www.jmscr.igmpublication.org **Impact Factcor-1.1147**

ISSN (e)-2347-176x

Journal Of Medical Science And Clinical Research
IGM Publication An Official Publication Of IGM Publication

Effects of Pregnancy on CD4 and CD8 T-Lymphocyte subsets and Determination of Best Parameter for Assessing Immunity in Pregnancy

Authors

Babah OA[1], Oluwole AA[1], Adeyemo TA[2], Afolabi BB[1], Oye-Adeniran BA[1] Okorie O[3], Aderolu M[1]

[1] Department of Obstetrics and Gynaecology, Lagos University Teaching Hospital, PMB 12003, Surulere, Lagos, Nigeria.
[2] Department of Haematology, Lagos University Teaching Hospital, PMB 12003, Surulere, Lagos, Nigeria.
[3]Department of Anaesthesia, Federal Medical Centre, Owerri, Nigeria

Correspondence Author
Babah OA.

P.O. Box 7065, Festac town, Lagos, Nigeria

E-mail: *ochuwab@yahoo.co.uk*

Tel: + 234802 359 1137

Abstract:

Objective: The aim of this study was to assess the effect of pregnancy on CD4+ and CD8+ cell counts, CD4/CD8 ratio and CD4 and CD8 percentages and also determine which of these parameters will be most reliable in assessing immunity in pregnancy.

Study design: This was a case-control study involving 80 healthy HIV seronegative pregnant women and 81 healthy HIV seronegative non-pregnant women. Their HIV status was determined after obtaining an informed consent. Their Full Blood Count, CD4+ cell count and CD8+ cell count were measured by standard laboratory methods. CD4%, CD8% and CD4/CD8 ratio were calculated from results obtained. All data collected were subjected to statistical analysis using Epi Info.

Key words: CD4+ cell count, CD8+ cell count, CD4%, CD8%, CD4/CD8 ratio, immunity, pregnancy, reference value

INTRODUCTION

Pregnancy is considered to be a state of physiological immunosuppression.[1] It is assumed to be associated with suppression of a variety of immunological functions in order to accommodate the foreign semi-allogenic foetal graft.[2] It may also partly explain why there may be improvement in some autoimmune disorders such as rheumatoid arthritis during pregnancy and why there may be increased susceptibility to certain infections such as vaginitis, urinary tract infection, malaria and influenza.

The immune system can be divided into two broad categories viz: the humoral and the cell-mediated immune system. This study dwelled basically on the cell-mediated arm of the immune system. Cellular immunity is conferred by the lymphocytes. All lymphocytes arise from stem cells in the bone marrow. There are two subsets of lymphocytes – the B-cells and the T-cells. The B-cells are precursors of plasma cells and they secrete specific antibodies whereas the T-cells are derived from the thymus.[3]

There are three subsets of T-cells namely the cytotoxic T-cells or natural killer (NK) cells which kill foreign or virus infected cells directly; the T-helper cells which assist the B-cells in antibody response and other T-cells in cell-mediated immunity; and the T-suppressor cells which inhibits the activities of the B-cells and other T-cells.

The helper T-lymphocytes express the cluster determinant 4 (CD4+) molecules while the cytotoxic T-cells express cluster determinant 8 (CD8+) molecules. Both CD4+ cell count and CD8+ cell count are used to measure the strength of an individual's immune response. Other parameters that can be used as indicators of immune status include CD4/CD8 ratio, CD4 and CD8 percentages.[1]

It is a well known fact that pregnancy alters functioning of most body systems such as the cardiovascular, respiratory, endocrine, renal and haematological systems. The immune system is not left out. Immunological changes in pregnancy include an increase in total leucocyte count by 30% basically due to an increase in neutrophils as lymphocyte count remains unchanged and there is a slight reduction in immunoglobulin G levels and an increase in immunoglobulin D levels with an increased susceptibility to infections.[3]

CD4+ and CD8+ molecules are members of the immunoglobulin superfamily and mediate adhesion to major histocompatibility complex class II and class I molecules respectively. CD4 and CD8 molecules also amplify stimulatory signals through the T-cell receptors.[4,5,6]

A lot is known about the effects of pregnancy on other body systems like the haematological system, but much less is known about the immunological changes. Hence, it becomes important to carry out more researches in this aspect of obstetrics.

Assessment of an individual's immune status, is essential in staging some diseases such as human immunodeficiency virus (HIV) infection and

acquired immune deficiency syndrome (AIDS), in determining when to commence treatment such as anti-retroviral therapy and in predicting risk of complications and debilitating infections. With the advent of HIV/AIDS, a state of pathological immunosuppression, a lot is now known about the effect of immunosuppression on the cluster determinant cells. Most widely studied of these cluster determinant cells being CD4 count. HIV is known to infect CD4 cells and cause a reduction in the absolute CD4 cell count.[7] In an attempt for the HIV-specific CD8 T-cells to help lower viraemia, there is an initial increase in the CD8 absolute cell count.[8]

Pregnancy is generally considered a state of physiological immunosuppression but very few researches have been done to determine its effects on the cluster determinant cells and most of these studies were conducted in foreign countries outside West Africa. Worse is the fact that interpretation of results of CD4 and CD8 counts in pregnant women with pathological immunosuppressive conditions such as HIV/AIDS is based on reference values for healthy non-pregnant women.

