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Research Article

Assessment of CD4, CD8 and White Cell Parameters Amongst HIV Seronegative Pregnant Subjects in Port Harcourt, Nigeria

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Abstract

Pregnancy involves maternal immunological adjustments to accommodate the fetus and maintain a strong immune defense against potential pathogens. The present study evaluated the changes in CD4, CD8, white blood cell (WBC) and total lymphocyte count (TLC) amongst HIV seronegative pregnant subjects in Port Harcourt, Nigeria. A total of 302 female subjects (18-39 years) were recruited for the study. They consisted of 205 pregnant subjects and 97 non-pregnant subjects which served as the control. All subjects were screened for HIV type 1 and type 2 using standard test kits. Total and differential white blood cell counts were determined using a haematology auto analyzer while the total lymphocyte count (TLC) was obtained by multiplying total white blood cell count (TWC) with percentage lymphocyte count. The CD4 and CD8 cell counts were analyzed using the automated flow cytometry analyzer while the CD4:CD8 cell count ratio was obtained by dividing the CD4 cell count value by that of CD8. The result of the study shows a statistically significant decrease in CD4 and CD8 cell counts, lymphocyte and total lymphocyte counts and an increase in neutrophil count in all the trimesters of pregnancy when compared to the non-pregnant control (p<0.05). Also, there was a significant increase in WBC during the third trimester and a similar decrease in monocyte count in the first and third trimesters of pregnancy. The evidence from the present study concludes that pregnancy modifies the maternal immune response to ensure fetal survival and the protection of the mother from invading pathogens as reported in the increase in total WBC, neutrophil and monocyte counts and a reduction in TLC, CD4 and CD8 counts. The study recommends routine assessments of these crucial cellular immune markers for pregnant women during antenatal visits.

Keywords

CD4 Count, CD8 Count, CD4:CD8 Ratio, Total Lymphocyte Count, Total White Blood Cell Count and Pregnancy

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1. Introduction

Pregnancy, also known as gestation, encompasses the period from fertilization of an embryo to childbirth, typically lasting about nine months and divided into trimesters of three months each. Throughout this period, a woman's body undergoes substantial anatomic, physiologic, and metabolic adaptations essential for accommodating the developing fetus and preparing for childbirth, lactation, and postnatal care [1-3]. These adaptations are intricately interlinked and impact all body systems, primarily influenced by the hormonal activities of the placenta [4, 5]. Maternal physiology experiences significant adjustments to facilitate the growth of the fetus, creating a unique challenge for the maternal immune system which must tolerate the presence of the fetus, and at the same time maintain a relatively strong immune response against invading pathogens [6, 7]. One essential aspect of the immune system is its role in safeguarding the host against pathogens. This capability relies on the innate immune system's ability to orchestrate cell migration for surveillance and to identify and react to incoming microorganisms. During normal pregnancy, the human decidua, the epithelial layer of the endometrium harbours a substantial population of immune cells such as natural killer (NK) cells (70%), macrophages (20 – 25%), dendritic (1.7%) and regulatory T cells (3 – 10%) [8-10].

The T lymphocytes, commonly known as T cells, are a type of white blood cell that plays a central role in the immune system. They are a key component of the adaptive immune response, which is the body's specialized defence mechanism against specific pathogens. Subtypes of the T lymphocytes include the helper T cells (CD4), the cytotoxic T cells (CD8) and the memory T cells. They work in coordination with other components of the immune system, such as the B cells, macrophages, and dendritic cells, to mount a targeted and specific defence against pathogens and the elimination of infected or abnormal cells [11, 12]. The CD4 cells are primarily responsible for orchestrating and coordinating the immune response via the interaction with other immune cells such as B cells and cytotoxic T cells and releasing signalling molecules called cytokines. These cytokines regulate the immune response, enhancing the activity of other immune cells. Similarly, CD8 cells, also known as cytotoxic T cells responsible for the recognition and destruction of infected cells and the activation of cell-mediated immunity. Both CD4 and CD8 cells are involved in immune "memory" as the cells "remember" previous encounters with specific pathogens, leading to a faster and more effective immune response upon subsequent exposure [12, 13].

