CD4, CD8 T-LYMPHOCYTES AND WBC PROFILES DURING PREGNANCY AMONG HIV-SERONEGATIVE WOMEN IN WUKARI, NORTH EAST, NIGERIA

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Abstract: This study aimed at instituting the standard values of CD4 and CD8 total counts among apparently healthy pregnant women in Wukari, Nigeria. Sixty (60) apparently healthy pregnant women age group of 18 – 40 years and thirty (30) non- pregnant women (control) age 18-40 were used as participants in the study. The pregnant individuals were divided into three groups, based on the trimester of pregnancy. CD4 and CD8 total counts were determined using flow cytometer. Automated blood analyzer was used to determine the leucocyte counts. The white blood cells (WBC) mean total count (x103/µl) of the pregnant women was 7.67±1.74 against 4.88 ± 0.64 of the control participants. The mean CD4 counts of pregnant women against the control individuals were 589.64 ±212.86 and 888.60 ± 296.66 cells/µl, respectively. This showed a significant decrease ($P < 0.05$) in the CD4 count of pregnant women compared to control individuals. The mean CD8 counts of pregnant women and control individuals were 387.18±122.33 and 316.66 ± 116.10 cells/µl, respectively. There was no significant difference ($P > 0.05$). The mean CD4 counts of pregnant women for the first trimester, second trimester and third trimester were 579.66 ± 269.33, 570.54 ±234.16 and 698.33 ± 254.74 cells/µl, respectively. This showed a significant difference from control individuals. For CD8 count, the results presented in mean ± standard deviation showed a non-significant difference ($P> 0.05$) in the three trimesters when compared with non-pregnant women.Comparisonshowed significance variations in the mean CD4 counts between the test and control but no significant difference in the total WBC and CD8 counts of both groups ($P>0.05$). In conclusion, the study showed that pregnancy significantly increased the WBC count but decreased CD4+ cell total count when compared tocontrol individuals while the mean CD8+ cell total count did not show any difference in the studied.

Keywords: CD4 counts, CD8 counts, leucocytes counts, healthy pregnant/non-pregnant women

Introduction

The Cluster of differentiation order wise known as CD is a protein expressed on the surface of the cells of the hematopoietic system. The expression of these proteins is used in lymphocyte nomenclature (Wingood, 2003). CD4 (cluster of differentiation 4) cells are a type of lymphocyte (white blood cell). They are an important part of the immune system. CD4 cells are sometimes called T-cellswhy CD8 (cluster of differentiation 8) is a trans-membrane glycoprotein that serves as a co-receptor for the T cell receptor (TCR). Like the TCR, CD8 binds to a major histocompatibility complex (MHC) molecule, but is specific for the class I MHC protein (Miri-Dashe *et al*., 2014). Over three hundred (300) "CD" molecules have been reported so far.CD for humans is numbered up to three hundred and seventy one (371) (as of 21 April 2016) (Zola *et al*., 2007; HCDM, 2016).

A healthy immune system normally has a CD4 count ranging from 500 to 1,600 cells per cubic millimeter of blood (cells/mm3) (Miri-Dashe *et al*., 2014). These proteins are often associated with the specific function of the cells. It is a protocol used for the identification and investigation of cell surface molecules providing targets for immunophenotyping of cells (Chan *et al*., 1998). Cells with different function express different CD molecules. For example, the CD3+ cells are total T-lymphocytes, CD4+ are T-helper cells and CD8+ cells are cytotoxic T-lymphocytes. The CD4 T-lymphocyte occupies the central position in regulating immune functions. The CD4 T-lymphocytes also known as T-helper cells, are coordinators of the body's immune response e.g. providing help to B-cells in the production of antibody, as well as in augmenting cellular immune response to antigens (Kapiga *et al*., 2009). CD8 is a trans-membrane glycoprotein that serves as co-receptors for the T cell receptor (TCR). CD8 T cells are an essential component of the adaptive immune system .Cytotoxic T cells (also known as TC, killer T cells, or cytotoxic T-lymphocytes (CTL) are a subgroup of T cells that induce the death of cells that are infected with viruses (and other pathogens), or are otherwise damaged or dysfunctional (Janeway *et al*., 2001). They display potent cytolytic activity against pathogen – infected host cells but are also involved during pregnancy when they aid in foetal implantation and prevention of foetal abortion. Activated CD8 T cells are expressed as part of the host regulatory response to control T-cell activity (Scaife *et al.,* 2006). Pregnancy is a unique state where the physiology of a woman is greatly altered to accommodate the newly developing "organ" the foetus (Lol *et al.,* 2004). Pregnancy leads to many functional (physiological) and structural (anatomical) changes in the body. They occur due to the needs of the developing baby, placenta and the uterus and the increasing levels of pregnancy hormones especially progesterone and estrogen (Geaghan, 2009).