In Nigeria, there are country-specific reference ranges for a number of haematological parameters but there are no national data for CD4 reference values yet.[9]

A number of conditions have been linked to immunosuppression. These include infective processes such as viral (for example, HIV infection), bacterial (for example, tuberculosis) or parasitic infections. Others are stressful conditions like burns, trauma, psychological stress, malnutrition, steroid use, physiological states such as pregnancy and even normal daily variations.[10]

Most of the pathological conditions listed above can occur in pregnancy and there may be a need to assess immune status of the pregnant woman in such situations. Pregnancy being a state of physiological immunosuppression, can be associated with an alteration in T-lymphocyte subsets.[1] However, baseline reference values for these T-lymphocyte subsets in pregnancy are not available and needs to be determined. Using reference range derived from healthy non-pregnant women may not be appropriate in pregnant women. Hence, there is a need to establish baseline values in healthy pregnant women.[1]

More so, apart from CD4 cell count, other parameters for assessing immunity are not being explored despite availability of the kits. These parameters include CD8 cell count, CD4/CD8 ratio, CD4% and CD8%. There is the possibility that these parameters might be more reliable than CD4 cell count in assessing cellular immunity in pregnancy.[11] This study therefore sought to:

- Determine the effect of pregnancy on CD4 cell count, CD8 count, CD4/CD8 ratio, CD4% and CD8%.
- Establish a reference range for each of these immunologic parameters (CD4 cell count, CD8 cell count, CD4/CD8 ratio, CD4% and CD8%) in pregnancy.

- Determine which of these parameters (CD4 cell count, CD8 cell count, CD4/CD8 ratio, CD4% and CD8%) best assessed immune status in pregnancy.

METHODOLOGY

Study design: This was a case-control study.

Study setting: This study was conducted at the Lagos University Teaching Hospital (LUTH), the largest tertiary health institution in Lagos state, Nigeria, after due approval by the hospital's Health Research and Ethics Committee (HREC) with approval number ADM/DCST/HREC/282. For this study, apparently healthy pregnant women were recruited from the hospital's ante-natal clinic and apparently healthy non-pregnant women were recruited from the hospital's family planning clinics. Blood samples were analyzed for HIV antibody, Full Blood Count, CD4+ cell count and CD8+ cell count at the Central Research Laboratory, College of Medicine, University of Lagos (CMUL).

Study population: This comprised 80 apparently healthy, HIV seronegative pregnant women who gave informed written consent, selected across the three trimesters of pregnancy. Gestational ages were calculated using the last menstrual period. Where a subject was unsure of her last menstrual period or there was discrepancy with the scan date, the earliest scan date was used. Eighty one (81) controls, matched for age and socio-economic status were selected from amongst apparently healthy, HIV seronegative non-pregnant women, who gave informed written consent. Age in the context of this study referred to a case and corresponding control that fell within the same 5 year age grouping designed for this study (that is, 20-25 years, 26-30 years, 31-35 years, 36-40 years and 41-45 years). Socio-economic status was determined using the occupation of subject and that of her husband, using the National Readership Survey (NRS) grades.[12]

Women excluded from the study were those with a history of clinical illness such as recent malaria, tuberculosis, renal disease, diabetes mellitus, sickle cell disease, asthma, rheumatoid arthritis, autoimmune disorders, pelvic inflammatory disease, etc; those with history of blood transfusion in the last one month or steroid use in the last one week, pregnant women in labour, those with history of membrane rupture, multiple gestations and congenital fetal anomaly.

Data collection: Prior to recruitment of eligible subjects for this study, each subject had individual counselling during which the purpose of the study was duly explained to the patient, voluntary counselling and testing for HIV was also given and an informed written consent obtained. Information was collected by direct questioning and/or from case notes using the proforma designed for this study. The information collected included sociodemographic data of the patient, last menstrual period and estimated gestational age (if pregnant), past medical, social and drug histories. The socioeconomic class of the subjects were determined using the patient's occupation,

her level of education and her husband's occupation.

Five millilitres (5mls) of blood was collected by venepuncture from the forearm of each subject into a vacutainer containing the anticoagulant EDTA, after the patients had rested for at least 15 minutes. Serial numbers only were assigned to each specimen for identification of the subjects (both cases and controls). Rapid HIV screening was conducted on all blood samples collected to ensure they were seronegative before subjecting them to further analysis. Full Blood Count was determined by standard method and the absolute CD4 cell count and absolute CD8 cell count were determined by flow cytometry. Their CD4%, CD8% and CD4/CD8 ratios were calculated from the total lymphocyte count obtained in the Full Blood Count and the CD4 and CD8 absolute counts.

