International Journal of Medical Science in Clinical Research and Review
Online ISSN: 2581-8945
Available Online at http://www.ijmscrr.in Volume 3|Issue 06 (November-December) |2020 Page: 201-208
Original Research Paper

A LONGITUDINAL STUDY OF CD4+ CELL COUNT AND LYMPHOCYTE (PERCENTAGE AND ABSOLUTE) COUNTS OF PREGNANT WOMEN ATTENDING ANTENATAL CLINICS AT NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL, NNEWI, NIGERIA.

Authors:
[1]Aloy-Amadi Oluchi C,[2]Akujobi Augustine U, [1]Nwadike Constance N, [1]Nnodim Johnkennedy, [3]Opara Chioma P,[4]Ahaneku Osuji and [5]Okafor Roseanne Adah
Department of Medical Laboratory Science, Imo State University, Owerri, Imo State, Nigeria.
Department of Optometry, Imo State University, Owerri, Imo State, Nigeria.
Department of Haematology and blood transfusion, NnamdiAzikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.
Department of Microbiology and Immunology, University of Abuja Teaching Hospital, Gwagwalada Abuja, Nigeria.
Department of medical Laboratory Science Rivers State University Port Harcourt Nigeria.
Corresponding Author:
Aloy-Amadi Oluchi C

Article Received 05-10-2020, Accepted 02-11-2020, Published 06-11-2020

ABSTRACT:
Background: Pregnancy is associated with changes in the hormonal and immunological environment in order to support a healthy pregnancy. The maternal immune system during pregnancy is altered to actively tolerate the semi-allogeneic fetus. Aim: To determine the levels of CD4+ cell and lymphocyte (percentage and absolute) counts of pregnant women attending antenatal clinics at Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria. **Methods**: One hundred and sixty (160) apparently healthy pregnant women who presented for booking in their first trimesters at the antenatal clinics of NAUTH, Nnewi constituted the study population. An equivalent number of age-matched non-pregnant women were used as the control groups. Five milliliters (5ml) of venous blood was collected from each subject by means of a hypodermic syringe and needle using a standard clean venipuncture technique. 2 mls was aliquoted into plain tubes for screening for HIV 1 & II, Hepatitis B surface Antigen (HBs Ag), Hepatitis C virus (HCV), while the remaining 3 mls was placed into potassium EDTA anticoagulated tubes for malaria parasite screening, CD4+ cell and lymphocyte (percentage and absolute) counts. The CD4+ cell counts were performed using parteccyflow counter, while the lymphocyte counts were done using automated haematology analyzer. The same procedures were conducted on non-pregnant controls and pregnancy test was also done to confirm they were not pregnant. The pregnant women were followed up till the last trimester. **Results**: The mean levels of CD4+ cell count in first (660.12± 484.92), second (625.45± 160.17), and third (621.92 ± 159.40) trimesters were significantly decreased compared to the control subjects (764.27 ± 182.58) ($F=11.3$, $P<0.001$).lymphocyte percentage showed a significant reduction in the first (33.83±35.05), second (31.15±32.48) and third (29.25±30.52), compared to controls (38.05±39.40) ($F=136.4$, $P<0.001$). Equally, absolute lymphocyte count was significantly decreased when the first (2.36±0.47), second (2.09±0.51) and third (1.85±0.52) were compared to the controls (3.10±0.76) ($F=181.5$, $P<0.001$). When compared across the trimesters the mean CD4+ cell count showed no significant decrease from the first (660.12 ±484.92), to the third (621.92±159.40) trimester ($F=1.0$, $P=0.386$) while the percentage and absolute lymphocytes were significantly decreased from the first trimester. (33.83 ± 35.05) and (2.36±0.47), to the last trimester (29.25 ± 30.52) and (1.85 ± 0.52 ($F=50.4$, $P< 0.001$) and ($F = 53.2$, $P<0.001$). **Conclusion**: This study has shown that levels of $CD4^+$ cell count and lymphocyte counts are decreased in pregnancy. Therefore, proper management of pregnancies at risk is needful to prevent unhealthy pregnancy outcome.