Pregnant women are not immune suppressed in the classic sense, but physiological changes induce a state of relative immune-suppression in cellular immune of response. Pregnancy induces a unique challenge for the maternal immune system, which must tolerate the presence of a semi allogeneicfoetus and still maintain a strong immune response against invading pathogens (Ogawa, 2003). In individuals with HIV infections, assessment of CD4 and CD8 cell counts is fairly common and they are routine indices for the evaluation of immune status and decision to initiate anti-retroviral drug therapy, ART (Gang *et al.,* 2003). Also with the advent of anti-retroviral therapy (ART) and other interventions to improve maternal and child health, pregnant women and infants are the focus of many health programs, including prevention of mother-to-child transmission (PMTCT). Recruitment of pregnant women into clinical trials and overall clinical management require accurate laboratory reference intervals for correct interpretation and decision making (Miri-Dashe *et al.,* 2014).

Data on normal ranges of CD4, CD8 and leucocyte counts in Wukari especially with reference to pregnant women are

generally lacking. It is therefore important to institute appropriate normal reference values for T cell subsets among the obstetric population of Wukari, North East, Nigeria.

Materials and Methods

Study area

This study was carried out in the Department of Microbiology, Federal University Wukari, Taraba State, Nigeria. Wukari metropolis is a large town which is the Headquarter of Wukari Local Government Area of Taraba State. Geographically, Wukari lies between latitude 7°55'42" North and longitude 9°47'59" East. It has an area of 4,308 km^2. Wukari is home to Federal University Wukari, Campus of Open University of Nigeria and Kwararafa University. The major languages spoken are Jukun, Kutep, Tiv, Hausa and Fulani (Imarenezor *et al.*, 2016).

Study population

This study was conducted in Wukari at the maternity section of General Hospital between Junes - December, 2017 on sixty (60) apparently healthy pregnant individuals. The individuals were aged between 18-40 years of age while thirty (30) non-pregnant women served as control individuals.

Study design

This study involved apparently healthy pregnant individuals. Only HIV 1/2, Hepatitis (B and C) viruses and VDRL seronegative individuals were engaged into this study. The participants were selected by simple random sampling. Each consenting participant was asked to fill a questionnaire and administered a consent form A and B to read and sign respectively. All participants were screened based on both the inclusion and exclusion criteria captured in the questionnaire.

Inclusion Criteria: Only apparently healthy pregnant women between 18 and 40 years and seronegative for HIV 1/2, Hepatitis (B and C) viruses and VDRL were recruited into the study.

Exclusion Criteria: Women who were breast-feeding, HIV-positive, menstruating or on any form of oral contraceptive and antiretroviral therapy at the time of study were excluded.

Blood Collection: Within the time frame of 8.00am – 12.00noon, four (4) milliliters of blood was collected through venepuncture from the antecubital vein into ethylene diaminetetraacetic acid (EDTA) tubes in accordance with biosafety precautionary measures. All the samples were transported immediately at cold chain temperature ranges of 20C to 80C to the laboratory and were analyzed within six hours of sample collection.

