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Parity and its effect on the CD4+ count of hiv-seronegative pregnant women attending Adeoyo hospital, Ibadan, Nigeria

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Abstract

The immune system of the pregnant woman is physiologically compromised and one of the parameters that reflect this status is the cluster of differentiation four (CD4) count levels. The CD4 count is frequently used as a surrogate marker for the cellular immune status of HIV seropositive persons but has also become a marker of adaptive immunity in other conditions. It is well established that certain factors affect the CD4+ count level in pregnancy. Our study sought to determine the influence of parity and gestational age on the CD4+ count of HIV-seronegative pregnant women. Pregnant women attending the antenatal clinic of Adeoyo Maternity Hospital Yemetu, Ibadan received voluntary counseling and testing for HIV, viral hepatitis B and C. Those who tested negative to these parameters had their CD4+ count assessed using the Cyflow method. Apparently healthy, non-pregnant women were also recruited as controls. A total of 60 pregnant women and 20 non-pregnant women randomly stratified, were recruited for this study. The mean CD4+ value of nulliparous pregnant women is 651.00 ± 53.84 cells/ml, the mean CD4+ count of primiparous women is 736.50 ± 47.51 cells/ml, and the mean CD4+ value of the multiparous group is 676.45 ± 41.62 cells/ml which are all significantly lower than the mean CD4+ count of 951.05 ± 47.08 cells/ml for the non-pregnant women (p=0.035). Comparison of mean absolute CD4+ cell count among women, based on trimester of pregnancy showed that the CD4+ count was highest in the first trimester than in subsequent stages of the pregnancy (second and third trimester), although the difference was not significant (p>0.05). Nulliparous pregnant women had similar level of mean absolute lymphocyte count as the non-pregnant women. Among the three parameters assessed (Total White blood cell count (TWBC), absolute lymphocyte count and absolute CD4+ count), only TWBC count and absolute CD4+ count was influenced by parity (p=0.0167, p=0.0351 respectively). Therefore, these two parameters may be used to assess the level of cellular immunity in pregnant women, based on parity.

Introduction

Pregnancy, also known as gestation or gravidity, is a state in which an embryo implants unto the maternal uterus and subsequently develops unto a fetus. Pregnancy which starts at conception, is a product of fertilization of an ovum by a spermatozoon to form a zygote, and ends in childbirth, abortion or miscarriage [1,2]. It lasts about 40 weeks from last menstruation or 38 weeks from conception date [3,4]. Since pregnancy can result in miscarriage, stillbirth or delivery of the baby, several factors each play a role in determining the fate of the unborn fetus. During pregnancy, there are significant maternal cardiovascular and hemodynamic changes which are aspects of maternal adaptation accompanying pregnancy. Such alterations may involve blood volume, heart rate, stroke volume, cardiac output, and systemic vascular resistance. These physiologic changes are usually well tolerated by the pregnant woman. Changes begin early in gestation and continue as pregnancy advances, and most of these changes are totally reversible after delivery. One key aspect of the dynamic state observed in gestation is the role played by the body's immunological status.

Aside the other physiological changes, pregnancy is normally associated with alterations in many hematological parameters. For instance, there is commonly an increase in white blood cell count (leukocytosis), which has been attributed to physiological stress and increased inflammatory response in pregnancy [5]. White blood cells or leukocytes are the cellular components of the body's immune system. White blood cell count increases during pregnancy typically has the lower limit of the reference range being 6,000/cu.mm in many populations [6]. In early pregnancy, leukocytes are abundant, comprising 30-40% of all human decidual stromal compartment cells. Leukocytosis is mainly due to neutrophilia; and immature forms like metamyelocytes and myelocytes (neutrophilic left shift) that may be present in the peripheral blood film.

Lymphocytes are a subset of the white blood cells with varied functions and include T, B, and natural killer (NK) cells. While T and B cells are the effectors of adaptive immunity, NK cells do not have recombined antigen receptors and are innate immune lymphocytes. T, B, and NK cells and their respective subsets all have their origin in the bone marrow – from derived progenitors. In the very early stage of pregnancy, there is no uniformity in the distribution of leukocyte subsets in the different compartments of the woman's uterus, a situation brought about by the extensive remodelling of the uterus in preparation for the foetus [7]. Studies on human tissues have shown that the majority of first-trimester human decidual leukocytes are NK cells, constituting about 70% of the total leukocyte population in the compartment, while macrophages make up approximately 20%. The

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population of T-cells is much lower, being between 10% and 20% of decidual leukocytes, of which CD4+ T cells fraction constitute 30-45% [8,9]. Dendritic cells (DCs) and B-cells are rarely encountered [7,10].

