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ROLE OF CELL MEDIATED CD4+ AND CD8+ T-CELLS IN PREGNANCY LOSS

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ABSTRACT

Background: Early pregnancy loss is commonly associated with immunological dysfunction. **Objective**: Analysis of CD4+ and CD8+ T-cell expression in patients with pregnancy loss in Abia State, South East, Nigeria. **Materials and Methods**: This was a cross-sectional study involving women in their reproductive years. Study population was stratified into 3 groups and the CD4 and CD8 concentrations measured and compared among the three groups. **Results**: A total of 130 apparently healthy Nigerian women of child-bearing age were enrolled in the study. The study groups consisted of 70 women who had just lost a pregnancy, 30 women with normally progressing pregnancy and 30 nonpregnant women. The mean CD8+ cell count of the pregnancy-loss subjects was significantly elevated relative to the normal pregnancy subjects. The mean CD4+ cell count value of the pregnancy-loss subjects was, however, significantly lower than that of the non-pregnant control group. **Conclusion:** Pregnancy loss is accompanied by reduction in CD4/CD8 ratio.

KEYWORDS: Pregnancy loss, CD4+ T-cell, CD8+ T-cell, CD4/CD8 ratio.

INTRODUCTION

Following the recognition of foetal antigens by the maternal immune system, the latter reacts by putting in place a wide range of protective mechanisms beginning with a leucocytic reaction at the level of the decidua (the endometrium of pregnancy) (Piccinni, 2006; Sanguansermsri and Pongcharoen, 2008). This immunological recognition of pregnancy is important for the maintenance of gestation, and a defective or inadequate recognition of foetal antigens by the maternal immune system may end up in failed pregnancy (Szekeres-Bartho, 2002). In the pregnant state, T-cells appear fairly abundant at the maternal-foetal interface, comprising about 10%–20% of the human decidual white blood cell population, of which 30%–45% are $CD4^+$ T-cells and 45%–75% are $CD8^+$ T-cell subset (Nancy and Erlebacher, 2014). T-cells originate from stem cells in haematopoietic tissue and undergo differentiation in the thymus triggered by thymopoietin (Yang *et al*., 2010). The main function of T-cells in the decidua, particularly of $CD4^+$ T-regulatory (Treg) cells, is generally thought to be the promotion of tolerance to the foetus (Heikkinen *et al*., 2004). Maternal T-cells are the source of cytokines that create a suitable microenvironment for preimplantation embryo development and pregnancy maintenance (Piccinni, 2005). Some studies have reported that T-cells exert a greater effect on patients with recurrent pregnancy loss (RPL) (Yang *et al*., 2010). Thus absence of this state of immune tolerance tends to loss of pregnancy. Secreted $CD8^+$ T-cells are thought to play a role in regulation of trophoblast invasion, though precise mediators have not yet been identified (Scaife, 2006). In this study we aimed at comparing the CD4+ and CD8+ T-cell concentrations in pregnancy loss, normal pregnancy and nonpregnant controls.

MATERIALS AND METHODS

A total of 130 apparently healthy women of childbearing age (18-45 years) were enrolled in this study, using a cross-sectional study design stratified into three groups of study as follows: pregnancy-loss (70 subjects), normal pregnancy (30 subjects) and non-pregnant control (30 subjects). Study lasted the period between December 2017 and March 2018.

Study Setting

The study took place at the following hospitals: Federal Medical Centre, Umuahia, Nazareth Specialist Hospital Aba, (a specialist gynaecological clinic), General Hospital, Aba and General Hospital, Ohafia, all in Abia state, Nigeria.

Blood collection

3mls of the blood was put into plain vacuum containers and the serum retracted after 30 minutes of clotting. The serum was used for the estimation of CD4+ and CD8+ T-cell count.

Laboratory Analysis

The manufacturers' standard operation procedure for each investigation was used during each assay, and the operational instruction for each machine was strictly followed. CD4+ and CD8+ T-cell counts were done using the ELISA technique with kit procured from Melsin Laboratories, China.

Ethical Approval

Ethical approval was obtained from the Ethics Committee of the Federal Medical Centre, Umuahia, Abia state Hospitals Management Board, and the Nazareth Specialist Hospital, Aba.

Statistical Analysis

Statistical analysis was done using SPSS windows version 20 (IBM Corporation, 2011). Data was grouped into pregnancy-loss, normal pregnancy and non-pregnant control subjects. The Kolmogirov-Smirnov test was used to determine normality. Parametric data were expressed as +/- SD. Immunological and haematologic parameters were skewed. Hence, they were expressed as median, 2.5^{th} (p2.5) and 97.5^{th} (p97.5) percentile, and were log-transformed prior to analysis. ANOVA and Games-Howell post hoc procedures were used to test for group differences. Data was considered significant at error probability *P*-level less than or equal to (≤) .05.

