 ± 126.50 cells/µl, respectively. The mean CD8 absolute counts of the two groups did not vary statistically significantly ($P > 0.05$).

The WBC (total), CD4, and CD8 counts of pregnant women according to trimesters were shown in Table 3. The WBC (total) in the first, second, and third trimesters did not change significantly ($P > 0.05$), with mean values of 7.02 ± 1.71, 7.14 ± 2.15, and 7.33 ± 1.37, respectively. For the first, second, and third trimesters, pregnant women's mean CD4 absolute counts were 578.53 ± 291.43 cells/µl, 563.70 ± 246.05 cells/µl, and 701.23 ± 247.62 cells/µl, respectively. Comparing this to the control participants' mean values revealed a statistically significant difference. When compared to non-pregnant women, the three trimesters' CD8 count findings, expressed as mean ± standard deviation, revealed a non-significant difference ($P > 0.05$).

TABLE 1: SOCIO-DEMOGRAPHIC PROFILE OF APPARENTLY HEALTHY PREGNANT SUBJECTS AND CONTROL

VARIABLES	PREGNANT WOMEN N=90	CONTROL SUBJECTS N=30
Age (mean ± SD in years)	31.70 ± 5.55	28.70 ±6.60
Gestation age (Mean ± SD in weeks)		
First Trimester	10.30 ± 0.72	-
Second trimester	20.10 ± 1.10	-
Third trimester	30.70 ± 0.70	-
Occupation		
Civil servants	35 (38.9)	16 (53.3)
Business women	25 (27.8)	9 (30.0)
Self employed	18 (20.0)	5 (16.7)
Others	12 (13.3)	-
Total	90 (100.0)	30 (100.0)

TABLE 2: THE WBC (TOTAL), CD4 AND CD8 COUNTS OF THE STUDY SUBJECTS

Parameter	Control subjects Mean ± SD N=30	Pregnant subjects Mean ± SD N=90	T-value	P-value
WBC	4.97 ± 0.82	7.17 ± 1.76	6.602	0.000(S)
CD4	935.40 ± 306.56	614.49 ± 266.87	5.492	0.000(S)
CD8	380.87 ±126.50	411.31 ± 161.76	0.939	0.350(NS)

TABLE 3: WBC (TOTAL), CD4 AND CD8 COUNTS OF PREGNANT WOMEN WITH RESPECT TO TRIMESTERS

	CONTROL MEAN ± SD N=30	1^{ST} TRIMESTER MEAN ± SD N=30	2^{ND} TRIMESTER MEAN ± SD N=30	3^{RD} TRIMESTER MEAN ± SD N=30	F – VALUE	P - VALUE
WBC	4.97 ± 0.82^{a}	7.02 ± 1.72^{b}	7.14 ± 2.15^{b}	7.33 ± 1.37^{b}	14.548	0.000
CD4	935.40 ± 306.56^{a}	578.53 ± 291.43^{b}	563.70 ± 246.05^{b}	701.23 ± 247.62^{b}	11.788	0.000

CD8	380.87±126.50[a]	418.73±210.58[a]	419.43±153.65[a]	395.77±109.44[a]	0.441	0.724

Values in rows with different superscripts are significantly different at $p < 0.05$

DISCUSSION

In order to monitor antiretroviral treatment (ART) and determine the extent of immunological damage in HIV-positive persons, CD4 and CD8 counts are often employed prognostic indicators. Changes in T lymphocyte subsets may happen during pregnancy since pregnancy is regarded as a physiologically impaired condition. Establishing baseline values for these counts is necessary, particularly for healthy pregnant women (Danyama et al., 2003).
The average CD4 absolute count in this investigation was 614.49 cells/µl. Compared to earlier authors' findings, Ekpoma's mean CD4 absolute count is lower. Tanjong et al. (2012) found that pregnant individuals in Buea, Cameroon, had a mean value of 851 cells/µl. Similar mean values of 771 cells/µl and 771.9 cells/µl were reported by Aina et al. (2005) and Akinbami et al. (2014) among seronegative pregnant individuals in Ilorin and Lagos, respectively, in Nigeria. Dayama et al. (2003) observed a mean value of 764 cells/µl in Pune, India, whereas Chama et al. (2009) discovered a mean value of 751.41 cells/µl in Maiduguri, Borno State, Nigeria. British pregnant women had higher CD4 counts, according to Makrydimas et al. (1994). Physiological leucocytosis brought on by recurrent infections might be the cause of these elevated levels.