Keywords: Pregnancy, CD4+ Cell Count, Percentage lymphocyte, Absolute Lymphocyte, Longitudinal.

INTRODUCTION:

Pregnancy is the term used to describe the period in which a fetus develops inside a woman's womb or uterus (spong, 2013). It is divided into three trimesters. The first trimester is from week one through week 12. The second trimester is from week 13 through 28, and the third trimester is from week 29 through 40 weeks (shriver, 2015). Pregnancy requires physiologic adaptations in all maternal systems, including the immune system. This process is complex and includes modifications at different levels and compartments of the maternal immune system (Luppi, 2003). The hormonal and immunological changes that occur over the course of pregnancy are necessary to support a healthy pregnancy, but also dramatically affects female susceptibility to autoimmune and infection diseases (Raghupathy, 1997). The maternal immune system during pregnancy is altered to actively tolerate the semiallogeneic fetus. These alterations include changes in local immune responses, that is in the uterine mucosa /decidua (Moffetand Colucci, 2014) and changes in peripheral immune responses (Veenstra Van Nieuwenhoven*et al*., 2003). After implantation, the uterine endometrium is rapidly infiltrated by fetal trophoblest cells. The endomentrium will then develop into the deciduas and ensure anchorage of the placenta and therefore proper fetal nutrition. However, this invasion needs to be properly regulated to protect the coporal integrity of the uterine wall of the mother, Both swallow and over-invasion will lead to problematic pregnancies (Moffet12 –king et al., 2002). Local decidual immune cells, such as uterine natural killer (uNK) cells and macrophages, are important regulators of this balance between tolerance of fetal trophoblasts and limitation of their invasion (Naruse 15 et al., 2009). When placental circulation is established, the peripheral blood also comes into close contact with fetal cells, specifically, villous trophoblasts. This may affect the peripheral maternal immune response.

Cluster of differentiation four (CD4+ cells)/ T-helper cells are white blood cells that fight infection. They are made in the spleen, lymph nodes and thymus gland, which are part of the lymph or infection, fighting system. They move throughout the body, helping to identify and destroy germs such as bacteria and viruses. Along with other tests, the $CD4^+$ T-lymphocytes synchronize the immune systems response to pathogens (Tsegaye*et al*., 2003). Previous studies have been published on $CD4^+$ cell counts during normal pregnancy. For example, Ufelle*et al*., (2017), showed that $CD4^+$ count decreased in pregnancy compared to non-pregnant females and decreased significantly as pregnancy progressed. Aina*et al*., 2005, also reported a lower mean $CD4^+$ count of 771 cells/µl in pregnancy compared to 828cells/µl for men and non-pregnant women. $CD4^+$ counts significantly correlates with lymphocyte percentage and number and this may be due to the fact that $CD4^+$ cell is a subset of T-lymphocyte and generally function as helper (induces) T-cells (Akinbami et al., 2014).

The decrease in $CD4^+$ cell counts at different gestational ages could be attributed to increasing physiological demand during pregnancy and the changes in the hormonal environment of pregnancy contribute to local suppression of cell mediated immunity at the maternal fetal interface (Bakalor et al., 2001). An earlier study among African women demonstrated reduced absolute values of $CD4^+$, $CD8^+$ and total lymphocytes in pregnancy (Dayama et al., 2003). According to Oladepo*et al*., (2009), immunity in pregnancy is physiologically compromised and may affect the $CD4^+$ cell count, as lower $CD4^+$ cell count was reported in pregnancy compared to non-pregnant females.

MATERILAS AND METHODS:

Study Site:

The study was carried out at the antenatal clinic of NnamdiAzikiwe University Teaching Hospital (NAUTH), Nnewi Nigeria, a government tertiary health-care institution serving Nnewi Local Government Area and its environs. The climate of Nnewi is of equatorial type, with temperature that ranges from 25-33^{o}c annually. Geographically, Nnewi falls within the tropical rain forest region of Nigeria, There are two main seasons- the rainy season, which starts from March to September and dry season, which starts from October to February. The population of Nnewi is about 1,047,309.

