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Elevated percentage of CD3$^+$T cells and pregnancy outcome in women with recurrent pregnancy loss

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ABSTRACT

Background: Even though the immune factor is not yet established as a cause of recurrent pregnancy loss (RPL), tons of other studies have shown that a significant proportion of immune abnormalities exist in RPL.
Methods: We conducted a retrospective cohort study with 850 women who were diagnosed with RPL. The percentages of CD3$^+$, CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$T cells of each participant, detected by flow cytometry, were obtained before pregnancy and at 6 weeks of gestation as part of their routine medical examination.
Results: Peripheral blood CD3$^+$ T cells prior to pregnancy (at baseline), increased significantly in women who had a miscarriage compared with the subsequent live birth group. Moreover, the percentage of CD3$^+$ and CD3$^+$CD4$^+$T cells during pregnancy increased significantly as compared with the baseline level. After adjusting for potential confounders, the multiple regression equation showed that the CD3$^+$ T cells < 67.84% was associated with the risk of miscarriage (OR 1.05, 95% CI, 1.01 to 1.11, p = .04). Additionally, a nonlinear relationship was observed between the percentage of CD3$^+$T cells and the risk of miscarriage.
Conclusions: The risk of miscarriage increased as the percentage of population with CD3+ value below 67.84% has increased, nevertheless, the miscarriage risk did not increase further when the level of CD3$^+$T cells was > 67.84%.

1. Introduction

Recurrent pregnancy loss (RPL), defined as the loss of three or more consecutive pregnancies in the same spouse, occur in approximately 1%–5% of couples attempting to bear children [1]. Many experts consider two consecutive losses as sufficient for the diagnosis of recurrent miscarriage [2]; this definition increases the percentage of RPL to 5% [3].

The latest review on RPL revealed that common indentified causes of RPL include uterine anomalies, anticardiolipin antibody syndrome (APS): an autoimmune, hypercoagulable state caused by antiphospholipid antibodies; hormonal and metabolic disorders, and cytogenetic abnormalities. Other etiologies have been proposed but are still considered controversial [4,5]. There is clear evidence to suggest that maternal T lymphocytes at maternal-fetal interface play an important role in the fetal survival and development [6,7]. It is gradually found in nearly 20 years of researches that 50% of the unexplained recurrent pregnancy loss (URPL) [8–10], is related with the immune factors, in which T lymphocytes are the most important cell populations and Vujaklija et al. also found that the peripheral blood T cell subsets in URPL women were abnormal [11].

Normal pregnancy is similar to allogeneic transplantation as embryo survives throughout pregnancy. This could be attributed to maternal-fetal immune tolerance; that is the failure of the organ graft recipient to express a graft destructive immune response [12–14]. Therefore, immunological mechanisms are involved in successful implantation [15]. T cells are 32% around the expected time of implantation (LH +7 days) and fewer (around 20%) in early pregnancy [16]. T cells are divided into different subsets according to the different CD markers on their surface. CD3$^+$ cells are expressed on the surface of all T cells and are common surface markers of T cells. CD4$^+$ exists on the surface of helper T cells (TH) to mediate cellular immunity and increasing the rate of CD4$^+$could enhance the function of the maternal immune system to increase the immune rejection towards embryo, this could lead to abortion. Human CD4$^+$ T cells can be classified into TH1, TH2, regulatory T (Treg) and Th17cells on the basis of their pattern of cytokine

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production [17,18], and they play different biological functions. Recent data from the human experience suggest that TH1 immune responses are detrimental for pregnancies and TH2 immune responses are necessary for successful pregnancies. Researches demonstrated that the ratio of TH1 and TH2 cell in the peripheral blood significantly increased in women with RPL [19]; T-cell abnormalities with increased TH1 and Th17 immunity, and decreased TH2 and T regulatory immune responses may play important roles in RPL [20]. Many studies had drawn similar conclusions [21–23]. Nevertheless, most published articles on RPL due to immunological causes, focusing on the percentage of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$T cells seems to have inconsistent findings.

Yoo et al. showed that there was no correlation between the percentage of $CD3^+$and $CD3^+CD4^+$cells in the peripheral blood of RPL and fertile women [24]. The percentage of $CD3^+CD4^+$ T cells and $CD3^+CD8^+$ T cells were significantly higher in women with RPL than fertile controls [15]. Some previous studies showed that there are no differences in the percentage of peripheral $CD3^+$T cells in RPL and normal fertile women [25–27]. Research by Ghafourian M indicated that the percentage of $CD3^+CD8^+$T cells was significantly higher in RPL women compared with the control group, and there was no statistical difference in the percentage of total $CD3^+$ and $CD3^+CD4^+$ T cells between the two study groups [25].

However, to the best of our knowledge, there is no research report that clearly defines the relationship between the dynamic changes in the percentage of T cell subsets and the pregnancy outcomes.