On the other hand, our mean CD4 absolute count number is greater than the mean of 578.3 cells/µl reported by Ekwempuet al. (2012) in another region of Nigeria. Mandala et al. (2017) found a mean value of 468 cells/µl in a recent research conducted in Malawi among pregnant women who were HIV-negative. Additionally, Towers et al. (2010) found that pregnancy dramatically reduces the mean absolute lymphocyte cell count, lymphocyte percentage, and absolute CD4 cell count. African women's absolute CD4+ counts decreased throughout pregnancy, according to Bisalkumi et al. (1992). A lower mean value for American women was also observed by Burns et al. (1996). In the absence of HIV infection, pregnancy alone has been linked to a decrease in T cell and lymphocyte counts across all subsets as well as neutrophilia, which is in line with findings from some studies (Pitkin and Witte, 1979; Valdimarsson et al, 1983; Ballock and Cauchi, 1993; Kuhnert et al., 1998; De Santis, 2011), but not in others (Tallon et al., 1984; Maclean et al, 1992; Watanabe et al., 1997). These cell subsets have previously been shown to be influenced by other variables, such as geography and ethnic group, which may account for the inconsistent changes in total lymphocytes and subsets during pregnancy seen in various studies (Mandala et al., 2010). The additional causes were identified by Ekwempu et al. (2012) as stress or weakened immunity. Pregnancy itself may be linked to the decrease of humoral and cellular immunity in HIV-negative women, according to certain research (Kumar et al., 1984; Weinberg, 1984; Biedermann et al., 1995).

The average CD4 absolute counts for the first, second, and third trimesters in this investigation were 578.53±291.43 cells/µl, 563.70±246.05 cells/µl, and 701.31±247.62 cells/µl, respectively. Gestational age and CD4 mean values were not significantly significant ($P > 0.05$). This result is consistent with earlier studies by Akinbami et al. (2014) and Ekuempu et al. (2012), who also discovered a statistically negligible correlation between CD4 and gestational age. Additionally, Temmerson et al. (1995) found no correlation between immunological markers and the length of pregnancy, and the pattern of immunological markers reported for HIV-positive women was comparable to that of uninfected controls. In contrast to our findings, Okeke et al. (2016) discovered that non-HIV-positive pregnant women had a substantial rise in CD4 from the second to the third trimester. According to Okeke et al. (2016), data demonstrated that pregnancy alone might affect the rise in CD4 cells during the third trimester and that the rise could be due to physiological endocrine changes during pregnancy.
Pregnancy hormones slow down the pace of CD4 cell count drop, however they are not sustainable, according to Lieve et al. (2007). Changes in the CD4 cell count, a subset of the total leucocyte count (TLC), were shown to not substantially affect the pregnant women's total lymphocyte count. However, Towers et al. (2010) noted a considerably reduced CD4 cell count throughout pregnancy and claimed that the trend during pregnancy was u-

shaped, whereas Chama et al. (2009) found that the mean CD4 count was greater in the first trimester than in later phases of pregnancy. According to Anglaret et al. (1994), physiological leucocytosis brought on by infections was the cause of the increased mean CD4 levels in various trimesters.

Pregnant women in Ekpoma had an average CD8 absolute of 411.31±161.76 cells/µl. This result differs from that of Dayama et al. (2003), who reported mean values of 547±56.49 cells/µl. Additionally, Mwinga et al. (2009)

found that the second and third trimesters had mean values of 513.5 and 506.8 cells/µl, respectively. It is important to note that Mwinga et al. (2009) only included HIV-uninfected pregnant women in the second and third trimesters of their research, whereas Danyama et al. (2003) only recruited participants in the third trimester. In contrast to our research, which calculated the mean of all of our individuals throughout the course of the three trimesters, the nature of these two studies may have distorted the results. However, these earlier authors' mean CD8 numbers for these two trimesters were greater than our study's mean values. The increased counts in these individuals may potentially be the result of physiological leucocytosis brought on by recurrent infections, according to Anglaret et al. (1997). Mandala et al. (2017), on the other hand, discovered that 54 pregnant women without HIV had a mean CD8 value of 270 cells/µl. Additionally, Towers et al. (2010) observed a mean absolute CD8+ cell count that did not alter substantially, suggesting that it is unaffected throughout pregnancy. According to other investigators, pregnant African and British women had lower CD8 counts (Bisalinkumi et al., 1992; Makrydimas et al., 1994). Consequently, it is clear that the T-lymphocyte subset count varies by area. variances may also result from the use of different tools and methods in various research, which might have led to instrumental and procedural mistakes in addition to geographical variances.

