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Lymphocyte subpopulations among pregnant women in Agbor, Delta state, Nigeria

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ARTICLE INFO

ABSTRACT

Objective: To determine the peripheral blood CD3 and CD8 T—lymphocytes subpopulation in healthy pregnant women in our environment. **Methods:** Pregnant women attending the antenatal care clinic (ANC) of the Central Hospital, Agbor , Nigeria, who gave informed consent were recruited for the study. Blood samples (10 mL) was collected by venipuncture from 121 healthy pregnant women (aged 17—47 years; mean 28.5 ± 0.43). 24 non—pregnant healthy women (aged 18—30years; mean 21.12 ± 0.64) were enrolled as controls. CD3+ and CD8+ counts were determined with the Partec flow cytometer. **Results:** The mean CD3+ count for pregnant women was 1311.10 ± 71.50 μ/L when compared to the controls which was 9.67 ± 0.99 μ/L. This was statistically significant(P<0.005). The mean CD8+ count for pregnant women was 340.91 ± 18.53 μ/L as against the 583.58 ± 47.30 μ/L (P>0.005). Primigravidas had a higher CD3+ mean count than multiparas while mean CD8+ count was higher in multiparas than in primigravidas. Similarly, the mean CD3+ count was significantly different between all the trimesters with the highest count of 1, 353.23 ± 43.90 μ/L in the first trimester as compared to the 9.67 ± 0.99 μ/L in the control. Mean CD3+ was highly significant in pregnant women with history of previous abortions while the CD8+ cells were not. **Conclusions:** The results suggest the need for routine determination of T lymphocyte subsets apart from CD4 in pregnant women attending ANC clinic to prevent any possible immunological /pregnancy disorder during the period of pregnancy.

1. Introduction

The lymphocytes play a central role in the regulation of immune response. Their distribution and function vary in different states. T— lymphocytes are required for the production of normal levels of antibodies by B—lymphocytes. Pregnancy is associated with the suppression of a clinical variety of humoral and cell mediated immunological functions in order to accommodate the fetus, which is a genetic makeup equally derived from both the father and the mother. During pregnancy the maternal immune system has to tolerate the persistence of 'non—self' (allogeneic) fetal cells[1].

Decidual macrophages and dendritic cells, which are found in close association with T— lymphocytes are the most potent activators of T— lymphocyte responses and could play a sentinel function for the immune system, initiating antigen—specific T— cell responses to fetal antigens. T— cell cytokines produced in response to fetal molecules could have a role in the maintenance or in the failure of pregnancy[2].

Any alteration in any parameter of the immune system can affect the health of the pregnant woman as well as the outcome of pregnancy. The immune status of the mother thus plays an important role in this process. Over fifty percent of gregnant women in the study environment suffer from either spontaneous abortion or miscarriages, many of which have undiagnosed causes. Since lymphocytes have been reported to play a role in the stability of the foetus, it became important to monitor maternal CD3 and CD8 counts as T— lymphocyte subset alterations in normal pregnant women in our population. This is the first study in our environment that reports on the CD3 and CD8 T— cell subpopulation in pregnant women.

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2. Materials and methods

2.1. Study population

At the Central Hospital, Agbor, all pregnant women who where registered for ante natal care (ANC) clinic received voluntary counselling and questionnaire was given to each of them who gave consent for the study. Immunological studies were carried out on peripheral blood CD3+ and CD8+ T lymphocyte subpopulations in samples obtained from 121 pregnant women (age 28.5±0.43, range 17 − 47 years) attending the Antenatal Clinic at the Central Hospital, Agbor Delta state were enrolled in this study. 24 non pregnant women (mainly student nurses) (aged 21.12±0.64, range 18–30 years) were included as controls.

Data such age, marital status, age at marriage, number of previous pregnancy, and assessment of gestation were based on information provided by the patients on questionnaires and data from the routine ANC Cards. Exclusion factors included those who were HIV positive, diabetics, hypertensive and sickle cell anaemia or any other disease that may affect the result of this study. Healthy non−pregnant women who were not on any hormonal contraceptives were used as controls.

Blood sample (10 mL) was collected from eligible participants by venipuncture into a vacutainer EDTA bottle. The blood sample was mixed and adequately labelled and stored at room temperature until used Ethical clearance was obtained from the Ethical and Research Committee. Of the Delta State Ministry of Health, Nigeria.

2.2. Enumeration of CD 3+ T−lymphocytes

Add 20 μL of well mixed blood in to a Partec tube then add 20 μL of CD3 mAb PE(UCUT−1, PE−conjugated IgG1) monoclonal antibody. The contents of the tubes are incubated in the dark at room temperature for 15min. Following incubation, 800 μL of dilution buffer is added to the tube to give a total volume of 840μL and a dilution factor of 42. The tubes are mixed gently for 5 seconds to re−suspend the cells immediately before counting.

