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RESEARCH PAPER

NORMAL CD4, CD8 T-LYMPHOCYTES AND LEUCOCYTE BASELINE IN HEALTHY HIV-SERONEGATIVE PREGNANT WOMEN IN EKPOMA, EDO STATE, NIGERIA

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ABSTRACT

This study was aimed at establishing the baseline values of CD4 and CD8 absolute counts among healthy pregnant women in Ekpoma, Edo State, Nigeria. Ninety (90) apparently healthy pregnant women between the age group of 18-39 years and thirty (30) non- pregnant women (control) were used as study subjects. The pregnant subjects were divided into three groups, depending on the trimester of pregnancy. CD4 and CD8 absolute counts were determined using flow cytometry. An automated blood analyzer was used to determine the leucocyte counts. The mean WBC (total) count ($x10^3/\mu l$) of the pregnant women was 7.71±1.76 against 4.97 ± 0.82 of the control subjects. Statistical comparison showed a statistical significance changes in the mean CD4 counts between the test and control subjects but no statistical significant difference in the total WBC and absolute CD8 counts of both groups (P>0.05). In contrast, the CD4 absolute count showed a statistical significant difference when compared with the mean values of the control subjects. In conclusion, 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 difference in the subjects studied.

Key words: CD4 counts, CD8 counts, Leucocytes counts, Healthy pregnant women, Ekpoma

INTRODUCTION

The "CD" or Cluster of differentiation is a protein expressed on the surface of the cells of the haematopoietic system. The expression of these proteins is used in lymphocyte nomenclature (Wingood, 2003). 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). 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*.,1988). 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 occupy the central position in regulating immune functions. The CD4 T-lymphocytes also known as T-helper cells, are co-ordinators 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 transmembrane 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 (Loh *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 (Murray and Mcjibbey, 2010). 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 semiallogeneic foetus 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 (Gange *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 Ekpoma especially amongst pregnant women are generally lacking. It would therefore be needful to establish appropriate normal reference values for T cell subsets among the obstetric population of Ekpoma, Nigeria.

MATERIALS AND METHODS

Study Area: This study was carried out in Ekpoma, the administrative headquarters of Esan West Local Government Area of Edo State, Nigeria. The area lies between latitudes $6^0$43N to $6^0$45N of the Equator and longitudes $6^0$6'E to $6^0$8'E of the Greenwich Meridian. It is the fourth largest town in Edo State and has an area of 502km^2 and a population of 89,628 in 1991 and 127,718 in 2006 majority of which are civil servants, traders, business men/women, farmers, teachers, lecturers and students by occupation. Ekpoma since its designation as headquarters and as the host of the state – owned university (Ambrose Alli University), has grown into an urban centre (Aziegbe, 2006).

Study Population: This study was a descriptive cross-sectional study conducted in Ekpoma at the maternity clinics of Osemudiamen Specialist Hospital and Esan West Local Government Area between January and June, 2017 on ninety (90) apparently healthy pregnant subjects. The subjects were aged between 18-39 years of age while thirty non-pregnant women served as control subjects.

Study Design: This study involved apparently healthy pregnant subjects. Only HIV 1/2, Hepatitis (B and C) viruses and VDRL seronegative subjects were recruited 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 in or out based on both the inclusion and exclusion criteria captured in the questionnaire.

Inclusion Criteria: Only apparently healthy pregnant women between 18 and 39 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 9.00am-12.00noon, four (4) milliliters of blood was collected through venepuncture from the antecubital vein into ethylene diamine tetraacetic acid (EDTA) tubes in accordance with biosafety precautionary measures. All the samples were transported immediately at cold chain temperature ranges of 2^0C to 8^0C to the laboratory and were analyzed within six hours of sample collection.

Specimen Analysis (A): 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, Chembio Diagnostics 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.

Specimen Analysis (B): The CD4 and CD8 counts were determined using Partec cyflow 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 University Ethics Committee in accordance with the code of ethics for biomedical research involving human subjects. 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 2.5^{th} and 95^{th} percentiles. Statistical significance was determined using the analysis of variance (ANOVA) or the Student's t-test as appropriate. $P<0.05$ was considered significant. All statistical analyses were done using SPSS version 21.0.

RESULTS

Table 1 summarizes the socio-demographic characteristics of pregnant and non-pregnant women in the study area. The mean age of the subjects (pregnant women) was 31.70 ± 5.55 years compared to control subjects (28.70 ± 6.60 years). According to gestational age, the mean age in weeks were 10.30 ± 0.72, 20.10 ± 1.10 and 30.70 ± 0.70 for the first, second and third trimesters respectively. Based on occupation, most of the pregnant subjects recruited for the study were civil servants while those who did not have any specific occupation (ie others) were the least.