Report on cyclical changes in CD4, total white blood cell and differential eosinophil, basophil and neutrophil as well as other haematological parameters during the menstrual cycle of healthy undergraduate students in Port Harcourt, Nigeria had been documented in our centre [14]. Meanwhile,

several reports on increased white blood cells and differential neutrophils and monocytes during pregnancy have been established [3, 15-17]. Though, the report on total lymphocyte count during the trimesters of pregnancy is relatively scanty, an increase in total lymphocyte count from the first to third trimester of pregnancy has been reported in Punjabi, India [18]. An increase in CD4 absolute count has been reported in Ekpoma, Nigeria [19]. However, despite its physiological importance, baseline data on CD4 and CD8 cell counts, total lymphocyte count and CD4:CD8 cell count ratio during pregnancy amongst HIV seronegative pregnant subjects in Port Harcourt, Nigeria is not readily available. The present study evaluates the changes in CD4, CD8, white blood cell and total lymphocyte count amongst HIV seronegative pregnant subjects in Port Harcourt, Nigeria. This will provide more insight into the pattern of change in these cell-mediated immune markers during pregnancy.

2. Materials and Methods

2.1. Study Population and Research Design

This was a cross-sectional descriptive study. A total of 302 female subjects (18-39 years) were recruited for the study. They consisted of 205 pregnant subjects and 97 non-pregnant subjects which served as the control. The pregnant subjects were further divided into three groups according to the trimesters of pregnancy consisting of 33, 79 and 93 subjects in their first, second and third trimester of pregnancy respectively. All pregnant subjects were attending antenatal care visits in tertiary and secondary health facilities in Port Harcourt, Nigeria. The non-pregnant subjects were recruited from the staff of the University of Port Harcourt, Nigeria. All subjects with known cardiovascular and metabolic diseases and all pregnant subjects with known pregnancy complication(s) were excluded from the study.

2.2. Data and Sample Collection

A basic questionnaire was employed to obtain details such as the participants' age and gestational age. The height and weight of the subjects were measured using a Secca scale. Body mass index (BMI) was calculated by dividing the weight (kg) of the subjects by the square of their heights (m). The diastolic and systolic blood pressures of all subjects were measured using a standard mercury sphygmomanometer after they were made to rest for about 30 minutes. The blood pressure measurements were done from the left arm of all subjects in a comfortably seated position. Successive measurements were done until two consecutive measurements with the same readings were obtained. The mean arterial pressure (MAP) of all subjects was determined from this formula: MAP = DBP +1/3 pulse pressure. About 5ml of

venous blood was collected from the antecubital vein of each subject, transferred into EDTA sample bottles and carefully mixed. Blood samples were collected each day within the hours of 9 AM to 12 PM.

2.3. Laboratory Analysis

All samples were screened for HIV type 1 and type 2 (HIV-1 and HIV-2) using Determine HIV-1/2 test kits (Abbot, USA). Total and differential white blood cell counts were determined using a haematology auto analyzer (Sysmex Corporation Kobe, Japan). Total lymphocyte count was obtained by multiplying total white blood cell count with percentage lymphocyte count [15]. CD4 and CD8 cell counts were analyzed using the automated flow cytometry analyzer (Partec GmbH Görlitz. Germany) while the CD4:CD8 cell count ratio was obtained by dividing the CD4 cell count value by that of CD8 [20].

2.4. Ethical Considerations

The procedures in this study adhered to the most stringent ethical guidelines outlined in the World Medical Association (WMA) Helsinki Declaration of 1963, updated in 2013 [34]. Before enrollment in the study, each potential participant signed a consent form. The research design and protocol received approval from the Research Ethics Committee of the University of Port-Harcourt (UPH/CEREMAD/REC/M66/008).

2.5. Data Analysis

Data from this study were analyzed using SPSS version 25. The mean and standard error were calculated for all parameters. The ANOVA followed by an LDS post hoc analysis was used to determine the differences among the non-pregnant controls and the pregnant subjects in their various trimesters. The differences between groups were considered statistically significant at a p-value less than 0.05 ($p < 0.05$).

3. Results

Table 1. Age, anthropometric parameters and blood pressure indices of pregnant and non-pregnant subjects.

Parameters	Non-pregnant (n=97)	1st trimester (n=33)	2nd trimester (n=79)	3rd trimester (n=93)
Age (years)	25.63 ±0.07	25.00 ±0.70	26.34 ±0.53	27.40 ±0.49
Body mass index (kg/m^2)	24.46±0.32	26.20±0.69	30.81±0.32*	33.89*±0.41
Systolic blood pressure (mmHg)	111.75±0.75	111.52±1.24	111.27±1.02	109.73±1.11
Diastolic blood pressure (mmHg)	72.62±0.78	71.67±1.42	73.42±0.92	72.85±0.93
Mean arterial pressure (mmHg)	85.29±0.70	84.95±1.32	86.03±0.89	84.39±1.08

Values are mean±SEM, * Showing a significant difference when compared to non-pregnant subjects ($p<0.05$).