Subjects:

One hundred and sixty(160) apparently pregnant women who presented for booking for antenatal care in their first trimester visit at NAUTH, Nnewi were enrolled into the study after approval from ethical committee of NAUTH and written informed consent obtained from the pregnant women. Their age range was between 20-40 years. Also 160 age-matched non-pregnant women served as the control group.

Study Design:

This research was a cohort study carried out at the antenatal care clinic of NAUTH, Nnewi, Anambra State, from February to December, 2016. All pregnant women who presented for booking for antenatal care in their first trimester visit were recruited for the study. They were enrolled after providing their informed consent. Questionnaires were administered to their medical and obstetrics history (age, parity, gestational age etc). Pregnancy and its duration were confirmed by ultrasound scan. During the first trimester visit, the blood pressure was measured using a sphygmomanometer, and at subsequent trimester. The weight and height measured were used to calculate the body mass index (BML).

These pregnant women were screened and those who were not eligible were excluded. One hundred and sixty (160) apparently healthy pregnant women were enrolled in the research as the study group, and their age range was 20-40 years. On the other hand, 140 age-matched non-pregnant women were enrolled in the research as the control group. Pregnancy test was conducted on the non-pregnant females to confirm they were not pregnant. These pregnant women were on iron supplements and were followed up till the last trimester. The same tests were conducted on the non-pregnant women at NAUTH laboratories. At the second trimester (5^{th} month), 156 pregnant women were followed up, and only 140 pregnant women completed the study at the third trimester (8^{th} month). These pregnant women could not complete the study because they miscarried had still birth, changed address or felt the study was a disturbance to them. Results from pregnant women were compared to the controls, and comparisons were also made across the trimesters.

Sample Collection:
A standard clean venipunture technique was used to collect fivemillilitres (5mls) of blood from each subject by means of a hypodermic syringe and needle. Twomillilitres (2mls) was aliquoted into gel tubes for screening for HIV I and II, Hepatitis B surface Antigen (HBsAg), Hepatitis (virus) (HCV) and VDRL, and the remaining 3mls was placed into potassium EDTA anticoagulated tubes for malaria parasite screening, and $CD4^+$ T-cell count and lymphocyte (percentage and absolute) counts. The $CD4^+$ T-cell absolute counts were done using the parteccyflow counter, while the lymphocyte counts were performed using the automated haematology analyzer.

Ethical Consideration:
The research study was approved by the Ethics committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, and Ethics Committee, an informed consent was obtained in writing before recruiting each subject into the study.

Statistical Analysis:
Statistical analysis was done using computer software package for social sciences (SPSS). All comparisons were performed using one-way analysis of variance (ANOVA). Statistical significance was calculated using post hoc test to analyse the results of the experimental data. Differences were considered to be significant at $p<0.05$.

RESULTS:

Table 1.0 Demographic Data Analysis of the Pregnant Women Studied

(N = 160)	M (SD) N(%)
Age (years	28.13±4.33
Bmi (kg/m^2)	28.17±4.15
Gravidity	
Primigravida)	98 (61.25)
Multigravida	62(38.75)
Occupation	
Civil Servants/Private employees	72(45.00)
Traders	48(30.00)
Housewives	29(18.13)
Students	11(6.87)
Education	
Tertiary	104(65.00)
Secondary	54(33.75)
Primary	2(1.25

Key:

BMI - Body Mass Index
N - No of Subjects
M - Mean
SD - Standard Deviation

Table 1.0, shows the characteristics of the pregnant women studied. The mean age of the pregnant women were 28.13 ± 4.23 and body mass index(BMI), 28.17 ± 4.15, 61.25% of the pregnant women were primigravidas, while 38.75% were multigravidas, 45.00% were civil servants/private employees, 30.00% traders, 18.13%, house wives and 6.87%, students.