The mean values for the first, second, and third trimesters were 418.73 cells/µl, 419.43 cells/µl, and 395.77 cells/µl, respectively. According to the statistical analysis, our research participants' gestational age and CD8 did not significantly correlate. Our results are consistent with those of Towers et al. (2010), who likewise discovered no statistically significant differences in the mean absolute CD8+ cell count. Our results, however, differed from those of Dayama et al. (2003), who reported mean values of 547.00 cells/µl among their individuals. Only pregnant women in their third trimester were recruited by Danyama et al. (2003). Furthermore, Mwinga et al. (2009) found that the participants' mean values in the second and third trimesters were 513.5 cells/µl and 506.8 cells/µl, respectively. This might also be caused by the previously stated factors. Mandala et al. (2017) found that the patients they examined had a lower mean value of 270 cells/µl. Reduced CD8 counts during pregnancy were also observed by Bisalinkumi et al. (1992) and Makrydimas et al. (1994). Reduced immunoreactivity during pregnancy is crucial for avoiding the fetus's rejection since, from an immune perspective, it is alien tissue.
Pregnant women in Ekpoma had a mean WBC total count (x103/µl) of 7.71, compared to 4.97 for the control group. In comparison to control patients, pregnant women had a statistically significant increase ($P < 0.05$). Our results are consistent with the findings of Towers et al. (2010), who found that the mean white blood cell (WBC) count was higher than the non-pregnant condition and that this parameter grew over the pregnancy up to and including parturition. According to a another research, African women's total WBC counts were lower throughout pregnancy, even though their leucocyte counts changed similarly to those of Caucasian women (Fleming and Harrison, 1985). On the other hand, Mandala et al. (2017) found that pregnant Malawian women had a mean WBC count of 12.3 (x103/µl), which was higher than our mean figure. According to Geaghan (2009), typical adult males and females have a WBC count between 4,500 and 11,000/µl. Pregnancy increases the white blood cell count; the reference range's lower limit is usually 6,000/cumm (Chandra et al., 2012). Pregnancy-related physiological stress is the cause of leucocytosis (Fleming, 1975). According to differential counts, neutrophils make up the majority of leucocytes (Gatti et al., 1994; Konijnenberg et al., 1997). This is probably because neutrophilic apoptosis is compromised during pregnancy (Gatti et al., 1994).

The WBC (total) count in the first, second, and third trimesters did not change significantly ($P > 0.05$), with mean values of 7.02, 7.14, and 7.33, respectively. This is comparable to the findings of Mwinga et al. (2009),

who discovered mean values of 6.7 in the third trimester and 6.9 in the second. There was no statistically significant difference found when the test and control participants' mean values were compared statistically. The prior study by Mandala et al. (2017), which revealed a mean value of 5.8 among 54 pregnant HIV-uninfected women, was in conflict with our findings. According to Milhorat et al. (1942), this might have resulted from the large rise in neutrophil count during pregnancy, which could have been caused by the stress response, redistribution of WBC during the marginal and circulation pools, or discomfort, nausea, vomiting, and anxiety in the absence of infection. In contrast, Okeke et al. (2016) found that HIV-uninfected pregnant women in another region of Nigeria had lower mean WBC total count (x103/µl) values in the second and third trimesters, respectively, of 2.93 and 3.26. Although the cause of these low numbers is unknown, genetic, environmental, or nutritional variables might be to blame (Shaper and Lewis, 1971; Ezeilo, 1974).

This research's lack of a longitudinal study that would have given a thorough knowledge of immune changes throughout pregnancy is one of its limitations (Gomo et al.

al. (2003). Second, it should be noted that when CD4+ and CD8 are expressed as total counts rather than absolute counts, they have been shown to be stable.
In conclusion, compared to non-pregnant controls, pregnancy considerably raised the mean WBC count but dramatically lowered the mean CD4+ cell count; however, there was no change in the mean CD8+ cell count among the participants examined.

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