Specimen Analysis: HIV sero-reactivity was determined according to the national algorithm II. Serial testing was carried out using Determine HIV – 1/2 test Kit in the first instance and Unigold HIV – 1/2 test was only used when Determine HIV 1/2 test was sero-reactive and discordant results resolved with the third kit, Stat Pak (tie-breaker). All the three test kits (Determine, Alere Medical Co. Ltd, Japan; Unigold, Trinity Biotech Plc, Ireland; and Stat Pak, ChembioDiagnostics systems, Inc., USA) were used according to the manufacturers' instructions. Participants were categorized as HIV non-reactive when they did not react with Determine HIV –1/2 rapid test kit.

The CD4 and CD8 counts were determined using Parteccyflow machine (SysmexPartec GmbH, Görlitz, Germany) according to the manufacturer's instructions. The cyflow counter is based on the simultaneous measurement of multiple physical characteristics of CD4 and CD8 T lymphocytes (at different times) in a single file as it flows through a light source usually a laser beam. The counter separated the CD4+ or CD8+ T cells from the monocytes CD4 or CD8 bearing cells and noise using a gating system. Leucocyte counts (total and differential leucocyte counts) were determined with Sysmex KX-21-N Haematology auto analyzer (Sysmex Corporation, Japan).

Ethical Approval: Approval for the study was obtained from the Ministry of Health, Jalingo, Taraba State in accordance with the code of ethics for biomedical research involving human subjects. Also, written informed consent of each participant was obtained. However, illiterate participants had their consent forms read and interpreted to them in their native languages by an interpreter.

Statistical Analysis: The data obtained were expressed as means ± standard errors of means (SEM). The medians were calculated and reference values were determined at 2.5th and 95th percentiles. Statistical significance was determined using the analysis of variance (ANOVA) $P<0.05$ was considered significant. All statistical analyses were done using SPSS version 21.0.

Results and Discussion

The results of the research for a six (6) months period are as shown on Tables 1 and 2. Table 1 show the WBC (total), CD4 and CD8 counts for pregnant and control individuals. The mean WBC (total) count (x103/µl) of the pregnant women was 7.67 ± 1.74 against 4.88 ± 0.64 of the control individuals. This comparison showed a statistical significant increase ($P<0.05$) between the pregnant women and the control individuals. The mean CD4 counts of pregnant women against those of the control individuals were 589.64 ±212.86 and 888.60 ± 296.66 cells/µl, respectively. This showed a statistically significant decrease ($P < 0.05$) in this CD4 count of pregnant women compared to control individuals. The mean CD8 counts of pregnant women and control individuals were 387.18 ±122.33 cells/µl and 316.66 ± 116.10 cells/µl respectively. There was no significant difference in the mean CD8 counts of both groups ($P > 0.05$). Table 2 portrayed the WBC (total), CD4 and CD8 counts of pregnant participants with respect to trimesters. There was no significant difference ($P > 0.05$) in the WBC (total) in the first trimester, second trimester and third trimester with respective mean values of 6.89 ± 1.51, 7.20 ± 1.98 and 7.39 ± 1.73. The mean CD4 counts of pregnant participants for the first trimester, second trimester and third trimester were 579.66 ± 269.33, 570.54±234.16 and 698.33 ± 254.74 cells/µl, respectively. This showed a statistical significant difference when compared with the mean values of the control individuals.

Table 1: The WBC (total), CD4 and CD8 counts of the study individuals

Parameter	Control individuals Mean ± SD=30	Pregnant individuals Mean ± SD=60	T- value	P- value
WBC	4.88±0.64	767±1.74	6.311	0.000(S)
CD4	888.60±296.66	589.64±296.66	5.231	0.000(S)
CD8	316.66±1.51	387.18±122.33	0.901	0.311(S)

Table 2: WBC (total), CD4 and CD8 counts of pregnant women with respect to trimesters

Parameter	Control Mean ± SD N=30	1st Trimester Mean ± SD N=20	2nd Trimester Mean ± SD N=20	3rd Trimester Mean ± SD N=20	F–value	P–value
WBC	4.88±0.64[s]	6.89±1.51[t]	7.20±1.89[s]	7.39±1.73[t]	13.464	0.000
CD4	888.60±296.66[s]	579.66 ±269.33[t]	570.54±234.16[t]	698.33±254.74[t]	10.564	0.000
CD8	316.66±1.51[d]	418.73±210.58[d]	419.43±153.65[d]	395.77±109.44[d]	0.421	0.622