65.00% attained tertiary education, 33.75%, Secondary, and 1.25%, primary.

Table 2.0 Levels of Immunological Parameters of Pregnant Women based on Trimesters compared to non-pregnant women.

Control/Trimester	$CD4^+$ (Cells/µl)	Percentage Lymphocyte (%)	Absolute Lymphocyte ($X10^3$/L)
Control[c] N = 160	764.27±182.58	38.05±39.40	3.10±0.76
First Trimester N = 160	660.12±484.92	33.83±35.05	2.36±0.47
Second Trimester N= 156	625.45±160.17	31.15±32.48	2.09±0.51
Third Trimester N =140	621.92±159.40	29.25±30.52	1.85±0.52
F(p-value)	11.3(<0.001)*	136.4(<0.001)*	181.5(<0.001)*
		Post Hoc	
C Vs 1st	0.001*	<0.001*	<0.001*
C Vs 2nd	<0.001*	<0.001*	<0.001*
C v 3rd	<0.001*	<0.001*	<0.001*
1st Vs 2nd	0.602	<0.001*	<0.001*
2nd Vs 3rd	0.999	<0.001*	<0.001*
1st Vs 3rd	0.522	<0.001*	<0.001*

Key:

Mean difference is significant at P<0.05
C = Control
SD = Standard Deviation
Trim = Trimester

The mean levels of $CD4^+$ cell count in first (660.12±484.92), second (625.45±160.17) and third (621.92±159.40) trimester were significantly decreased compared to the control subjects (764.27±182.58) (F=11.3, P<0.001). Lymphocyte percentage showed a significant reduction in the first (33.83±35.05), second (31.15±32.48) and third (29.25±30.52), compared to controls (38.05±39.40) (f=136.4, p<0.001). Equally, absolute lymphocyte was significantly decreased when the first (2.36±0.47), second (2.09±0.51) and third (1.85±0.52) were compared to the controls (3.10±0.76) (f=181.5, P<0.001).

Post Hoc Analysis:

The mean levels of CD4+ cell count in non-pregnant women (764.27±182.58) was significantly higher compared to the first trimester (660.12±484.92) (p=0.001). The mean level of $CD4^+$ cell count in second trimester (625.45±160.17) was statistically decreased significantly when compared to that of the controls (764.27±182.58) (P<0.001). Similarly, that of third trimester (621.92±159.40) was significantly decreased when compared to controls (764.27±182.58) (P<0.001). When the mean level of CD4+ cell count in the first trimester (660.12±484.92) was compared to the second trimester (625.45±160.17), no statistical significant difference was observed (p=0.602). That of third trimester (621.92±159.40) also showed no statistical significant decrease compared to the second (625, 45±160.17) (p=0.99).Equally, the mean levels of CD4+ cell count in first trimester (660.12±484.92), showed no statistical significant difference when compared to the third trimester (p=0.522). The lymphocytes showed a statistically significant decrease when the first when the first (33.83±35.05), second (31.15±32.48), and third (29.25±30.52) trimesters were compared to the controls (38.05±39.40), the second (31.15±32.48) compared to the first (33.83±35.05), the third (29.25±30.52) compared to the second (31.15±32.48) and the first (33.83±35.05),the third (29.25 ± 30.52) compared to the second (31.15±32.48) and first (33.83±35.05),(P<0.001).A statistically significant decrease was observed in the absolute lymphocyte values when the first (2.36±0.47), second (2.09±0.51) and third (1.85±0.52) trimesters were compared to the controls (3.10±0.76), the second (2.09±0.51) and third (1.85±0.52) trimesters compared to the first (2.36±0.47), and when the third trimester (1.85±0.52) was compared to the second (2.09±0.51) (p<0.001) (Table 2.0).

Table 3.0: Levels of Immunological Parameters in Pregnant Women at Different Trimesters (Mean ± 8D).

