INTRODUCTION

The transition of motherhood is a challenge for women, which results in not only a physical burden but also mental disorders. Postpartum depression (PPD) and postpartum anxiety (PPA) are common psychological problems that occur in up to approximately 20% of women, which result in detrimental effects on postpartum women and children, subsequently resulting in physical and psychological problems among children of women with PPD and PPA. 

Abstract

Background: T-helper (Th) cells regulate inflammation and immunity, which is implicated in psychological disorders. The current study aimed to explore the clinical role of blood Th1, Th2, and Th17 cells and their main secreted cytokines in postpartum depression (PPD) and postpartum anxiety (PPA).

Methods: A total of 226 postpartum women were included. At 6 weeks postpartum, Edinburgh Postnatal Depression Scale (EPDS) and State Trait Anxiety Inventory 6 item version (STAi6) scores were assessed; meanwhile, blood Th1, Th2, and Th17 cells were detected by flow cytometry, serum interferon-gamma (IFN-γ), interleukin-4 (IL-4), and IL-17A were detected by enzyme-linked immunosorbent assay.

Results: The incidence of PPD and PPA were 24.3% and 27.9%, respectively. Th17 cells and IL-17A were positively correlated with EPDS score and STAi6 score (all \(p < 0.001\)). Besides, Th17 cells (\(p < 0.001\)) and IL-17A (\(p = 0.002\)) were increased in PPD cases vs. non-PPD cases, and they were also elevated in PPA cases vs. non-PPA cases (both \(p < 0.05\)). However, Th1 cells, Th2 cells, IFN-γ, and IL-4 were not linked with EPDS score or STAi6 score (all \(p > 0.05\)); besides, they did not vary in PPD cases vs. non-PPD cases or in PPA cases vs. non-PPA cases (all \(p > 0.05\)). Multivariate logistic regression model analysis showed that Th17 cells were independently associated with an elevated risk of PPD (odds ratio [OR] = 1.600, \(p = 0.001\)) and PPA (OR = 1.371, \(p = 0.022\)).

Conclusion: Blood Th17 cells and IL-17A are positively linked with the risk of PPD and PPA, indicating which may be involved in the development of PPD and PPA.

KEYWORDS
cytokines, postpartum anxiety, postpartum depression, risk factors, T-helper cells
PPA. Thus, it is vital to explore factors affecting PPD and PPA to promote their management.

The abnormal differentiation of T cells plays a crucial clinical role in anxiety and depression by dysregulating the immune system. For instance, the T helper 1 (Th1)/Th2 cells ratio is elevated in people with depression; moreover, it has also been reported that Th17 cells are increased in people with depression; furthermore, another interesting study also presents that Th17 cell-secreted cytokine (interleukin-17 [IL-17]) are positively correlated with the severity of anxiety and depression among lung cancer survivors. Notably, accumulating studies have shown that Th1, Th2, and Th17 cells are abnormally regulated during the postpartum period. Taken together, we deduced that Th1, Th2, and Th17 might play an important clinical role in PPD and PPA, while related information is scarce.

Thus, the current study aimed to explore the correlation of blood Th1, Th2, and Th17 cells, as well as their main secreted cytokines with PPD and PPA.

2 | METHODS

2.1 | Participants

Between March 2021 and October 2021, 226 postpartum women were enrolled in this study. The inclusion criteria (1) aged ≥18 years; (2) within 6-week postpartum; (3) voluntarily participated and was able to complete the questionnaire or scale independently; (4) willing to provide peripheral blood (PB) samples for scientific research. The exclusion criteria (1) had complications within 6-week postpartum; (2) history of mental disorders before pregnancy; (3) history of severe central nervous system disease; (4) history of psychoactive drug abuse; (5) with insufficiency of important organs or malignant tumors. The study ethics was approved by the Institutional Review Board of Shanghai Key Laboratory of Maternal Fetal Medicine, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University. All patients signed the informed consent before enrolment.

2.2 | Demographic and perinatal characteristics

After enrollment, demographic and perinatal characteristics were recorded by case report form. The demographic features included the following: age; smoke history (yes/no); drink history (yes/no); education level (primary school or less, high school, undergraduate, graduate or above); annual family income; previous gravidity number; previous birth number; previous abortion number. The perinatal features included the following: gestational week; unplanned pregnancy (yes/no); planned maternity leave time (≤128 days or >128 days); satisfaction of labor experience (unsatisfied or satisfied); breastfeeding difficulty (yes/no); number of caregivers (<2 or ≥2).