Table 2 revealed the WBC (total), CD4 and CD8 counts of the pregnant and control subjects. The mean WBC (total) count ($x10^3/\mu l$) of the pregnant women was 7.71 ± 1.76 against 4.97 ± 0.82 of the control subjects. This comparison showed a statistical significant increase ($P<0.05$) between the pregnant women and the control subjects. The mean CD4 absolute counts of pregnant women against those of the control subjects were 614.49 ± 266.87 cells/µl and 935.40 ± 306.56 cells/µl respectively. This showed a statistically significant decrease ($P < 0.05$) in thse CD4 count of pregnant women compared to control subjects. The mean CD8 absolute counts of pregnant women and control subjects were 411.31 ± 161.76 cells/µl and 380.87 ± 126.50 cells/µl respectively. There was no statistical significant difference in the mean CD8 absolute counts of both groups ($P > 0.05$).

Table 3 depicted the WBC (total), CD4 and CD8 counts of pregnant women 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 7.02 ± 1.71, 7.14 ± 2.15 and 7.33 ± 1.37. The mean CD4 absolute counts of pregnant women for the first trimester, second trimester and third trimester were 578.53 ± 291.43 cells/µl, 563.70 ± 246.05 cells/µl and 701.23 ± 247.62 cells/µl respectively. This showed a statistical significant difference when compared with the mean values of the control subjects. 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.

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	1ST TRIMESTER MEAN ± SD N=30	2ND TRIMESTER MEAN ± SD N=30	3RD 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

CD4 and CD8 counts are widely used prognostic markers to assess the degree of immune impairment in HIV seropositive individuals and to monitor antiretroviral therapy (ART). Pregnancy is considered as a physiologically immunocompromised 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 study, the mean CD4 absolute count obtained was 614.49 cells/µl. The mean CD4 absolute count recorded in Ekpoma is lower than those found by previous authors. Tanjong *et al* (2012) reported a mean value of 851 cells/µl among pregnant subjects residing in Buea, Cameroon. In Nigeria Aina *et al* (2005) and Akinbami *et al* (2014) both reported similar mean values of 771 cells/µl and 771.9 cells/µl among seronegative pregnant subjects they studied in Ilorin and Lagos respectively. In Pune, India, Dayama*et al* (2003) reported a mean value of 764 cells/µl while Chama *et al.* (2009) found a mean value of 751.41 cells/µl in Maiduguri, Borno State, Nigeria. Makrydimas *et al* (1994) also 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 absolute count value is higher than the mean value of 578.3 cells/µl reported in another part of Nigeria by Ekwempu*et al.* (2012). In a recent study also among HIV – uninfected pregnant women in Malawi, Mandala *et al* (2017) reported a mean value of 468 cells/µl. Towers *et al.* (2010) also reported that the mean absolute lymphocyte cell count, lymphocyte percentage and absolute CD4 cell count are significantly lower in pregnancy. Bisalkumi *et al.* (1992) also reported that absolute numbers of CD4+ were reduced during pregnancy in African women. Burns *et al.* (1996) also reported a reduced mean value for American women. 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 (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). 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.*, 2010). Ekwempu *et al* (2012) attributed the other factors to be stress or decreased immunity. There is some evidence to suggest that in HIV – uninfected women, pregnancy per se is associated with suppression of humoural and cellular immunity (Kumar *et al.*, 1984; Weinberg, 1984; Biedermann *et al.*, 1995).

Also in this study, the mean CD4 absolute counts in the three trimesters (ie 1st, 2nd and 3rd) were 578.53±291.43 cells/µl, 563.70±246.05 cells/µl and 701.31±247.62 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 Ekuempu *et al* (2012) who both found a statistically insignificant association between CD4 and gestational age. Temmerson *et al* (1995) also reported no association between duration of pregnancy and immune markers and the pattern of immunological markers described for HIV-infected women were similar to those of uninfected controls. Contrary to our finding, Okeke *et al* (2016) reported that CD4 of non-HIV infected pregnant women increased from second trimester through third trimester and was found to have a significant increase. Okeke *et al* (2016) suggested that this indicated that pregnancy alone can influence increase in CD4 cell through 3rd trimester and attributed the increase might be a result of physiological endocrine changes in pregnancy.

Lieve *et al* (2007) noted that pregnancy hormones slowdown the decline in the rate of CD4 cell count though not sustainable. It was observed that the changes in the CD4 cell count which is a subset of total leucocyte count (TLC) does not significantly reflect in total lymphocyte count of the pregnant women. However, Chama *et al* (2009) reported that the mean CD4 count was higher in the 1st trimester than in later parts of pregnancy while Towers *et al* (2010) observed a significantly lower CD4 cell count during pregnancy and stated that the progression through pregnancy was u-shaped. Anglaret *et al* (1994) attributed the higher mean CD4 counts in some trimesters to be due to physiological leucocytosis due to infections.