All specimens were transported to the laboratory within 2 hours of collection and were stored at 2 to 8^{o}C until analyzed. All samples were however analyzed on the same day the specimens were taken after excluding Human Immunodeficiency Virus (HIV) infection.

For determination of CD4 and CD8 absolute cell counts, 20µl of CD4 PE antibody and CD8 PE antibody respectively (depending on what parameter was being analyzed) were added into a Partec test tube (Rohren tube) and 20µl of well mixed whole blood (in EDTA bottle) were added. These were mixed gently and incubated in the dark for 15 minutes at room temperature. Eight hundred microlitre (800µl) of the CD4 or CD8 buffer respectively were then added and mixed gently. The CD4 absolute count and CD8 absolute count were then analyzed using the Cyflow counter.

Full Blood Count was performed for each subject and the total lymphocyte count derived from this. The total lymphocyte count, CD4 absolute cell count and CD8 absolute cell count obtained were used in calculating the CD4%, CD8% and the CD4/CD8 ratio.

Data management: The data obtained were analyzed using the Epi Info statistical software (August 2008, version 3.5.1). The mean values of CD4 cell count, CD8 cell count, CD4/CD8 ratio, CD4% and CD8% of each group and the standard deviations were also determined. Student t-test and analysis of variance (ANOVA) were used where applicable in comparing values between groups. A p-value of less than 0.05 was considered to be statistically significant.

The population reference ranges[13,14] were established using the 95% prediction interval.

Study limitations: The limitations of this study were:

1. A variety of other factors which might influence CD4 and CD8 cell levels such as micronutrient deficiencies and psychological stress were not taken into consideration in this study.
2. Some patients might have been on drugs such as steroids, antibiotics etc that can

alter the CD4 and CD8 cell levels without being aware and thus give inappropriate information.

3. Some patients who tested negative to Human immunodeficiency virus (HIV) by rapid test might actually be in the window period.

RESULTS

Socio-demographic characteristics of patients: A total of 161 apparently healthy, HIV seronegative women were recruited for this study after giving their informed consent. Of these 80 were pregnant women while 81 were non-pregnant women. The mean age ± S.D of all subjects was 32.3 ± 4.59 years, with a range of 22 – 45 years and a median of 32.0 years. The mean age± S.D of the pregnant women was 31.7 ± 4.16 years while that of the non-pregnant women was 32.9 ± 4.94 years. There was no statistically significant difference in the mean age in both groups (t = 1.6341, p = 0.1042). They were predominantly married women (96.3% of study population), of low parity (predominantly para 0 – 2), who attained tertiary level of education (78.9% of study population) and belonged to middle social class C (65.8% of study population). There was no statistically significant difference in the age group and socioeconomic class of the two populations studied, that is healthy pregnant and non-pregnant women, p = 0.9999 and 0.9968 respectively (*Table I*).

Distribution pattern of study population: In order to test the normality of the study population, a histogram was constructed using the CD4 count of the subjects. To further enhance clarity of the distribution pattern, the histogram constructed was converted to a regression curve using Microsoft office excel 2007 version. The distribution curve obtained for the study population was bell shaped similar to a classical Gaussian curve with minimal skewing. The study population may therefore, be assumed to have a normal distribution (*Figures 1a and 1b*).

Effects of pregnancy on leucocytes and T-lymphocytes subsets: The mean ± S.D values of CD4 count in non-pregnant and pregnant women were 920 ± 255 cells per microlitre and 729 ± 232 cells per microlitre respectively. The mean ± S.D values of CD8 count on the other hand for non-pregnant and pregnant women were 429 ± 160 cells per microlitre and 405 ± 186 cells per microlitre respectively. The mean ± S.D values of CD4% and CD8% for non-pregnant were 39.5 ± 8.4% and 18.3 ± 5.2% respectively and for pregnant women 36.5 ± 12.2% and 20.0 ± 9.07% respectively. The mean ± S.D value of CD4:CD8 ratio was 2.40 ± 0.77 in non-pregnant women and 2.01 ± 0.76 in pregnant women (*Table II*). None of the women in both arms (pregnant and non-pregnant) had CD4 count below 350 cells per microlitre.

This study showed a mean ± S.D value of total leucocyte count of 5822 ± 1794 cells per microlitre in the non-pregnant women and 7752 ± 2017 cells per microlitre in pregnant women; giving a 33% increase in total leucocyte count in pregnancy from the non-pregnant value (t =

6.4177, p = 0.0000). On the other hand, the mean ± S.D value of absolute neutrophil count rises from 2885 ± 1307 cells per microlitre in the non-pregnant to 5140 ± 1659 cells per microlitre in pregnancy and this translates to a 78.2% increase in absolute neutrophil count in pregnancy from non-pregnant values (t = 9.5860, p = 0.0000). The mean ± S.D value of absolute lymphocyte count was found to be 2375 ± 628 cells per microlitre in the non-pregnant and 2068 ± 504 cells per microlitre in pregnancy, translating to a decrease in lymphocyte count by 12.9% in pregnancy from the non-pregnant value (t = 3.4257, p = 0.008).