Trimester	$CD4^+$ (Cells/µl)	Percentage Lymphocyte (%)	Absolute Lymphocyte ($X10^3/L$)
First Trimester N=160	660.12±484.92	33.83±35.05	2.36±0.47
Second Trimester N=156	625.45±160.17	31.15±32.48	2.09±0.51
Third Trimester N=140	621.92±159.40	29.25±30.52	1.85±0.52
F(P-value)	1.0(0.386)	50.4(≤0.001)*	53.2(<0.001)*
Post Hoc			
1st Vs 2nd	0.494	<0.001*	<0.001*
2nd Vs 3rd	0.993	<0.001*	<0.001*
1st Vs 3rd	0.425	<0.001*	<0.001*

Key:
*** Significant at P<0.05**
Trim = Trimester

The mean levels of CD4+ cell count compared across the first (660.12 ± 484.92), second (625.45 ± 160.17) and third (621.92±159.40) trimesters were not significantly decreased statistically (F=1.0, P=0.386). The mean levels of lymphocyte was significantly reduced statistically from the first (33.83 ± 35.05) to the third (29.25 ± 30.52) trimester (F=50.4, P≤0.001). Absolute lymphocyte was significantly lowered statistically from the first (2.36±0.47) to the third (1.85 ± 0.52) trimester (F=53.2, P<0.001).

Post Hoc Analysis:
There was no significant decrease in the $CD4^+$ cell count when the second (625.45 ± 160.17) and the third (62.92 ± 159.40) trimesters were compared to the first trimester (660.12±484.92) (P=0.494) and (P=0.425), and when the third trimester (621.92±159.40) was compared to the second (625.45 ±160.17)(p=0.993). The lymphocyte wassignificantly lowered when the second (31.15±32.48) and third (29.25±30.52) trimesters were compared to the first (33.83 ± 35.05) (p<0.001). Similarly, absolute lymphocyte was significantly reduced when the second (2.09±0.51) and third (1.85±0.52) trimesters were compared to the first (2.36±0.47) (p<0.001) (Table 3.0)

DISCUSSION:

This study revealed that, the mean $CD4^+$ cell count was significantly lower at all trimesters compared to the non-pregnant women. This could be probably due to the fact that during pregnancy, the immune system is compromised. Changes in the hormonal environment may play a role in the suppression of maternal immune response. It agrees with the study done by Aina*et al*., (2005) and Ufelle*et al*., (2017), who reported a lower mean CD4+ count in pregnancy compared to non-pregnant women. According to Bakalor*et al*., (2001), the decrease in CD4+ cell counts at different gestational ages could be attributed to increasing physiological demand during pregnancy and the changes in the hormonal environment of pregnancy contribute to local suppression of cell medicated immunity at the maternal-fetal interface. The decrease in CD4+ cell count in pregnancy maybe due to the fact that pregnancy is an immune-compromised state which alters T-lymphocyte subsets (Tanjong*et al*., 2012). In pregnancy, immune function is suppressed and the state of pregnancy represents an extreme challenge for the immune system. The maternal immune system during pregnancy is altered in order to tolerate the semi-allogeneic fetus (Chen *et al*., 2012). This study also showed that the $CD4^+$ cell count progressively declined from the first to the third trimesters significantly. This decrease might be because of the increasing physiological demand associated with pregnancy.It agrees with the work done by Ufelle*et al*., (2017), who stated that CD4+ cell count was significantly decreased, when compared to non-pregnant women and decreases as gestational age increases, but disagrees with the work of Akinbami, (2014), who reported an insignificant association between CD4+ count and gestational age. The progressive decrease in CD4+ cell count at different gestational ages could be attributed to increasing physiological demand during pregnancy and the changes in hormonal environment. Gomo*et al*., (2004), also reported that no relationship exists between gestational age and CD4+ cell count in HIV negative women.