The mean CD8 absolute of pregnant women in Ekpoma was 411.31±161.76 cells/µl. This finding is at variance with the previous report of Dayama *et al* (2003) who found the mean values of 547±56.49 cells/µl. Mwinga *et al* (2009) also

reported mean values of 513.5 cells/µl and 506.8 cells/µl in the second and third trimesters respectively. It is noteworthy to mention here that Danyama *et al* (2003) recruited participants in third trimester only whereas 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 our study that determined the mean of all our subjects in the three trimesters. Nevertheless, the mean CD8 values obtained by these previous authors in these two trimesters were higher than the mean values we obtained in our study. According to Anglaret *et al.* (1997), the higher counts found in these subjects might also be due to physiological leucocytosis due to repeated infections. In contrast, Mandala *et al* (2017) found a mean CD8 value of 270 cells/µl among the 54 HIV-uninfected pregnant women they studied. Towers *et al* (2010) also reported a mean absolute CD8+ cell count that was not significantly different and therefore appears to be unaffected during pregnancy. Other authors (Bisalinkumi *et al.* (1992) and Makrydimas *et al.* (1994)) both reported decreased CD8 counts during pregnancy in African and British women respectively. Therefore, regional diversity in the T-lymphocyte subset count is evident. In addition to the regional changes, variations could also be due to use of different equipment and techniques in different studies and could have given rise to procedural and instrumental errors.

With respect to trimesters, the mean values obtained in the three trimesters (1^{st}, 2^{nd} and 3^{rd}) were 418.73 cells/µl, 419.43 cells/µl and 395.77 cells/µl respectively. The statistical analysis revealed that there was no significant association between CD8 and gestational age in our study subjects. Our finding is in line with the report of Towers *et al* (2010) who also found that the mean absolute CD8+ cell count is not significantly different. In contrast, our finding disagreed with the previous report of Dayama *et al*. (2003) who found mean values of 547.00 cells/µl among the subjects they studied. Danyama *et al.* (2003) recruited only third trimester pregnant subjects. In addition, Mwinga *et al.* (2009) reported mean values of 513.5 cells/µl and 506.8 cells/µl in the second and third trimesters of the subjects they studied. The same reasons earlier mentioned might also be responsible for this. However, Mandala *et al* (2017) reported a lower mean value of 270 cells/µl among the subjects they studied. Bisalinkumi *et al* (1992) and Makrydimas *et al* (1994) also found decreased CD8 counts during pregnancy. Diminished immunoreactivity during pregnancy is important in preventing rejection of the foetus, which from an immunological point of view is foreign tissue.

The mean WBC total count ($x10^3$/µl) of pregnant women in Ekpoma was 7.71 against 4.97 of the control subjects. This showed a statistically significant increase ($P < 0.05$) in pregnant women compared to control subjects. Our finding is in tandem with the report of Towers *et al.* (2010) who 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. Another study reported that althsough changes in leucocyte counts during pregnancy in African women were similar to those reported in Caucasian women, the total WBC counts were lower in the African women (Fleming and Harrison, 1985). In contrast, our WBC count mean value was lower than the mean value of 12.3 ($x10^3$/µl) reported by Mandala *et al.* (2017) among pregnant Malawian women. The WBC count for normal male and female adults is 4,500 – 11,000/µl (range is estimate of 95% confidence limits) (Geaghan, 2009). White blood cell count is increased in pregnancy with the lower limit of the reference range being typically 6,000/cumm (Chandra *et al.*, 2012). Leucocytosis occurring during pregnancy is due to physiologic stress induced by the pregnant state (Fleming, 1975). Neutrophils are the major type of leucocytes on differential counts (Gatti *et al.*, 1994; Konijnenberg *et al.*, 1997). This is likely due to impaired neutrophilic apoptosis in pregnancy (Gatti *et al*., 1994).

There was no significant difference ($P > 0.05$) in the WBC (total) count in the first trimester, second trimester and third trimester with respective mean values of 7.02, 7.14 and 7.33. This is close to the report of Mwinga *et al* (2009) who found mean values of 6.9 and 6.7 in the second and third trimesters respectively. Statistical comparison between the two mean values of the test and control subjects did not show any statistical significant difference also. Our finding contradicted the previous report of Mandala *et al.* (2017) who found a mean value of 5.8 among 54 pregnant HIV-uninfected women. Milhorat *et al* (1942) observed that this may have been due to the significant increase in neutrophil count during pregnancy, possibly related to stress response, redistribution of WBC during the marginal and circulating pools or pain, nausea, vomiting and anxiety in the absence of infection. On the contrary, Okeke *et al* (2016) reported lower mean WBC total count ($x10^3$/µl) values of 2.93 and 3.26 in the 2^{nd} and 3^{rd} trimesters respectively among HIV-uninfected pregnant women in another part of Nigeria. The reason for these low counts is not clear but this may be attributed to genetic, environmental or dietary factors (Shaper and Lewis, 1971 ; Ezeilo,1974).

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). Secondly, it is worth mentioning that CD4+ and CD8 have been observed to be stable when they are presented as total counts than absolute counts.

In conclusion, 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 difference in the subjects studied.

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AUTHORS' CONTRIBUTIONS

The contributions of the authors/co-authors are briefly stated as follows:
Babatope, I.O. – Research idea and design; drafted the work
Isabu, P.A. – Medically certified the subjects and reviewed the write-up
Marenezor, E.P.K. – Data analysis, also reviewed the write-up
Adesanya, T.M. and Ikhimiukor, A.P. – Field work, administration of questionnaires, sample collection and also contributed reagents/materials