It was observed that the mean CD4 and CD8 cells, their percentages and the CD4:CD8 ratio change in pregnancy from the non-pregnant values. Although this alteration was statistically significant for CD4 count and CD4/CD8 ratio ($p < 0.05$), there was no statistically significant difference in the changes that occur in the CD8 count and CD4 and CD8 percentages ($p > 0.05$) as shown in *Table II*.

This change was more marked with the CD4 count which decreased by 20.8% in pregnancy from the non-pregnant value. The immune parameter that was least affected by pregnancy was the CD8 count which decreased by 5.8% from non-pregnant value. The CD4:CD8 ratio decreased by 16% from the non-pregnant value.

Reference values for immune parameters in pregnancy: The reference ranges established for the population in this study for the various immune parameters analysed using the 95% prediction interval are as shown in *table II*. The standard reference range for CD4 count for non-pregnant women was 466 – 1373cells per microlitre while for pregnant women it was established to be 262 – 1195 cells per microlitre. The reference ranges for the CD4/CD8 ratio for non-pregnant and pregnant women were 0.85 – 3.95 and 0.48 – 3.54 respectively.

Variations in levels of immune parameters during pregnancy: This study showed that during pregnancy there is a significant decrease in total lymphocyte count as pregnancy progresses (p = 0.0063). A progressive decrease in the absolute CD4 cell count and CD4% during pregnancy was also observed and these changes were statistically significant (p = 0.0000 and 0.0492 respectively). However an initial rise in the CD8 and CD8% percentage was observed and this reached a peak in the second trimester (with the value of the CD8% rising slightly above the non-pregnant value all through gestation) and subsequently falls but these changes are not statistically significant (p = 0.7353 and 0.4436 respectively). The mean CD4/CD8 ratio increases slightly in the first trimester above the non-pregnant value and thereafter falls progressively as pregnancy progresses and this change was found to be statistically significant (p = 0.0006). *Table III and figures 2a – 2d* summarizes the effect of pregnancy on the immunologic parameters assessed in this study.

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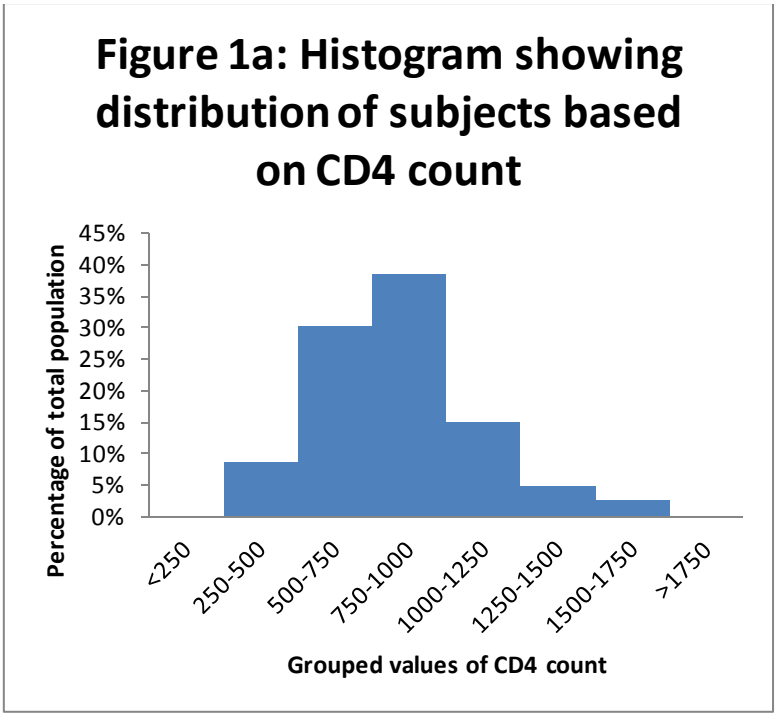
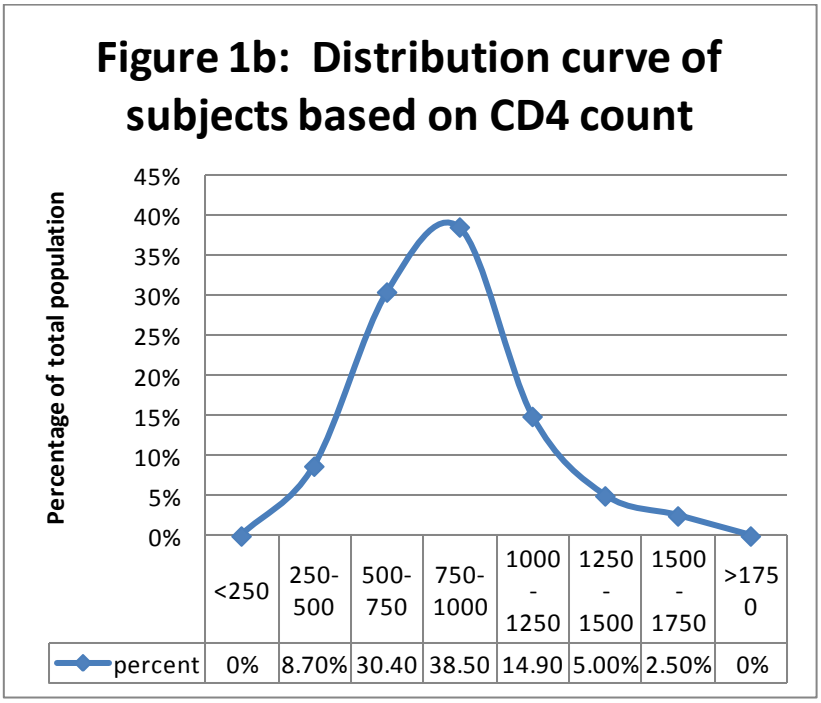
Table I: Sociodemographic characteristics of study population