RESULTS

Table 1: Comparison of CD4+ and CD8+ T-cell concentrations between Pregnancy-Loss, Normal Pregnancy, and Non-pregnant Subjects.

Parameter		Pregnancy Loss (n=70)	Normal Pregnancy (n=30)	Non-pregnant (n=30)	*F*	*P*
CD8 (U/ml)	*Mdn*	14.00	10.15	12.00	5.77	.00**
	P2.5 – P97.5	8.65 – 58.17	7.60 – 10.15	8.60 – 12.00		
CD4 (U/ml)	*Mdn*	1.00	1.20	1.50	4.61	.01**
	P2.5 – P97.5	0.60 – 7.53	0.80 – 1.20	0.70 – 1.50		

Key: n = number of subjects, *Mdn* = median, P2.5 = 2.5^{th} percentile, P97.5 = 97.5^{th} percentile, *F* = F-test statistic, *p* = error probability, CD8 = Cluster Differentiation 8, CD4 = Cluster Differentiation 4, *Significant difference observed at $p \leq .05$, **Significant difference observed at$p \leq .01$, using ANOVA.

Table 2: Post hoc Testing of CD4+ and CD8+ T-cell concentrations between Pregnancy-Loss, Normal Pregnancy, and Non-pregnant Subjects.

Immunological Parameter	*Post hoc* Pair	95% CI	*P*
CD8 (U/ml)	PL vs Norm P.	0.06 – 0.31	.00**
	Norm P. vs Non-P.	-0.35 – -0.00	.05*
	PL vs Non-P	-0.15 – 0.17	.99
CD4 (U/ml)	PL vs Norm P.	-0.19 – 0.10	.78
	Norm P. vs Non-P.	-0.37 – 0.03	.12
	PL vs Non-P	-0.40 – -0.02	.03*

Key: PL = Pregnancy Loss, Norm P. = Normal Pregnancy, Non-P. = Non-pregnant, *p* = error probability, 95% CI = 95% Confidence Interval of the Difference, CD8 = Cluster Differentiation 8, CD4 = Cluster Differentiation 4, **Significant difference observed at $p \leq .01$, *Significant difference observed at $p \leq .05$. *Post hoc* testing were done using Turkey HSD and Games-Howell methods as applicable.

Table 3: Comparison of CD4-CD8 Ratio between Pregnancy-Loss, Normal Pregnancy, and Non-pregnant Subjects.

Derived Parameter		Pregnancy Loss (n=70)	Normal Pregnancy (n=30)	Non-pregnant (n=30)	*F*	*P*
CD4:CD8	*Mdn*	0.08	0.11	0.10	12.91	.00**
	P2.5 – P97.5	0.02 – 0.38	0.08 – 0.11	0.06 – 0.10		

Key: n = number of subjects, *Mdn* = median, P2.5 = 2.5^{th} percentile, P97.5 = 97.5^{th} percentile, *F* = F-test statistic, *p* = error probability, CD4:CD8 = CD4-CD8 ratio, *Significant difference observed at $p < .05$, **Significant difference observed at $p < .01$, using ANOVA.

Table 4: Post hoc Testing of CD4-CD8 Ratio between Pregnancy-Loss, Normal Pregnancy, and Non-pregnant Subjects.

Derived Parameter	*Post hoc* Pair	95% CI	*P*
CD4:CD8	PL vs Norm P.	0.44 – 0.08	.00**
	Norm P. vs Non-P.	-0.00 – 0.07	.99
	PL vs Non-P	0.44 – 0.10	.00**

Key: PL = Pregnancy Loss, Norm P. = Normal Pregnancy, Non-P. = Non-pregnant, *p* = error probability, 95% CI = 95% Confidence Interval of the Difference, CD4:CD8 = CD4-CD8 ratio, **Significant difference observed at$p < .01$. All *post hoc* testing were done using Turkey HSD and Games-Howell methods as applicable.

DISCUSSION

There are varied reports regarding changes in CD4+ and CD8+ T-cell concentrations in pregnancy loss (Vidali, 2012; Sehmsdoorf *et al*., 2004; Ghafourian *et al*., 2014). Normal pregnancy is generally associated with lowered CD4+ and lowered CD4/CD8 ratios (Oladepo *et al*., 2009; Akinbami *et al*., 2014). Chama and colleagues reported a decrease of CD4+ cells especially in the first trimester (Chama *et al*., 2009). Babatope *et al*, (2018), found significantly lower serum concentrations of CD4 levels during pregnancy when compared to nonpregnant controls but found no significant difference in CD8+ levels when pregnant and nonpregnant states are compared.