In this study the lymphocytes were significantly decreased in pregnancy compared to non-pregnant women. This could be as a result of immune suppression associated with pregnancy. *Osonuga et al., (2011)* and *Obeagu et al., (2013)* showed the same

pattern. When compared in all the trimesters, they were also significantly reduced from the first to the third trimester. *Osinuga et al., (2011) and Obeagu et al., (2013)* observed percentage lymphocytes to increase in second trimester with a decrease in the third trimester. *Duria et al., (2017),* showed that lymphocyte counts were comparable during the first two trimesters, but dropped significantly over the last one, while *Chandra et al., (2012)* observed lymphocyte counts to be decreased through the first and second trimesters and increased during the third trimester. The decrease in lymphocyte count can be attributed to change that occur during pregnancy due to the development and growth of a fetus and may be because, pregnancy is an immunocomprised state which results in a weakened immunosystem.

REFERENCES:

1. Abuye c, Tsegaye A, West C.E, Versloot P, Sanders E. J and Wolday D (2005). Determinants of $CD4^+$ counts among HIV negative Ethiopians: Role of body mass index, gender, cigarette smoking, Khat (Catha edulis) chewing, and possibly attitudes. *Journal of Clinical Immunology*; **25**:127-133.
2. Aina O, Dadik J, Charurat M, Amangaman P, Gurundi S, and Mang E (2005). Reference of values of $CD4^+$ T Lymphocytes in human immunodeficiency virus negative adult Nigerians. *Clinical diagnostic Laboratory Immunology*; **12**:525-530.
3. Akinbami A.A, DosunmuA. O, Adediran A, Adenunmi A. A, Rabiu K.A, Osunkalu V, Ajibola S, Uche E. L and Adelekan A (2014). Cluster of differentiation 4 cell count mean value, reference range and its influencing factors in Human Immunodeficiency Virus Seronegative Pregnant women in Lagos*; Nigerian Medical Journal*; **55(**2): 116-120
4. Audu R. A, Idigbe E.O, Akamu A.S, Maje A.G, and Onyemide J (2007). Values of CD4 T lymphocyte in apparently healthy individuals in Lagos; *Nigerian European Journal of Scientific Research***16**:168-173.
5. Bakalor V.K, Link A.A and Dimiton M.A (2001). IL-12, TNF-Alpha and hormonal changes during late pregnancy and early post-partum; implications for autoimmune disease activity during these times. *Journal of Endocriminology*; **86**:4933-4938.
6. Bisalinkumi E, Nawrocki P, Chao A, Bu Iterys M, Dushimimana A, Mugabo E and Saah A (1992). T-cell subset changes during and after pregnancy in a cohort of HIV – Sero (+) and Sero (-) African mothers; 8th International Conference. AIDS. Abstract No. PoA 2086.
7. Bussmann H, William W.C, Masupu K.V, Peter I Gaolekwe S.M, Mim S, Reit A.M, Ahns, Wu I, Thior I, Essex mand Richard M (2004). Low $CD4^+$T-Lymphocytes values in Human Immunodeficiency Virus negative adults in Botswana; *Clinical Diagnostic Laboratory of Immunology*; **11** (5); 930-935.
8. Chama C.M, Morrupa J.U, Abuja U.A, and Kayode A (2009). Normal CD4+-Lymphocyte baseline in healthy HIV negative pregnant women. *Journal of Obstetrics And Gynecology*; **29**:702-704.
9. Charles A.J, Jr Paul Travers, Mark Walport and Mark J.S hlomchick (2001). Imunobiology: The immune system in health and disease. New York, Garland Science; 5th edition; 1-10.
10. Chen S.J, Liu Y.L and Sytwu H.K (2012). Imunologic regulation in pregnancy; from mechanism to therapeutic strategy for imuno modulation. *Clinical Developmental Immunology*; **183**-184.