PARAMETER	Non-pregnant, n = 81	Pregnant, n = 80	Total
Age (years)			
20 – 25	5 (3.1%)	5 (3.1%)	10 (6.2%)
26 – 30	26 (16.1%)	26 (16.1%)	52 (32.3%)
31 – 35	31 (19.3%)	31 (19.3%)	62 (38.5%)
36 – 40	16 (9.9%)	15 (9.3%)	31 (19.3%)
41 – 45	3 (1.9%)	3 (1.9%)	6 (3.7%)
Total	81 (50.3%)	80 (49.7%)	161 (100%)
$\chi^2 = 0.0260, df = 4, p = 0.9999$			
Marital status			
Single	5 (3.1%)	0 (0.0%)	5 (3.1%)
Married	75 (46.6%)	80 (49.7%)	155 (96.3%)
Divorced	1 (0.6%)	0 (0.0%)	1 (0.6%)
Total	81 (50.3%)	80 (49.7%)	161 (100%)
$\chi^2 = 6.1553, df = 2, p = 0.0461$			
Educational status			
None	1 (0.6%)	0 (0.0%)	1 (0.6%)
Primary	2 (1.2%)	1 (0.6%)	3 (1.9%)
Secondary	20 (12.4%)	10 (6.2%)	30 (18.6%)
Tertiary	58 (36.0%)	69 (42.9%)	127 (78.9%)
Total	81 (50.3%)	80 (49.7%)	161 (100%)
$\chi^2 = 5.6134, df = 3, p = 0.1320$			
Socioeconomic class			
A	0 (0.0%)	0 (0.0%)	0 (0.0%)
B	9 (5.6%)	8 (5.0%)	17 (10.6%)
C	53 (32.9%)	53(32.9%)	106 (65.8%)
D	17 (10.6%)	17 (10.6%)	34 (21.1%)
E	2 (1.2%)	2 (1.2%)	4 (2.5%)
Total	81 (50.3%)	80 (49.7%)	161 (100%)
$\chi^2 = 0.0526, df = 3, p = 0.9968$			
Parity			
0	33 (20.5%)	34(21.1%)	67 (41.6%)
1	19 (11.8%)	17 (10.6%)	36 (22.4%)
2	14 (8.7%)	17 (10.6%)	31 (19.3%)
3	6 (3.7%)	7 (4.3%)	13 (8.1%)
4	6 (3.7%)	5 (3.1%)	11 (6.8%)
5	2 (1.2%)	0 (0.0%)	2 (1.2%)
6	1 (0.6%)	0 (0.0%)	1 (0.6%)
Total	81 (50.3%)	80 (49.7%)	161 (100%)
$\chi^2 = 3.5781, df = 6, p = 0.7335$			

NOTE: Figures are presented as frequency (percentage of total population), $p < 0.05$ is considered to be statiatically significant. Grade A is the highest social class while grade E is the lowest social class.





NOTE: Although there is a positive skew, this is minimal and the study population may be assumed to have a normal distribution.