2.3. Enumeration of CD 8+ T−lymphocytes

Add 20 μL of whole blood to a test tube then add 20 μL of CD8 (MEM−31, PE− conjugated IgG2a) monoclonal antibody. The contents of the tubes are incubated in the dark at room temperature for 15 min. Following incubation, 800 μL of dilution buffer is added to the tube to give a total volume of 840 and a dilution factor of 42. The tubes are mixed gently for 5 sec to re−suspend the cells immediately before counting.

2.4. Flow cytometric analysis

CD8 and CD3 PE fluorescence are analysed by the Partec

flow cytometer with an excitation light source of 488 nm or 532. The tubes containing 840 μL was transferred to the equipment for counting and the results for T cells were displayed automatically as T−cells per μL of whole blood.

2.5. Statistical analysis

Data were analysed by the Turkey multiple comparison test using Graph Pas Prism Version 5

3. Results

Table I shows mean ±SEM CD3+ and CD8+ T lymphocytes in pregnant women and control subjects in the study. The mean ±SEM CD3+ for entre pregnant women was 1311.10 ±71.50 this was found significantly higher (*P*<0.05). While the mean±SEM CD8 among pregnant women was 340.91± 18.53 non significant (*P* >0.05) when compared with control subjects.

Table 1
Mean ±SEM CD3+ and CD8 T lymphocytes in pregnant women and control subjects in the study.

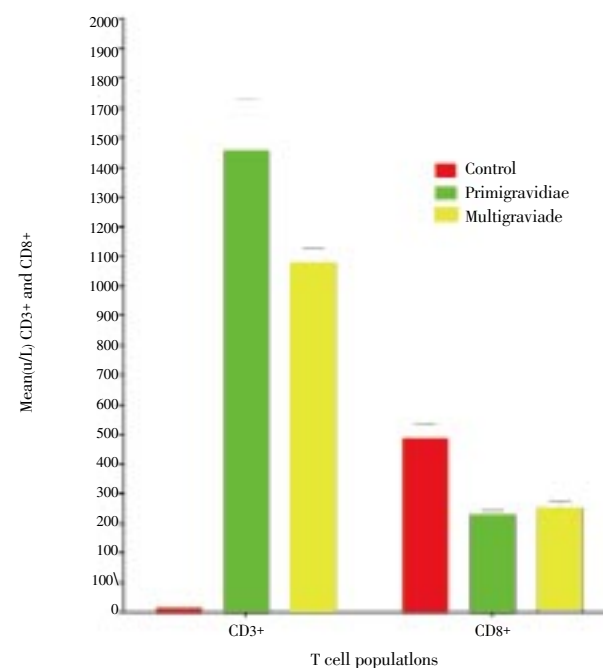| T−lymphocytes	Control (n=24)	Pregnant women (n=121)	*t*−cal	*P* values
CD3+	9.67±0.99	1311.10±71.50	17.47	<0.005
CD8+	583.58±47.30	340.91±18.53	4.78	>0.005

Figure 1. Mcan CD3+ and CD8+ accroding to gravidity.

Figure 1 shows the mean±SEM of T− lymphocytes amongst pregnant women based on gravidity. The mean±SEM CD3+ for primigravidae was (1554.81±175.53), while multigraviade had (1176.74±49.27) as mean±SEM for CD 3+. Primigravidae recorded a mean±SEM CD8+ T cells of (327.09±14.99), with multigraviade having (348.53± 27.57) (*P*<0.05).The mean CD3+

and CD 8+ T lymphocyte values based on the gestational age was found to be significant ($P<0.05$) when compared with controls (Figure 2). Figure 3 shows that CD3+ was highly significant in women with history of previous abortion ($P<0.05$) while CD8+ cells was not significant for women with history of abortion. Significant CD3+ and CD8+ counts were obtained in pregnant women without history of previous abortion (Figure 4).
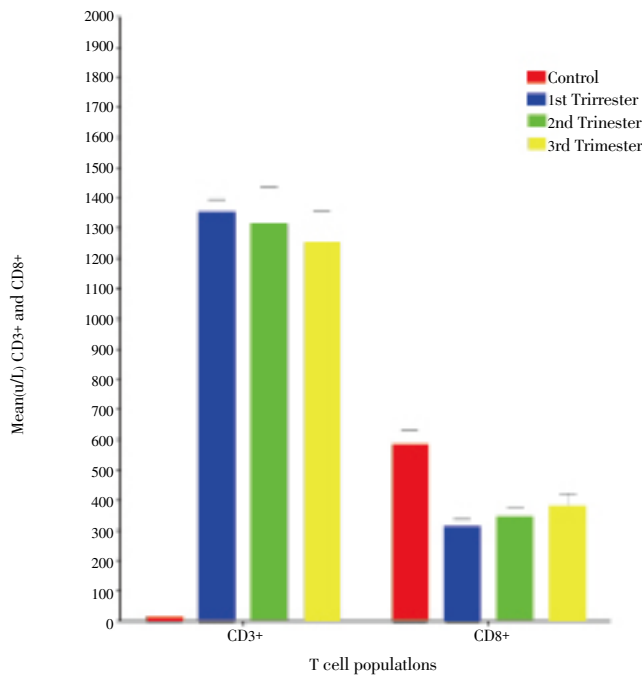