2. Materials and methods

2.1. Study subjects

This is a retrospective cohort study performed at the Reproductive Immunology department of Shanghai First Maternity and Infant Hospital. Approval of the study protocol was obtained from the institutional research ethics committee. All participants provided written informed consent before their enrollment. Total of 7380 women who have had a minimum of 2 consecutive pregnancy losses (early or late miscarriage versus stillbirth/neonatal death) were enrolled between December 2014 and January 2016. The previous pregnancy losses were all confirmed by either urine hCG/serum hCG level and ultrasound or uterine curettage and histology. Exclusion criteria included: chromosomal, endocrine, anatomical, infectious disease and thrombophilia or autoimmune diseases factors, such as the presence of antiphospholipid antibodies, lupus anticoagulant or anticardiolipin antibodies. A total of 3632 women had no record of the factors listed above; however, 37 women were excluded due to their inability to conceive during our study period. The remaining 3595 women who got pregnant were followed-up. The additional exclusive criteria were as follows: (i) ectopic pregnancy, (ii) biochemical pregnancy, (iii) gestational trophoblastic disease, (iv) early spontaneous abortion (< 6 weeks), (v) lost to follow-up. In the end, a total of 850 participants were included in the study. These women were classified, based on their pregnancy outcome, into 2 main groups; live birth and miscarriage group (Fig. 1). The flow cytometry was used to detect the CD3+, CD3 + CD4+ and CD3 + CD8+ T cells level in the peripheral blood. Further analysis of the relationship and correlation between the percentage of T cells and pregnancy outcomes were conducted.

2.2. Laboratory tests and sample collection

Using the standardized clinical laboratory protocol, 2 ml of peripheral blood were drawn before pregnancy and on the 6th week of pregnancy into heparinised tubes. Flow cytometric assay for analysis of cell viability was performed. Following lysing of red blood cells, leukocytes were stained with the fluorochrome-conjugated monoclonal antibodies specific for cell surface antigens. Anti-CD3-a fluorescein isothiocyanate conjugate (FITC), CD4-APC CD8-PE (mouse monoclonal antibodies, Dako, Denmark) were used to detect specific lymphocyte subsets. Appropriate isotype controls were used for each antibody. Four-colour flow cytometric analysis was performed using fluorescence-activated cell sorting (FACS) flow cytometry (Becton Dickinson). The gate was considered on the lymphocyte region by characteristic forward and side scatter parameters. For each sample, at least 10,000 cells were analyzed and finally, the percentage of the cells expressing CD3+, CD4+ and CD8+ markers were evaluated. The normal range of circulating lymphocytes obtained, according to our laboratory reference values, were 63.04% to 77.98% for CD3 + (%); CD3 + CD4 + Tcells (%) were 25.80% to 38.74%; CD3 + CD8 + T cells (%) were 21.30% to 34.38%.

2.3. Statistical analysis

The analysis was performed with R (http://www.R-project.org) and EmpowerStats software (www.empowerstats.com, X&Y solutions). We first described women' baseline characteristics before pregnancy and 6 weeks of pregnancy in Table 1. Then we used the Kolmogorov-Smirnov test to determine the distribution of the data of T lymphocytes subsets. Data are presented as mean ± SD and proportion in parentheses. Comparisons between groups were performed using chi-square tests for categorical variables and two-sample *t*-tests for continuous variables. We then applied univariate logistic regression analyses of age, BMI, the number of miscarriages, CD3+, CD3 + CD4+, CD3 + CD8 + T cells and pregnancy outcomes (Table 2). We further applied a 2-piecewise linear regression model to examine the threshold effect analysis of the percentage of CD3+ T cells on the various pregnancy outcomes (Fig. 2). Multivariable logistic regression models were fitted while adjusting for age, BMI and number of miscarriage. Adjusted OR and 95% CIs were obtained from these models (Table 3). Probability values of < 0.05 were considered statistically significant.

3. Results

3.1. Participants' characteristics

During the study period, 850 women who were diagnosed with RPL were recruited for assessment of the relationship between the T lymphocyte subset levels and pregnancy outcomes. The clinical characteristics of all women enrolled in our study are as follows (Table 1). Of all the 850 women, the pregnancy outcome of 323(38.0%) women had a miscarriage, 188(58.2%) of those women were < 35 years of age while 135(41.08%) women were ≥35 y (advanced age). The age of the miscarriage group was significantly higher than that of the live birth group ($p < .001$). The number of miscarriages ($p < .001$) and the level of CD3+ (%) ($p = .02$) before pregnancy were also significantly higher in the miscarriage group than the live birth group. The mean BMI was 21.76 ± 2.98, 219(67.80%) women who miscarried were < 23 kg/m^2, nevertheless, no statistical differences were observed between the BMI of the 2 groups (p = NS).

3.2. Comparison of the percentage of $CD3^+$, $CD3^+CD4^+$and $CD3^+CD8^+$ T cells of live birth and miscarriage groups

The comparison between the live birth group and miscarriage group before pregnancy demonstrates that the level of $CD3^+$T cells in the miscarriage group was significantly higher than that in the live birth group ($p = .02$), while the level of $CD3^+CD4^+$and $CD3^+CD8^+$ T cells did not show any statistical differences between the two groups. Moreover, during pregnancy, the level of $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$T cells between the two groups were statistically insignificant. Furthermore, the changes in the T-lymphocyte subsets at gestation were compared. The difference value (DV) means the change in the T lymphocyte subsets before and during pregnancy, (DV = Level of T lymphocyte subsets $_{(\text{during pregnancy})}$ - Level of T lymphocyte subsets