Table II: Mean values and reference ranges of immunologic parameters in healthy non-pregnant and pregnant Nigerian women

IMMUNOLOGIC PARAMETER	MEAN VALUE IN NON PREGNANT WOMEN (n = 81)	MEAN VALUE IN PREGNANT WOMEN (n = 80)	PERCENTAGE CHANGE IN PREGNANCY	t test	p-value
Total leucocyte count (cells/ml)	5822 ± 1794 (2212 - 9432)	7752 ± 2017 (3693 - 11812)	33.2% increase	6.4177	0.0000
Neutrophil count (cells/ml)	2885 ± 1307 (255 - 5515)	5140 ± 1659 (1801 - 8479)	78.2% increase	9.5860	0.0000
Lymphocyte count (cells/ml)	2375 ±628 (1111 - 3639)	2068 ± 504 (1053 - 3083)	12.9% decrease	3.4257	0.0008
CD4 count (cells/ml)	920 ± 255 (466 - 1373)	729 ± 232 (262 - 1195)	20.8% decrease	4.9837	0.0000
CD8 count (cells/ml)	429 ± 160 (107 - 752)	405 ± 186 (30 - 779)	5.8% decrease	0.7630	0.4469
CD4%	39.5 ± 8.4 (22.6 – 59.4%)	36.5 ± 12.2 (11.9 – 61.1%)	7.6% decrease	1.8196	0.0707
CD8%	18.3 ± 5.2 (7.9 – 28.7%)	20.0 ±9.07 (2.0 – 38.6%)	9.3% increase	1.4720	0.1434
CD4:CD8 ratio	2.40 ± 0.77 (0.85 – 3.95)	2.01 ± 0.76 (0.48 – 3.54)	16.0% decrease	2.5750	0.0113

NOTE: Figures are presented as mean ± standard deviation and figures in paracentesis are established population reference ranges for immune parameters using 95% prediction interval.

Table III: Alteration in level of immunologic parameters in various trimesters of pregnancy

IMMUNOLOGIC PARAMETERS	NON-PREGNANT STATE (n = 81)	FIRST TRIMESTER (n = 24)	SECOND TRIMESTER (n = 29)	THIRD TRIMESTER (n = 27)	P-value
Mean lymphocyte count (cells/ml)	2375 ± 628	2166.7 ± 542.7	2048.3 ± 491.1	2000 ± 488.3	0.0063
Mean CD4 count (cells/ml)	920 ± 255	821.3 ± 189.5	759.4 ± 299.8	662.1 ± 241.7	0.0000
Mean CD8 count (cells/ml)	429 ±160	379.1 ± 189.8	418.3 ± 193.1	413.2 ± 181.8	0.7353
Mean CD4%	39.5 ± 8.4	38.6 ± 7.0	38.1 ± 17.2	33.0 ± 8.4	0.0492
Mean CD8%	18.3 ± 5.2	19.7 ± 13.9	21.6 ± 11.9	20.7 ± 8.0	0.4436
Mean CD4/CD8 ratio	2.40 ± 0.77	2.44 ± 0.91	1.88 ± 0.56	1.77 ± 0.66	0.0006

NOTE: Figures are presented as mean ± standard deviation. Analysis of variance (ANOVA) was used to test for statistical significance between the means of all four groups.