In pregnancy the immune response directed majorly by the T cells is known to be segmented into stages. Different subsets of T cells exist, each of which play one role or the other at the appropriate stages of pregnancy. The cytotoxic T cells (CD8) are the effector cells which carry out phagocytic destruction of infected cells. T helper cells (CD4+) help in boosting the immune response while the T regulatory (Treg) cells act to balance the activities of these groups of immune cells to prevent auto immunity. In addition, the Treg cells and the CD4+ HLA-G T cells ensure tolerance of the mother to the allogenic foetus [11]. Subsets of T helper cells include T helper1 cells (Th1) which when activated, trigger inflammation by secreting pro-inflammatory cytokines and T helper2 (Th2) cells which aid wound healing and immune tolerance by secreting anti-inflammatory cytokines [12]. Studies suggest a pro-inflammatory (Th1) profile at the onset of pregnancy. Implantation and placentation result in the invasion of, and remodeling of the woman's endometrium so as to accommodate the new fetus. Pro-inflammatory cytokines appear vital for successful pregnancy in the early stages of implantation [7]. A careful balance of the pro- and anti-inflammatory response is however required to preserve pregnancy. Depressed inflammatory responses at the beginning of pregnancy may cause failure of implantation, while high inflammatory responses lead to acute rejection.

At mid-gestation, when foetal antigens begin to develop, an anti-inflammatory Th2 profile guarantees tolerance of the foreign foetal antigens [13]. The immune cells believed to aid this dynamic phenomenon are the T regulatory (T reg) cells which are a unique specialized subset of the T cell compartment whose main role in pregnancy is to suppress alloreactive immune responses and promote tolerance to the foetus [14]. The period immediately preceding labour and up until delivery is often associated with a pro-inflammatory state in which there is reversal back to Th1 profile from the previously Th2 predominating levels. Studies indicate increases in Th1 proinflammatory cytokines and reduction in Th2 cytokines in those women who are undergoing active labour [7]. Thus, normal pregnancy is associated with a pro-inflammatory Th1 profile at early and late pregnancy stages, with these two periods being important for a proper blastocyst implantation and initiation of labour respectively. While the number of NK cells remains stable as pregnancy advances, those of T cells increase with advanced gestation [15]. As for CD4+ cells in particular, the absolute CD4 count may decrease at the early stage and then increase later in pregnancy [16]. However, whether there will be increase or decrease of the absolute CD4+ count during gestation is highly variable and unpredictable.

In different studies carried out on pregnant women in Nigeria, baseline CD4+ count of HIV-seronegative women was established [17-20]. There is paucity of data on the influence of parity on the immune status of pregnant women. Identifying the effects of parity and gestational age on the CD4+ lymphocyte count of pregnant women in the population studied should help to validate previous studies and further aid the assessment of the physiological changes that occur in the course of pregnancy; with a view to determining additional factors that influence cellular immunity during the child-bearing period. In addition, the outcome should aid comparability of data with other populations and assist in improved monitoring of the pregnant woman during this stage of the woman's reproductive life.

Materials and methods

Methodology

This is a grouped, randomized survey in which nulliparous pregnant women were randomized with primiparous and multiparous pregnant women. The study was conducted in Adeoyo Hospital, Yemetu Ibadan, Nigeria. Ethical approval was obtained from the Research Ethical Committee of Adeoyo Hospital, Ibadan and informed consent was obtained from the subjects prior to the study. A total of 60 non-complicated pregnant women were randomly stratified into three groups of 20 subjects each and were purposively enrolled into this study from those pregnant women attending the antenatal clinic at the hospital. Twenty (20) non-pregnant women were selected for the control group from among the healthy hospital female staff in the same age range as the subjects. Respondents that tested positive for HIV, Hepatitis B and C and those with severe complications were excluded. Demographic data which included parity status, maternal age, previous hormonal contraceptive use and gestational age were obtained via verbal questioning and records documented. The study lasted for four months. Samples were collected on each antenatal clinic day from the subjects at the point of booking for 3 months. The fourth month was used for sample analysis of control subjects.

All participants were within the age range of 17-42 years. For each subject, 3ml of venous blood was dispensed into EDTA bottle. After sample collection, total white blood cell count and differential count (thin film) were done and absolute CD4+ count was also carried out. The CD4+ count was done with Cyflow counter and Partec CD4 easy count kit. Fluorescent-labeled antibodies directed against the human cell surface marker CD4 were used for labeling cells in whole blood samples, applying the CD4+ easy count kit (Partec Code No. 05-8401) and the Cyflow® Counter (Partec Code No. CY-S-3022).