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

For CD8 count, the results presented in mean ± standard deviation showed a non-significant difference (P> 0.05) in the three trimesters when compared with non-pregnant participants.CD4 and CD8 counts are widely used predictive markers to assess the degree of immune impairment in HIV seropositive individuals and to monitor antiretroviral therapy (ART). Pregnancy is considered as a physiologically immune-compromised state, hence alterations in T lymphocyte subsets may occur during pregnancy. There is need to establish baseline values of these counts, especially in healthy pregnant women (Danyama *et al.,* 2003). In this research, the mean CD4 count obtained was 589.64 cells/µl. This mean CD4 count recorded in Wukari is lower than those found by previous authors. Tanjang *et al.* (2012); Babatope *et al.* (2018) who reported a mean value of 851 and 614.49 cells/ul, respectively among pregnant individuals residing in Buea, Cameroon and Ekpoma, Nigeria. Other researchers in Nigeria such as Aina *et al.* (2005) and Akinbami *et al.* (2014) both reported similar mean values of 771 and 771.9 cells/µl among seronegative pregnant subjects in a study carried out in Ilorin and Lagos respectively. Chama *et al*. (2009) in their research found a mean value of 751.41 cells/µl in Maiduguri, Borno State, Nigeria. Also Makrydimas *et al*. (1994) reported increased CD4 count during pregnancy in British women. These higher counts may be due to physiological leucocytosis due to repeated infections. In contrast, our mean CD4 count value is higher than the mean value of 578.3 cells/µl reported in another part of Nigeria by Ekwempu *et a.* (2012). It has been observed that pregnancy alone, in the absence of HIV infection, was associated with a reduction in lymphocytes and T cell numbers across all subsets and with a neutrophilia consistent with what has been observed in some studies (De Santis*et al*., 2011). The lack of consistency in changes in total lymphocytes and subsets in pregnancy found in different studies could be because these cell subsets have previously been shown to be affected by other factors such as location and ethnic group (Mandala *et al.,* 2017). The other factors were also attributed to be stress or decreased immunity (Ekwempu *et al.,* 2012). Also in this research, the mean CD4 counts in the three trimesters (1st, 2nd and 3rd) were 579.66±269.33 cells/µl, 570.54±234.16 cells/µl and 698.33±254.74 cells/µl, respectively.

The CD4 mean values and gestational age were not statistically significant (P > 0.05). This finding is in consonance with the previous reports of Akinbami *et al.* (2014) and (Ekwempu *et al.* (2012) who both found a statistically insignificant association between CD4 and gestational age. The mean CD8 count of pregnant women in Wukari was 387.18±122.33 cells/µl. This finding is at variance with the previous report of (Babatope *et al*., 2018), with mean values of 411.31±161.76 cells/µl and Towers *et al.* (2010) who also reported mean values of 513.5 and 506.8 cells/µl in the second and third trimesters, respectively. It is noteworthy to mention here (Mwinga *et al.,* 2009) enrolled into their study only second and third trimesters of HIV-uninfected pregnant women. Therefore, the nature of these two studies might have skewed the statistics in comparison to this research that determined the mean of all our individuals in the three trimesters. According to Anglaret *et al*. (1992), the higher counts found in these subjects might also be due to physiological leucocytosis due to repeated infections. In contrast, a mean CD8 value of 270 cells/µl among the 54 HIV-uninfected pregnant participants was found in a previous research work studied (Mwinga *et al.,* 2009). Also, it was reported that a mean absolute CD8+ cell count that was not significantly different and therefore appears to be unaffected during pregnancy (Towers *et al*., 2010). With respect to trimesters, the mean values obtained in the three trimesters (1st, 2nd and 3rd) were 446.65, 457.43 and 418.88 cells/µl, respectively.