Figure 2a: Changes in lymphocyte count during pregnancy

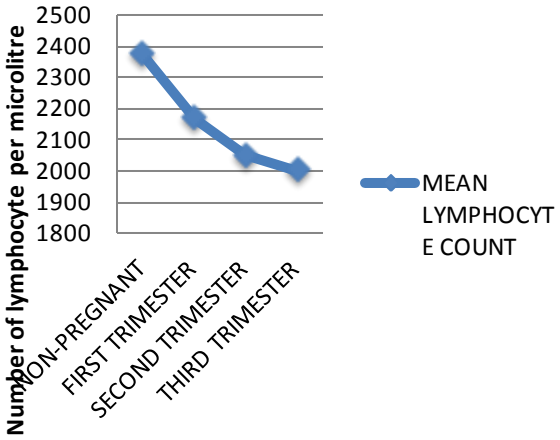


Figure 2c: Changes in CD4% and CD8% during pregnancy

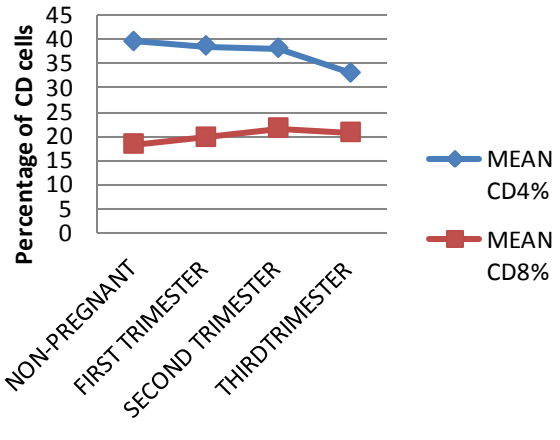


Figure 2b: Changes in CD4 and CD8 counts during pregnancy

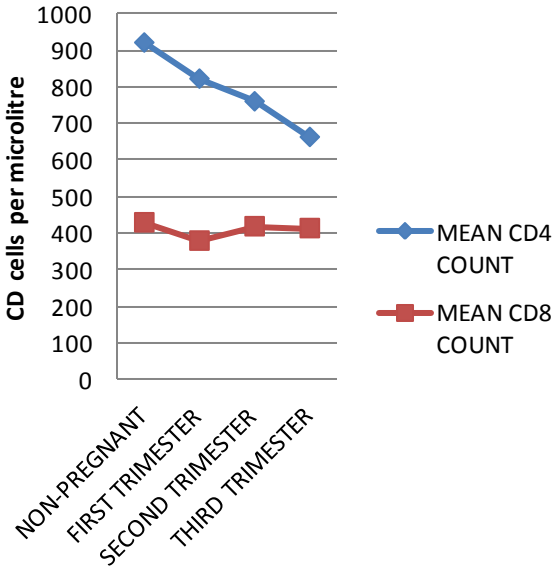
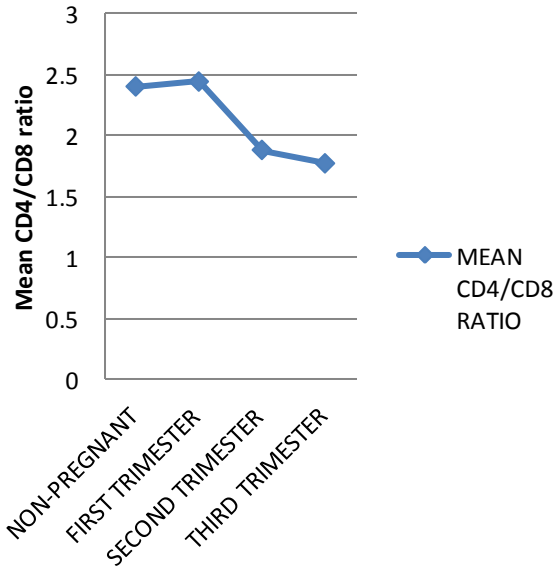


Figure 2d: Changes in CD4/CD8 ratio during pregnancy



DISCUSSION

Pregnancy is considered a state of physiological immunosuppression. This study has further re-emphasized this phenomenon. The population studied may be assumed to have a normal distribution, considering the distribution curve which is quite similar to a Gaussian curve. We observed in this study that the total leucocyte count increases in pregnancy by 33% from the non-pregnant value. This is similar to findings in earlier study.[3] This increase in total leucocyte count in pregnancy observed in this study could be attributed to the neutrophil count which increases markedly in pregnancy by 78%. A slight decrease in lymphocyte count by 13% in pregnancy was noted in this study.

As in earlier studies, this study showed a significant decrease in CD4 count in pregnancy.[1,15,16] There was a 20.8% decrease in absolute CD4 count in pregnancy, with mean values of 919.5 cells per microlitre in non-pregnant women and 728.6 cells per microlitre in pregnant women. A mean CD4 count of 771 cells per microlitre in pregnant women and 828 cells per microlitre in non-pregnant women were obtained in the study by Olumuyiwa A et al in Plateau state, Nigeria and 751.4 cells per microlitre for pregnant women and 869 cells per microlitre for the non-pregnant in the study by Chama CM et al in Maiduguri, Nigeria. When the figures obtained from this study was compared with those from the local studies cited above, it was observed that the mean CD4 count in pregnant women in southern Nigeria was lower than those obtained in Northern Nigeria while the values were higher in non-pregnant women in southern Nigeria.[15,16] Could there be a geographical variation in the levels of these immunological parameters? If this be the case, then there would be a need to conduct similar research work in other states of the federation.

The change in CD8 count, on the other hand is minimal. This study revealed a mean value of 429 cells per microlitre for absolute CD8 count in non-pregnant women and 405 cells per microlitre in pregnant women. In India a reduction in CD8 count has also been documented.[1] Similar observation was also made by Watanabe et al who found that the numbers of CD4 and CD8 cells decrease during pregnancy but did not change significantly after delivery.[17]

When this haemodilution effect of pregnancy is taken into consideration by comparing the percentage counts of these cells (CD4% and CD8%), it was found that the CD4% falls by 7.6% in pregnancy while the CD8% shows a 9.3% rise in pregnancy. This trend (a decrease in CD4% and increase in CD8% in pregnancy) was also observed in a study by Dayama A. et al in India.[1] A reduction in CD4 cells (helper cells of the immune system) and an initial increase in CD8 cells (which has been known to be a reflection of the activities of the cytotoxic T-cells following an infection) has been seen in immunosuppressed states such as in human immunodeficiency virus (HIV) infected people. This further buttresses the fact that pregnancy is a state of physiological immunosuppression.