Table 1 displays the age, body mass index (BMI), and blood pressure parameters (systolic, diastolic, and mean arterial pressure) of both pregnant and non-pregnant subjects in the study. As anticipated, a notable difference in BMI was observed among pregnant participants during the second and third trimesters compared to the non-pregnant control ($p<0.05$). BMI was highest during the third trimester, followed by the second trimester. The mean values for blood pressure parameters (systolic, diastolic, and mean arterial pressure) in the pregnant groups were comparable to those in the non-pregnant group.

Table 2. Values of total white blood cell and differential lymphocyte, neutrophil, and monocyte cell counts and total lymphocyte count of pregnant and non-pregnant subjects.

Parameter	Non-pregnant (n=97)	1st trimester (n=33)	2nd trimester (n=79)	3rd trimester (n=93)
Total white blood cell (cell/μl)	7.79 ±0.05	7.50 ±0.31	7.81 ±0.18	9.67* ±0.18
Lymphocyte count (%)	64.09±0.60	24.14*±0.83	25.05*±0.58	20.84*±2.33
Neutrophil count (%)	26.01±0.51	63.38*±0.79	65.34*±0.40	61.35*±.83
Monocyte count (%)	8.25±0.25	12.44*±0.55	9.61±0.31	12.11*±2.07

Parameter	Non-pregnant (n=97)	1st trimester (n=33)	2nd trimester (n=79)	3rd trimester (n=93)
Total lymphocyte count (%cell/µl)	501.00±6.35	178.46*±3.47	184.10*±3.08	201. *±6.43

Values are mean±SEM, * Showing a significant difference when compared to non-pregnant subjects (p<0.05).

Table 2 illustrates the values of total white blood cell count, differential lymphocyte, neutrophil, monocyte and total lymphocyte count of both pregnant and non-pregnant subjects in the study. The data shows a statistically significant increase in neutrophil count in all trimesters of pregnancy compared to the non-pregnant control (p<0.05). The total white blood cell count was significantly increased during the third trimester compared to the non-pregnant control (p<0.05) while monocyte count was significantly increased in the first trimester of pregnancy compared to the non-pregnant control. However, a significant decrease in differential lymphocyte and total lymphocyte count was observed in all trimesters of pregnancy when compared to the non-pregnant control group (p<0.05).

Table 3. Values of CD4 count, CD8 count and CD4:CD8 cell count ratio parameters of pregnant and non-pregnant subjects.

Parameters	Non-pregnant (n=97)	1st trimester (n=33)	2nd trimester (n=79)	3rd trimester (n=93)
CD4 (cell/µl)	957.51±17.67	630.58*±26.52	795.92*±22.04	692.65*±0.65
CD8 (cell/µl)	908.43±17.78	471.33*±20.01	543.46*±16.17	545.43*±28.35
CD4:CD8 count ratio	1.13±0.04	1.36*±0.04	1.50*±0.03	1.38*±0.04

Values are mean±SEM, * Showing a significant difference when compared to non-pregnant subjects (p<0.05).

Table 3 shows the values of CD4 and CD8 cell counts and CD4:CD8 cell count ratio of both pregnant and non-pregnant subjects in the study. The data indicates a statistically significant decrease in the CD4 and CD8 cell counts in all trimesters of pregnancy when compared to the non-pregnant control group (p<0.05). It also shows a statistically significant increase in the CD4:CD8 cell count ratio when compared to the non-pregnant participants (p<0.05).

4. Discussion

The maternal body undergoes significant physiological changes to support fetal growth, posing a distinct challenge to the maternal immune system. It must both accommodate the presence of the fetus and sustain a robust immune defence against potential pathogens. In this study, we examined the alterations caused by pregnancy in the maternal immune system, specifically examining changes in circulating CD4, CD8, white blood cell, and total lymphocyte count across the trimesters of pregnancy among HIV seronegative pregnant individuals.

Data from the study indicates a significant increase in the BMI of pregnant subjects during the second and third trimesters compared to the non-pregnant control (p<0.05) (Table 1). Weight gain during pregnancy is a natural occurrence primarily attributed to several factors, including the formation of the placenta, expansion of fluid volume, increased uterine weight, development of amniotic fluid, and accumulation of maternal fat, protein, and other essential nutrients [21, 22]. Reports from previous studies relate preterm delivery to reduced weight gained before pregnancy [23, 24]. The weight gained by the population under study is apparently normal. This is however expected as the present study excluded all metabolically compromised subjects. Though preeclampsia and eclampsia in normal pregnancy leading to maternal and fetal mortality and morbidity had been reported [25, 26], the present study does not indicate any gestational complication. There was no significant difference in all blood pressure parameters between the pregnant and non-pregnant subjects. This could be due to the exclusion of all subjects with known cardiovascular disease.