Results

Table 1 shows the demographic data of participants. Enrolment was by stratification with 20 subjects in each of the nulliparous, primiparous and multiparous groups. The age of respondents ranged between 16 and 42 years with a mean age of 27.9 years. The highest frequency of 32 was in the age range 20-29years. Nine (9), 33 and 18 subjects were in their first, second and third trimesters respectively. Forty-seven were married while thirteen were single. Twenty-nine (29) of the pregnant women had previously been on hormonal contraceptive before conception. Twelve had adopted other non-hormonal contraceptive methods while nineteen had never used any form of contraceptive.

Table 2 displays the average span for child spacing in the women and effect of parity on the total white blood cell count of pregnant women. While non-pregnant and nulliparous women had nil spacing, the primiparous and multiparous groups had average19.7 months and 28.2 months of spacing respectively. Comparison of total white blood cell count shows a significant difference in which highest total white blood cell (TWBC) count ($7.68 \pm 0.53 \times 10^9$ cells/L) was observed in nulliparous women group when compared with other groups of women ($F = 12.60$, $p = 0.017$).

Table 3 shows the effect of parity on both the absolute CD4+ count and the absolute lymphocyte count. Comparison of mean absolute CD4+ count showed a significant difference in which higher absolute CD4+ count (951.05 ± 47.08 cells/μL) was observed in non-pregnant women when compared with other groups of pregnant women ($F = 8.17$, $p = 0.035$). The table also shows the influence of parity on the

Table 1. Showing the demographic data of the respondents. It features the marital status, gestational age and the pre-conception contraceptive use of the participants

Age	Frequency	Percentage
< 20	8	13.3%
20-29	32	53.3%
30-39	13	21.7%
40 and above	7	11.7%
Total	**60**	**100%**
Parity Status	**Frequency**	**Percentage**
Nulliparous	20	25%
Primiparous	20	25%
Multiparous	20	25%
Non-Pregnant (Control)	20	25%
Total	**80**	**100%**
Gestational age		
1st trimester	9	15%
2nd trimester	33	55%
3rd trimester	18	30%
Total	**60**	**100%**
Marital status		
Married	47	78.7%
Single	13	21.3%
Total	**60**	**100%**
Pre-conception contraceptive use		
Previous hormonal contraceptive use	29	48.3%
Other contraceptive methods	12	20.0%
Never used contraceptive	19	31.7%
Total	**60**	**100%**

Table 2. Displayed the mean child-spacing period and effect of parity on the total white blood cell count of the pregnant women. F = 12.60; p = 0.017

Total white blood cell counts (x 10^9 cells/L)		
Status of women	**Average span of child-spacing**	**mean TWBC ± SEM**
n		
Nulliparous 20	Nil	7.68 ± 0.53
Primiparous 20	19.7 months	7.38 ± 0.51
Multiparous 20	28.2 months	6.83 ± 0.30
Non-pregnant 20	Nil	4.46 ± 0.23

Table 3. Displayed the effects of parity on the absolute lymphocyte and the absolute CD4+ count of pregnant women. F = 8.17, p = 0.035; F = 0.89, p = 0.513

Absolute CD4+ count (cells/µL)	Absolute lymphocyte count (x 10^9 cells/L)	
Status of women n	**mean ± SEM**	**mean ± SEM**
Nulliparous 20	651.00 ± 53.84	1.90 ± 0.13
Primiparous 20	736.50 ± 47.51	2.16 ± 0.11
Multiparous 20	676.45 ± 41.62	2.04 ± 0.11
Non-pregnant 20	951.05 ± 47.08	1.95 + 0.12

Table 4. Shows the effects of gestational age on the total white cell count, absolute lymphocyte count and absolute CD4+ cell count of pregnant women. Abs: Absolute; Lymph: Lymphocyte; TWBC: Total White Blood Cell Count. F = 2.22, p = 0.256; F = 2.29, p = 0.237; F = 0.96, p = 0.476.

TWBC	Abs Lymph count	Abs CD4+ count (cells/µL) (x 10^9 cells/L	
Trimester n	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
1st 09	7.69 ± 0.35	2.10 ± 0.78	714.6 ± 34.24
2nd 33	6.13 ± 0.60	1.69 ± 0.21	603.8 ± 97.87
3rd 18	7.15 ± 0.48	2.08 ± 0.14	681.2 ± 47.11

absolute lymphocyte count. Although higher absolute lymphocyte count (2.16 ± 0.11 x 10^9 cells/L) was observed in primiparous women when compared with other women, the difference was insignificant (F =0.89, p = 0.513). There is a close similarity in the mean count of non-pregnant women and that of nulliparous women.