Although none of the women recruited for this study had CD4 count less than 350 cells per microlitre, the reference ranges established for CD4 count in healthy HIV seronegative pregnant women (262.3 – 1194.9 cells per microlitre in this study) showed that in the general population, some healthy HIV seronegative pregnant women might have values below 350 cells per microlitre whereas this is less likely in the non-pregnant population with an established reference range of 466 – 1373.0 cells per microlitre. Similar observations have been made in earlier studies. In India a CD4 count range of 250 – 1776 cells per microlitre was obtained for healthy HIV seronegative pregnant women and 539 – 1627 cells per microlitre for non-pregnant women.[1] In Maiduguri Nigeria, CD4 count range was 267 – 1318 cells per microlitre in healthy HIV seronegative pregnant women and 401 – 1713 cells per microlitre in the non-pregnant.[16]

However the current National Guideline for Prevention of Mother to Child Transmission of HIV (PMTCT) has recommended the commencement of antiretroviral drugs in all pregnant women with CD4 count below 350 cells per microlitre irrespective of the clinical stage of the disease.[18] Bearing in mind that even pregnant women without the disease can have values that meet this criteria, it therefore follows that using 350 cells per microlitre might cause unnecessary intervention in women with the disease with its cost implication and attendant consequences such as development of toxicity and resistance to the drugs. This further buttresses the need to establish specific reference ranges for immune parameters in pregnancy in our locality.

When a comparative study was made to know the changes that occur in the levels of these immune parameters as pregnancy progresses by trimesters, it was found that the absolute lymphocyte count, CD4 count and CD4% falls progressively in pregnancy while the CD4/CD8 ratio rises slightly above non-pregnant value in first trimester and thereafter falls progressively as pregnancy advances. These changes may be accounted for by the physiological and psychological stress pregnancy poses as it advances. On the other hand, the CD8 count and CD8% rises and peak in the second trimester and then falls slightly in the third trimester. This might be as a result of initial rise in the CD8 cells in response to the decrease in CD4 cells. The CD8% remains slightly elevated above the non-pregnant level all through gestation. Although the changes in CD8 count and CD8% by trimester as pregnancy progresses do not show any statistically significant difference, that in CD4 count, CD4% and CD4/CD8 ratio show statistically significant difference, probably because of the marked alteration in levels of CD4 cells in pregnancy.

The observation of an increase in CD8 count at midgestation is similar to an earlier finding in mice, in which it was shown that there is an increased turnover of CD8 T-cells during pregnancy and that the number of maternal CD8 T-cells in both the spleen and the uterine draining lymph nodes are transiently increased at mid-gestation and this correlates with enhanced CD8

T-cell proliferation and an increase in relative expression of both pro-survival and pro-apoptotic molecules[19]. In another study also conducted in mice, it was found that the sustenance of pregnancy is exceedingly CD8 dependent as depletion of CD8 T-cells led to a termination of the pregnancy protective effect of progesterone substitution[20].

Although a study in Zimbabwe[21] had revealed an association between gestational age and CD4 count, this study showed that gestational age does not significantly affect CD4 count in healthy seronegative pregnant women.

As regards which of the immune parameters studied might be best used in assessing immunity in pregnancy, considering the fact that CD4% and CD8% counts do not show change significantly in pregnancy compared to their absolute counts, they may be better used in assessing immunity. This study also showed that amongst the immune parameters studied, CD4/CD8 ratio show narrow range of values and bearing in mind the alterations that occur in the absolute CD4 and CD8 counts in pregnancy and that CD4/CD8 ratio is an immunologic parameter that combines the two major subsets of T-lymphocytes (the helper cells and the cytotoxic cells) that impact on immunity, it might be best to use this in assessing immunity in pregnancy. However, further longitudinal studies will be necessary to further explore these findings.

CONCLUSION

Pregnancy is indeed a state of physiological immunosuppression. There is an appreciable reduction in the CD4 count in pregnancy and this alteration is less with the CD8 count. Reference ranges established in pregnancy in this study showed that some healthy pregnant women in the general population might have CD4 count below 350 cells per microlitre, hence it might not be appropriate to use 350 cells per microlitre in determining when to start antiretroviral drugs in HIV seropositive pregnant women as is the current practice as per the national guideline. More so, there seem to exist a geographical variation in CD4 counts even within country. These further buttresses the need for a meta-analysis of data from different parts of the country to establish a country specific reference ranges for the immunologic parameters especially in pregnancy.

The CD4/CD8 ratio which showed narrow range of values and combines the two major categories of immune cells (the helper and the cytotoxic T-lymphocytes) might be the best parameter in assessing immunity in pregnancy, even in regions like ours where no country-specific reference range exist.

RECOMMENDATIONS:

1. It is important to bear in mind that pregnancy is a state of physiological immunosuppression and so there might be a need to lower cut-off values of immune parameters such as CD4 count in

pregnancy when planning intervention, rather than use same cut-off derived for the non-pregnant women or men in the general population.

2. Values of immune parameters varies from country to country, hence there is a need to establish a reference range in Nigeria.
3. The CD4/CD8 ratio which showed narrow range of values and assesses the two major cell types of the immune system (helper and cytotoxic T-cells) might be the best parameter for assessing immunity in pregnancy.

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