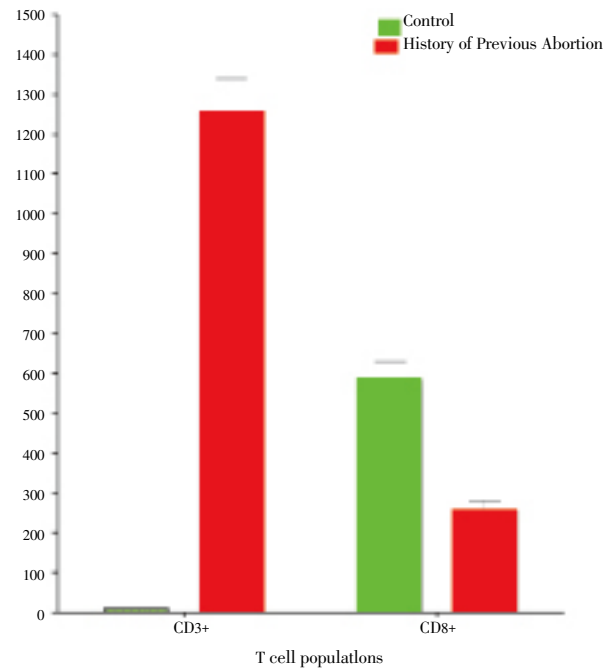**Figure 2.** CD3+ CD8+ Tlymphocytes according to gestational age.

Figure 3. CD3, and CD8+ Tlymphocytes based on study population with History of abortion.
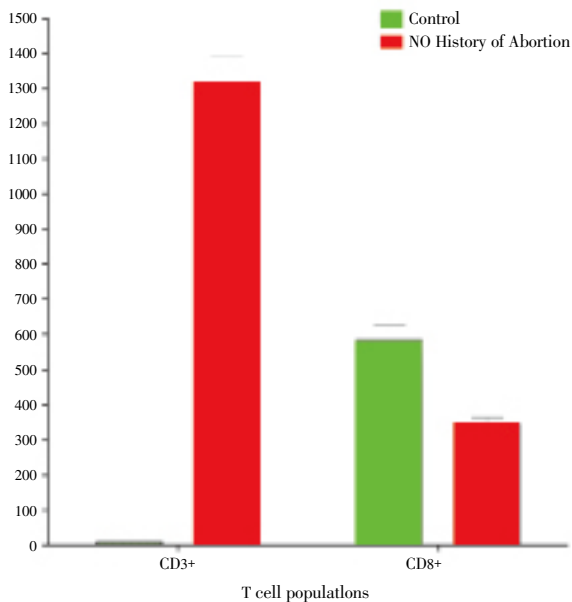

Figure 4. CD3, and CD8+ Tlymphocytes based on study pupulation with NO History of abortion.

4. Discussion

The present study was carried out to investigate CD3+ and CD8+ T–lymphocyte subpopulations in pregnancy. The immune responsiveness of women is altered during pregnancy in order to retain protective properties against disease and at the same time to allow tolerance of the foetus [3]. T– Cells can be characterised by their expression of surface molecules and by their capacity to produce various cytokines. Studies done in women with unexplained pregnancy losses suggest T– cell alterations that may be involved in the pathogenesis of recurrent pregnancy losses.

In this study, there was a gradual decrease in CD3+ T– cells as the pregnancy progresses. This finding is in agreement with previous reports[4, 5].It has been reported that in peripheral blood there was no difference in the CD3+ T cells in non pregnant women with a history of recurrent pregnancy loss as compared to those of normal non– pregnant women[6]. However, when the CD3+ T cells levels measured during the first trimester of women who miscarried the levels are significantly lower than those who have successfully delivers a live infant[6].

In this study there was a gradual increase in the CD8+ counts within the gestational age of the pregnant women. This is higher than those in the study conducted on women in New York[7]. Previous studies have reported little or no changes in CD8+ cell levels during pregnancy[4, 8–10]. This has been attributed to the compensatory mechanism that demonstrates an intact capacity of recovery after the adaptation to pregnancy[11].

Spontaneous abortion is the most common adverse reproductive outcome. Generally, immunocompetent cells and mediators such as CD3+ and CD8+ T cells have

been reported to contribute to maintenance or failure of pregnancy[12]. Hence from these observations CD8+ cells are very likely to be pregnancy protective. Depletion of CD8 could lead to a termination of the pregnancy protective effect of progesterone substitution[13]. In this study women with previous abortion had lower values for CD8+ compared with those with history of no previous abortion. Furthermore, CD8+ was higher in the control group than in the study population. This result is in line with previous reported where lower CD8+ T lymphocyte in women with history of recurrent spontaneous abortion was observed[14–16].

Given that CD8+ cells appear to be critical in the defense against infections, an increase in their levels may at or near delivery reduce the risk of perinatal infections. We therefore recommend that pregnant women who attend antenatal clinic be evaluated for the CD4 and CD8 counts in addition to the CD4 count which is only occasionally screened for to check for HIV and AIDS patients who are pregnant.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

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