The statistical analysis revealed that there was no significant association between CD8 and gestational age in these research participants. The finding is in line with the report which found out that the mean absolute CD8+ cell count is not significantly different (Towers *et al*., 2010). The mean WBC total count (x103/µl) of pregnant participants in Wukari was 7.67 against 4.85 of the control individuals. This showed a significant increase ($P < 0.05$) in pregnant participants compared to control participants. The finding is in tandem with the report that observed an elevated mean white blood cell (WBC) count above the non-pregnant state and this parameter increased throughout the pregnancy to and including parturition. A limitation of this study is that it did not include a longitudinal study tracking changes in individual women throughout pregnancy which would have provided a comprehensive understanding of immunological changes in pregnancy (Gomo *et al.,* 2003). Conclusively, pregnancy significantly increased the mean values of WBC count but significantly decreased CD4+ cell count when compared to non-pregnant controls while the mean CD8+ cell count did not show any significant difference in the individuals studied.

Conflict of Interest

Authors declare that there is no conflict of interest.

References

Aina O, Dadik J, Charurat M, Amangaman S, Gurundi E, Mang R, Guyit N, Lar P, Datong C, Daniyam P, Kanki A & Abimiku A 2005. References of CD4 T-lymphocytes in human immunodeficiency virus-negative adult Nigerians. *ClinDiagn Lab Immunol.,* 12(4): 525-530.

Akinbami AA, Dosunmu AO, Adediran A, Adewunmi A, Rabiu KA, Osunkalu V, Ajibola S, Uche EI & 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. *Nig. Med. J.,* 55(2): 116-120.

Anglaret X, Diagbouga S, Mortier E, Meda N, Vergelalette V & Sylla-Koko F 1997. CD4+ T-lymphocyte counts in HIV infection: are European standards applicable to Africanpatients. *J Acquir Immune DeficSyndr HumRetrovirol.,* 14: 361-367.

Babatope IO, Isabu PA, Imarenezor EPK, Adesanya TM & Ikhimiukor AP 2018. Normal CD4, CD8 T-lymphocytes and leucocyte baseline in healthy HIV-seronegative pregnant women in Ekpoma, Edo State, Nigeria. *Int. J. Basic, Appl. and Innovative Res.,* 7(1): 18 – 28.

Chama CM, Morrupa JY, Abja UA & Kayode A 2009. Normal CD4 T-lymphocyte baseline in healthy HIV negative pregnant women. *J. Obstetrics and Gynaecol.*, 29(8): 702-704.

Chan JKC, Ng CS & Hui PK 1988. A simple guide to the terminology and application of leucocyte monoclonal antibodies. *Histopathology*, 12(5): 461-480.

Danyama A, Pandit D, Mudaliar S & Bharadwaj R 2003. A pilot study on CD4 and CD8 cells in healthy HIV seronegative pregnant women. *Indian J. Med. Res.*, 117: 198-200.

De Santis GC, Brunetta DM &Vilar FC 2011. Haematological abnormalities in HIV-infected patients. *Int. J. Infect. Dis.*, 15: e808-e811.

Ekwempu AI, Ekwempu CC, Ikeh E, Olabode A & Agaba E 2012. Conparison of CD4 cell counts in pregnant HIV-seropositive and HIV-seronegative Nigerian women. *Lab. Medicine*, 43(5): 168-171.

Gange SJ, Lau B, Phair J, Riddler SA, Detels R & Margolick JB 2003. Rapid declines in total lymphocyte count and haemoglobin in HIV infection begin at CD4 lymphocyte counts that justify antiretroviral therapy. *AIDS*, 17: 119-121.

Geaghan SM 2009. Appendix B, Normal blood values: Selected health – associated values in neonatal, paediatrics, and adult populations. In Wintrobe's clinical Haematology 12th edition. Edited by: Greer JP, Foerster J & Lukens JN. Philadelphia: Lippincott Williams & Wilkins, pp. 2584-2586.