11. Dayama A, Pandi D, Muddiar S, BlaraDway R, Bharcha K.E and Schrotri A.N (2003). A Pilot study on $CD4^+$ and $CD8^+$ cell counts in healthy HIV sero-negative pregnant women. Indian *Journal of Medical Research*; **117**:198-200.
12. Dayama A, Pandi D, Mudeliar S, Blaradway R, Bharucha K.E and Shrotri A.N (2003). A pilot study on CD4+ and CD8+ Cell counts in healthy HIV sero negative pregnant women. *Indian Journal Of Medical Research*: **117**:198-200.
13. Dekel N, Gnainsky T, Granot 1 and Mor G (2010). Inflammation and implantation. *American Journal OF Reproductive Immunology*; **63**:17-21.
14. Leber A, Teles A and Zenchussen A.C (2010). Regulatory T-cells and their role in pregnancy. *American Journal of Reproductive Immunology*; **63**:445-459.
15. Luppi P (2003). "How Immune mechanisms are affected by pregnancy". Vaccine; **21**(42), 3352-3357.
16. Moffet A, and Colucci F. (2014). Uterine NK cells: active regulators at the maternal fetal interface. *Journal of Clinical Investigation*: **124** (5): 1872-1879.
17. Moffett A, and Loke C (2006), Immunology of placentation in eutherian mammals. Nature Reviews Immunology: **6(8)**:584-594.
18. Moffett- King A, Entrican G, Ellis S, Hutchison J, and Bainbridge D (2002). Natural Killer cells. *Trends Immunology*; **23(7):** 332-333.
19. Moffett-King A (2002). Natural Killer cells and pregnancy. *Nature Reviews Immunology*; **2 (**9) 656-663.
20. Mor G (2008). Inflammation and Pregnancy: The role of toll-like receptors in trophoblastic immune interaction. *Annals of New York Academic Science*; **1127**:121-128.
21. Mor G and Cardenas I (2010). The immune system in pregnancy: a unique complexity. *American Journal of Reproductive Immunology*; **63**:425-433.
22. Nanzigu S, Wacko P, Petzold M, Kiwanuka G, Dungah it and Makumbi F (2011). CD4+ T-Lymphocyte reference ranges in Uganda and its influencing factors. Laboratory Medicine; **42**:9-10.
23. Naruse K, Lash G.E, Innes B.A et al., (2006). Localization of matrix metalloproteinase MMP)-2, MMP- a and tissue inhibitors for MMPs (TIMPs) in uterine natural killer cells in early human pregnancy. Human Reproduction. **24(3)**:553-561.
24. Oladepo D.K, Idigbe E.O, Audu RA, Inyang U.S, Made E and Philip A. O (2009). Establishment of reference values of CD4 and CD8 lymphocytes subsets in healthy Nigerian adults. Clinical Vaccine Immunology; **16**: 1374-1377.
25. RaghupathyR(1997). This type of immunity is compatible with successful pregnancy. *Immunology Today*: **18**:478-482.
26. Shriver E.K (2015). Pregnancy condition information; National Institute of child health development.
27. Spong, C. Y(2013) Defining "term" pregnancy :Recommendations from the Defining :"Term

Pregnancy workgroup. JAMA, 309(13), 2445-2446. Retrieved October 28, 2013. From http://jama.jamanetwork.com/article.aspx? article 1D= 1685467.

28. Tsegaye A, Wolday D, Otto S, Petros B, Assefa T and Alebachew T (2003). Immuno phero typing of blood lymphocytes at birth, during childhood, and during adulthood in HIV -1- in infected Ethiopians. *Clinical Immunology*; **109**:338-364.
29. Ufelle S.A, Onyekwelu K.C, Ezeh R.C, Ikekpeazu J.E and Ibegbu D.M (2017). The CD4+ T Lymphocyte values of HIV-Positive and HIV Negative pregnant women in Enugu, South-East Nigeria. *Biomedical Research*; **28** (14): 6282-6286.
30. Venstra Van Nieuwenhoven A.L, Heineman M.J and Fass M.M (2003). *The Immunology of Successful Pregnancy*; **9(4):** 347-357.