The study also showed a statistically significant increase in the values of total white blood cell count in the third trimester, neutrophil in all trimesters, and monocyte in the first and third trimesters. However, there was a significant reduction in differential lymphocyte and total lymphocyte count when compared to apparently healthy non-pregnant participants (p<0.05) (Table 2). These findings are fairly in tandem with previous reports from Nigeria [3, 16, 27-30] and other parts of the world [31, 32]. Pregnancy has traditionally been linked to leukocytosis, characterized by an enhanced inflammatory response marked by elevated levels of circulating neutrophils which have been detected as early as the second month of gestation [33, 34]. Additionally, the rise in neutrophils could stem from a stress reaction triggered by the

redistribution of white blood cells between marginal and circulating pools. There's also a suggestion that a decrease in neutrophil apoptosis, chemotaxis, and phagocytic activity contributes to their increased numbers in circulation [35, 36]. Previous studies have documented a decline in overall lymphocyte count during pregnancy [37-39], a trend that aligns with our results. However, contrary to other findings, our study did not detect an elevation in lymphocyte count during the third trimester. The possible reason for these variations is not very clear, however, the immune system may have adapted to the presence of the fetus, which is genetically different from the mother by reducing the number of circulating lymphocytes to avoid rejecting the fetus, which could lead to miscarriage or preterm birth, while still maintaining the ability to fight off infections that could harm the mother or the fetus [40].

Significant reductions in the CD4 and CD8 counts and an increase in the CD4:CD8 cell count ratio were observed among the pregnant subjects when compared to the non-pregnant control ($p < 0.05$) (Table 3). This decrease in absolute CD4 and CD8 cell counts is corroborated by reports from other parts of Nigeria [19, 41], Japan [42], USA [43] while the increase in CD4:CD8 cell count ratio is in tandem with a report from Lagos, Nigeria [41]. The reasons for the decrease in the immune elements are not clear but adaptation and downregulation of cell-mediated maternal immune response to the developing fetus have been indicated to be a possible factor [40, 44]. However, this apparent downregulation of cell-mediated immunity as proposed by the classical model of immunity [45] leading to the maternal immune tolerance of the fetus has also been indicated as a major factor causing vulnerability to infections during pregnancy [46].

5. Conclusion

Pregnancy necessitates maternal immunological adjustments to support the fetus while preserving robust immune defences against potential pathogens. Due to the lack of local data on the dynamics of white blood cell, CD4, and CD8 counts during pregnancy, this study was initiated. The present study reports a significant increase in total white blood cell count and differential neutrophil and monocyte counts with an increase in CD4:CD8 cell count ratio and a significant reduction in total lymphocyte count (TLC), differential lymphocyte count, absolute CD4 and CD8 cell count during pregnancy. Based on the findings of this study, we recommend the incorporation of regular assessments of CD4 and CD8 levels during antenatal appointments. The research underscores the importance for physicians in our setting to take into account the pregnancy status of their female patients when interpreting the results of these crucial cellular immune markers. This study can lay the groundwork for cohort studies investigating both cellular and non-cellular immune variations throughout pregnancy. This could lead to a better understanding of how these immune markers impact maternal health, fetal development, and pregnancy outcomes, ultimately contributing to improved prenatal care and therapeutic interventions.

Abbreviations

CD4: Cluster of Differentiation 4
CD8: Cluster of Differentiation 8
WBC: White Blood Cell
TLC: Total Lymphocyte Count
HIV: Human Immunodeficiency Virus
SEM: Standard Error of the Mean
DBP: Diastolic Blood Pressure
MAP: Mean Arterial Pressure
BMI: Body Mass Index

Author Contributions

Solomon Ovomarani Akevwighome: Data curation, Investigation, Methodology, Writing - original draft

Bruno Chukwuemeka Chinko: Data curation, Formal Analysis, Supervision, Writing - original draft, Writing - review & editing

Sunday Ogbu Ojeka: Funding acquisition, Methodology, Investigation, Project administration

Kinikanwo Innocent Green: Funding acquisition, Investigation, Methodology, Project administration

Datonye Victor Dapper: Conceptualization, Project administration, Writing - review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Field

Solomon Ovomarani Akevwighome: Haematology-1, Body fluid-2, Immunology-3, Biomedical Sciences-4, HIV/AIDS-5

Bruno Chukwuemeka Chinko: Blood and Body fluid Physiology-1, Immune Physiology-2, Ethno Pharmacology-3, Biomedical Sciences-4, General physiology-5

Sunday Ogbu Ojeka: Immune Physiology-1, Blood Physiology-2, HIV/AIDS-3, Reproductive Physiology-4, Antioxidants-5

Kinikanwo Innocent Green: Obstetrics-1, Gynaecology-2, Reproductive Health-3, Endocrinology-4

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