Gomo E, Vennervald BJ, Ndlouu PD, Kaestel P, Nyazema NZ & Fris H 2003. Reference values and predators of white blood cell subset counts: a cross-sectional study among HIV seronegative pregnant women in Zimbabwe. *Eur. J. Obstet. Gynaecol. Reprod. Biol.*, 107: 156-162.

HCDM 2016. Responsible for HLDA Workshop and CD molecules. Human Cell Differentiation Molecule Council (successor to the HLDA workshops). *Retrieved* 2016-04-21.

Imarenezor EPK, Brown STCL, Yakubu OE & Soken DC 2016. Survey of hepatitis B and C among students of Federal University Wukari, Taraba State, Nigeria. *Int. Res. J. Med. and Med. Sci.*, 4(3): 31-37.

Janeway CA, Travers P, Walport M & Shlomchik MJ 2001. Immunobiology 5th ed. New York and London: Garland Science. ISBN 0-8153-4101-6.

Kapiga SH, Pluto S, Msamanga GI & Spiegelman D 2009. Risk factors for cervical squamous intraepithelial lesions among HIV-1 seropositive women in Dares salaam, Tanzania. *Int. J. Gynaecol. and Obstetrics*, 9(6): 87-94.

Lol FH, Aralkumaran S, Montan S & Ratnam SS 2004. Maternal mortality: evolving trends. *Asia Oceania J. Obstetrics and Gynaecol.*, 20(3): 301-304.

Makrydimas G, Plachouras N, Higueras MT, Thilaganathan B & Nicolaides K 1994. Maternal peripheral blood lymphocyte subpopulations in normal and pathological pregnancies. *Fetal DiagnTher.*, 9: 371-378.

Mandala WL, Gondwe EN, Molyneux ME, MacLenna JM & McLenna CA 2017. Leucocytes counts and lymphocyte subsets in relation to pregnancy and HIV infection in Malawian women. *Am. J. Reprod. Immunol.*, 78: e12678.

Miri-Dashe T, Osawe S, Tokdung M, Daniel N, Choji RP & Mamman I 2014. Comprehensive references ranges for haematology and clinical chemistry laboratory parameters derived from normal Nigeria adults. *PLoS ONE*, 9(5): e93919.

Mwinga K, Vermund SH, Chen YQ, Mwatha A, Read JS, Urassa W, Carpenetti N, Valentine M & Goldenberg RL 2009. Selected hematologic and biochemical measurements in African HIV-infected and uninfected pregnant women and their infants: The HIV Prevention Trials Network 024 protocol. *BMC Paediatrics*, 9(49): 1-14.

Ogawa M 2003. Differentiation and proliferation of haemopoietic stem cells in pregnancy. *Blood*, 93(81): 2844-2853.

Scaife PJ, Bulmer JN, Robson SC, Innes BA & Searle RF 2006. Effect or activity of decidual CD8+ T lymphocytes in early human pregnancy. *J. Biol. and Reproduc.*, 7(5): 56-57.

Tanjong RA, Atashili J, Kamga HL, Ikomey G, Akenji NT & Ndumbe MP 2012. Reference values of CD4 lymphocyte counts in HIV seronegative pregnant women in Buea, Cameroun. *Afr. J. Exper. Microbiol*., 13: 28-34.

Towers CV, Rumney P & Ghamsary MG 2010. Longitudinal study of CD4+ cell counts in HIV-negative pregnant patients. *J. Maternal-Feto & Neonatal Med*., 23(10): 1091-1096.

Wingood GM 2003. Feminization of the HIV epidemic in the United States: Major research findings and future research needs. *J. Urban Health*, 8014(Suppl. 3): iii 67 - iii76.

Zola H, Swart B, Banham A, Bargy S, Beare A, Bensussan A, Boumsell LD, Buckley C, Bühring HJ, Clark G, Engel P, Fox D, Jin BQ, Macardle PJ, Malavasi F, Mason D, Stockinger H & Yang X 2007. CD molecules 2006-human cell differentiation molecules. *J. Immunol. Methods*, 319(1-2): 1-5.