In our work, the mean CD8+ T-cell count of the pregnancy-loss subjects (*Mdn*: 14.00 U/ml, p2.5 – p97.5: 8.65 – 58.17 U/ml) was significantly elevated relative to the normal pregnancy subjects (*Mdn*: 10.15 U/ml, p2.5 – p97.5: 7.60 – 10.15 U/ml, $p = .00$) (Table 1). This value was also higher than that of the non-pregnant control subjects (*Mdn*: 12.00 U/ml, p2.5 – p97.5: 8.60 – 12.00 U/ml), but was however not significant ($p > .05$). The increase in the levels of peripheral CD8+ T cells in pregnancy loss agrees with the finding of Ghafourian *et al*, (2014) who even postulated that CD8+ cells were involved in the pathogenesis of recurrent pregnant loss. In their work, Babatope *et al*, (2018) found no significant difference in CD8+ T-cell levels when pregnant and nonpregnant states were compared.

The mean CD4 count of the pregnancy-loss subjects (*Mdn*: 1.00 U/ml, p2.5 – p97.5: 0.60 – 7.53 U/ml) was slightly reduced when compared with that of the normal pregnancy subjects (*Mdn*: 1.20 U/ml, p2.5 – p97.5: 0.80 – 1.20 U/ml) but this difference was however not significant ($p > .05$). (Table 1). In line with earlier reports by Oladepo *et al*, (2009) and Akinbami *et al*, (2014), the difference in the CD4+ count between the normal pregnancy and non-pregnant control subjects was not significant, either ($p > .05$). The pregnancy-loss CD4 count value in our study was, however, significantly lower than that of the non-pregnant control group (*Mdn*: 1.50 U/ml, p2.5 – p97.5: 0.70 – 1.50 U/ml, $p = .03$). This is at variance with the reports by Vidali (2012) and Gao *et al*, (2014). Gao *et al*, (2014), found higher values of CD4+ levels among those with RPL than among sporadic aborters and normal nonpregnant controls while they found no significant difference in the CD8+ levels among the groups. Thus in their work, CD4/CD8 ratio was significantly higher in patients with RPL than with subjects of sporadic spontaneous abortion and healthy nonpregnant controls. Vidali (2012) also suggests that an increased CD4/CD8 ratio (i.e. increased CD4 and decreased CD8) and not lowered CD4/CD8 ratio (i.e. reduced CD4 and elevated CD8), is indicative of an increased tendency to miscarry. But in our work, the CD4/CD8 ratio of the pregnancy-loss subjects (*Mdn*: 0.08, p2.5 – p97.5: 0.02 – 0.38) was, however, significantly lower than that of the normal pregnancy (*Mdn*: 0.11, p2.5 – p97.5: 0.08 – 0.11) and non-pregnant control groups (*Mdn*: 0.10, p2.5 – p97.5: 0.06 – 0.10) ($p < .01$). (Table 3). Ghafourian *et al*, (2014) also found a lower CD4/CD8 ratio in recurrent pregnancy loss (RPL) women compared to controls as well as null statistical difference in the percentage of CD4+ in the groups studied. They also found a significant higher CD8+ count in RPL women over normal pregnant controls (as in our study). However, Sehmsdoorf *et al*, (2004) found no significant difference in the numbers of CD4+ and CD8+ T-cells between women who had just suffered pregnancy loss and women with normally progressing pregnancies.

Norton *et al*, (2010) suggested that pregnancy does not fundamentally alter or suppress the tissue-specific mechanisms that maintained T-cell homeostasis even though pregnancy may modify local environment. They reported that the proliferative capacity of CD8 T-cells appears to be maintain ned throughout pregnancy without resulting in foetal demise and that early tolerance of the foetal tissue does not demand a state of reduced proliferation of foetal-antigen-specific CD8 T-cells.

Regarding miscarriages, there are varied reports concerning the values of both CD4+ and CD8+ T-cell count. What constitutes normal CD4/CD8 ratio varies and this has been attributed to gender, age, ethnicity, genetics, exposures and infections (Wikby *et al*., 2008). Alteration or inversion of this ratio said to be at it's normal range between 1.5-2.5 (McBride and Strikers, 2017) is related to altered immune function, among others (Appay and Sauce, 2008) and this is via isolated apoptotic or targeted cell death of circulating CD4 cells, expansion of CD8 cells or a combination of both phenomena (Vidali, 2012; McBride and Strikers, 2017).

CONCLUSION

The study highlighted that the proportion of serum CD8+ T cells was significantly higher in pregnancy loss women when compared with the control groups. Also the CD4/CD8 ratio was found to be lower in pregnancy loss subjects when compared with the control groups. Increased levels of CD8 T –cells might therefore be contributory to the pathogenesis of pregnancy loss.

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