Table 4 shows the effects of gestational age on the total white cell count, absolute lymphocyte count and absolute CD4+ cell count of pregnant women. Comparison of mean Total white blood cell count among women, based on trimester of pregnancy showed no significant difference (F = 2.22, p = 0.256). Comparison of mean absolute lymphocyte count among women based on trimester of pregnancy showed no significant difference (F = 2.29, p = 0.237). Similarly, no significant difference was observed in the mean absolute CD4+ cell count among women, based on trimester of pregnancy (F = 0.96, p = 0.476).

Discussion

The main objective of this study was to determine the effect of parity on the CD4 lymphocyte count of pregnant women and generally to assess the cellular immune response levels in pregnant women by gestational age. The population studied consisted of pregnant women between the ages 16 and 42, with a mean age of 27.9 years and all being in the child-bearing stage of womanhood. The age range with the highest frequency is 21-25 years and the age range with the lowest frequency is 36-40years. In regard to gestational age, the category of women in their second trimester had the highest frequency while those in the first trimester had the lowest. Multiparous women had mean longer child-spacing period than primiparous women.

Physiological changes in pregnancy are principally influenced by changes in the hormonal milieu. Hormonal shifts in value tend to align with the maternal immune status at any point in time and pregnancy is associated with subtle immunological changes at different stages of the pregnancy [10]. In our study, the total white blood cell count performed on nulliparous pregnant women, primiparous pregnant women, multiparous pregnant women and non-pregnant women (control), had a significant difference (p = 0.0001). Comparison of the mean total white blood cell count (TWBC) between pregnant women and non-pregnant women showed that pregnant women (nulliparous, primiparous and multiparous) had higher total white blood cell count when compared with non-pregnant women. White blood cell count is usually increased in pregnancy with the larger number consisting of neutrophils. Other changes in the leukocyte profile may include display of toxic granulation by neutrophils; while there is depression of chemotaxis and phagocytic activity, more likely due to inhibitory factors present in the serum of the pregnant woman [5,21]. Additionally, increased oxidative metabolism is observed in neutrophils during pregnancy. Immature forms that do not have any pathological significance such as myelocytes and metamyelocytes may also be found in the peripheral blood film of normal women during pregnancy.

However, we found that inflammatory responses (as determined by the TWBC) decreased with subsequent parity. Parity is the classification of a woman by the number of live-born children and stillbirths she has delivered at more than 20 weeks of gestation. It indicates the number of pregnancy that has reached the legal age of viability [21]. Parity has been known to be a factor that influences some variables in women of child-bearing age. Comparison of the mean total white blood cell count showed nulliparous pregnant women having higher total white blood cell count when compared with other groups of women (primiparous and multiparous pregnant women). Being new to the pregnancy process, there is an altered physiology in the body of the nulliparous woman; with a consequence that her body recognizes the fetus as being an invading object. The invasion of the decidua by the fetus brings about physiological stress and inflammation hitherto not experienced in the nulliparous woman; and her body's innate

immune system responds by inducing leukocytosis which essentially is due to neutrophilia. However, by the time of another pregnancy, some physiological and immunological adaptation must have taken place, culminating in improved innate immune tolerance to the fetus, and consequently diminished inflammation; and this phenomenon seems to get improved with parity. Hence, the total white blood cell count in the nulliparous pregnancy is higher than that of primiparous (second pregnancy) while that of primiparous is greater than those of multiparous women.

No significant change was observed between the pregnant and non-pregnant women's absolute lymphocyte counts in our study, implying that changes in the TWBC observed in this study is essentially brought about by increase in the granulocytes (mainly neutrophils) fraction. In addition, absolute lymphocyte count performed on pregnant women and the non-pregnant women, showed no significant difference between the two groups, signifying that gestation has no influence on the absolute lymphocyte count of women. While the highest absolute lymphocyte count was observed in primiparous pregnancies; there is an insignificant difference when compared with other groups of women. A notable observation is the level of absolute lymphocyte count similarity seen in both the non-pregnant women and the nulliparous women. The aggregate finding then is that while there are changes in the total number of leukocytes, no significant change occurs in the number of lymphocytes. This again buttresses the assertion that neutrophils and not lymphocytes are the leukocyte subset most affected in the leucocytosis of pregnancy, signifying an increased level of inflammation in pregnancy as compared to the non-pregnant woman.

Our data also showed that the absolute CD4+ count performed on pregnant women by parity showed a significant difference (p= 0.0351) with higher absolute CD4+ count being observed in non-pregnant women, when compared with pregnant women; indicating that the T helper cells are a group of immune cells depressed in pregnancy. A relatively high CD4+ count usually connotes a state of adaptive immune competence since CD4+ T cells play a key role in immune protection in the normal person. The functions of these cells include assisting B cells to make antibodies, recruitment of other immune cells and enhancing the capacity of macrophages to develop more potent microbial destructive activities, among others [23]. However, because there is a level of immune-modulation in pregnancy, the low CD4+ count among the subjects in our study is not surprising. Abbassi-Ghanavati et al. [24] had posited that blood parameter values found in pregnant women may differ from those in the non-pregnant state. Moreover, the comparatively low levels of (677.6/ml) CD4+ cells in our study subjects are comparable to the ones found in normal HIV sero-negative pregnant women in other studies [17,19,20,25] carried out in Nigeria. While Aina *et al*., reported a mean CD4+ count of 771 cells/ml, for pregnant women in their study, Chama et al. [25] had a mean count of 754/ml, while Akinbami et al. [20] had 771cells/ml. Lower counts than were found in our study were obtained in other centres within Nigeria [26,27]. Differences in count may be influenced by a variety of factors in different study populations but it appears that geographical location, genetics, education and diet pattern may contribute.

We observed that the absolute CD4+ count of the pregnant women in this study was most depressed among the nulliparous women. Being their first time of exposure to the immune modulating condition of pregnancy, the level of CD4+ count depression is understandable. However, the CD4+ value went up again by the time of the second pregnancy and fell in subsequent pregnancies. It is not yet clear what is responsible for this phenomenon. However, in their study, Kieffer et al. [28] found that effector memory T cells persisted for about 18 months post-partum, during which the population of T cells were generally boosted. In our study population, the time span between delivery of the first baby and a subsequent pregnancy averages about 20 months. This time frame and the 18 months of T effector memory cell persistence in the Kieffer et al. [28] study is comparatively similar. The relative equivalence in the average child spacing period of 20 months post-delivery for women who are primiparous in our study population to conceive after previous delivery; and the 18 months span in which the Kieffer et al. [28] study found a general boosting of the T cell population among women post-partum, may explain the increased CD4+ count observed among the primiparous group. This suggests that T cell numbers are expanded after the first baby, by the mechanism of effector memory T cells persistence. However, it is not yet clear why there is a decline in the CD4 count among multiparous subjects as observed herein. However, since immune cells respond to hormonal changes [13], the use of hormonal contraceptives pre-conception, for birth control after a few babies; as is now the common practice in the study population, may be responsible for the observed depressed CD4+ count at this stage.

From our data, comparison of mean value of all parameters assessed (Total white cell count, Absolute lymphocyte count, and Absolute CD4+ count) among women, based on gestational age of pregnancy showed no significant difference. Previous studies have established that the CD4+ count of pregnant women generally follow a pattern in which there is absolute CD4+ count decrease at the early stage and then increase later in pregnancy [16]. The pattern in our study suggests a raised CD4+ count in the first trimester followed by a decline in mid-gestation and another moderate increase in the third trimester. It should however be noted that the data here shows that of the absolute CD4+ count and not the subsets. We did not carry out studies on the T helper cells (Th1 and Th2) subsets. The variations seen in the cellular parameters tested for gestational age may be explained by the hormonal changes that occur from trimester to trimester in the pregnant woman. Except for the absolute lymphocyte count which alterations were not significant, there was generally a similar pattern of variation in the blood cellular components in accordance with gestational age in our study. In the three parameters assessed and especially the Total white blood cell count and absolute CD4+ count, the trend follows the same pattern in that, within the first trimester the values are relatively high, decreases remarkably in second trimester and is raised a little in the third trimester. This pattern aligns with the concept that normal pregnancy is associated with a pro-inflammatory profile at early and late stages, while mid-gestation is anti-inflammatory.

Conclusion

We were able to establish the influence of parity on the CD4+ count of pregnant women as well as on their total white blood cell counts. The WBC count result depicts an improved innate immune tolerance with each subsequent pregnancy. From the pattern of CD4+ counts by parity in this study, hormonal interplay, possibly caused by pre-conception contraceptive use in the multiparous women, may depress the CD4+ T cells level after the first one or two babies. More research efforts should elucidate the obstetric linkage responsible for this immune response pattern.

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