2.3 | PPD and PPA evaluation

The participants assessed PPD and PPA at 6-week (±1 week) postpartum. The Edinburgh Postnatal Depression Scale (EPDS) was used to evaluate PPD. The EPDS included 10 items, and the total score of EPDS ranged from 0 to 30. The EPDS score > 12 was considered as PPD. The State Trait Anxiety Inventory 6 item version (STAI6) was used to evaluate PPA. The STAI6 included 6 items, and the total score of STAI6 ranges from 0 to 24. The STAI6 score > 15 was considered as PPA.

2.4 | Sample detections

A total of 226 PB samples of participants were collected at 6-week postpartum. Within 24 h after collection, 226 fresh PB samples were analyzed using flow cytometry to detect Th1 cells (% in CD4+ T cells), T-helper 2 (Th2) cells (% in CD4+ T cells), and T-helper 17 (Th17) cells (% in CD4+ T cells) with the employment of BD Pharmingen™ Human Th1/Th2/Th17 Phenotyping Kit (BD Biosciences, San Diego, CA, USA). Serum was isolated from 226 PB samples by centrifuge and stored in liquid nitrogen for further detection. The levels of interferon-gamma (IFN-γ), interleukin-4 (IL-4), and interleukin-17A (IL-17A) in serum were detected in batch by enzyme-linked immunosorbent assay (ELISA) Kit, Human IL-4 ELISA Kit, and Human IL-17A ELISA Kit (BD Biosciences, San Diego, CA, USA), respectively. All procedures were strictly implemented according to the product protocol.

2.5 | Statistical analysis

The distributions of continuous variables were assessed by Kolmogorov–Smirnov (KS) test. With a p < 0.05 for the KS test, the variables were non-normally distributed. The correlation of T helper cells and their related cytokines with EPDS score and STAI6 score was determined using Spearman’s rank correlation test. The comparison of T helper cells and their related cytokines between two groups was evaluated using the Mann–Whitney U test. Multivariate logistic regression analysis was used to assess relating factors of PPD and PPA, with all potential factors were included by the step forward method. Statistical analysis was performed by SPSS V.22.0 (IBM Corp., Armonk, NY, USA), and figures were plotted by GraphPad Prism V.7.02 (GraphPad Software Inc., San Diego, CA, USA). The p < 0.05 was considered as statistical significance.

3 | RESULTS

3.1 | Study flow

A total of 323 women within 6-week postpartum were screened for eligibility, then 97 women were excluded (including 46 women who did not meet inclusion criteria or met exclusion criteria, and 51
women who declined to participate). Subsequently, 226 women were eligible, then PPD evaluation (using EPDS scale) and PPA evaluation (using STAI6 scale) were conducted at 6-week (±1 week) postpartum among them. Then, PB samples of participants were collected at 6-week postpartum, and serum was isolated from PB samples; afterward, Th1 cells, Th2 cells, and Th17 cells in PB were detected by flow cytometry; IFN-γ, IL-4, and IL-17A in serum were detected by ELISA. Finally, all 226 women were included in the analysis (Figure 1).

### 3.2 | Clinical characteristics

Among 226 participants, the mean age was 27.7 ± 3.6 years; meanwhile, 32 (14.2%) and 50 (22.1%) participants had a smoke history and drink history, respectively. Furthermore, the mean previous gravidity, birth, and abortion numbers were 1.4 ± 0.9, 0.3 ± 0.5, and 1.1 ± 0.7, accordingly. Moreover, 30 (13.3%) participants had unplanned pregnancies; meanwhile, 64 (28.3%) participants had planned maternity leave time > 128 days; besides, there were 173 (76.5%) participants with satisfied labor experience; furthermore, 153 (67.7%) participants had caregivers ≥ 2 (Table 1).

### 3.3 | PPD and PPA evaluation

The mean ± standard deviation (SD), median (interquartile range [IQR]), and range of EPDS score were 9.2 ± 4.6, 8.0 (6.0–12.0), and 0.0–25.0, respectively (Figure 2A); besides, the rate of PPD was 24.3% (Figure 2B). In addition, the mean ± SD, median (IQR), and range of STAI6 score were 13.5 ± 3.4, 13.0 (11.0–16.0), and 6.0–23.0, accordingly (Figure 2C); besides, the rate of PPA was 27.9% (Figure 2D).

### 3.4 | Correlation of T helper cells and their related cytokines with EPDS score

Th1 cells were not correlated with EPDS score ($r_s = 0.097, p = 0.147$) (Figure 3A). Th2 cells were not correlated with EPDS score either ($r_s = −0.062, p = 0.355$) (Figure 3B). Th17 cells were positively correlated with EPDS score ($r_s = 0.306, p < 0.001$) (Figure 3C). Furthermore, no correlation was found in IFN-γ ($r_s = 0.104, p = 0.118$) (Figure 3D) or IL-4 ($r_s = −0.061, p = 0.359$) (Figure 3E) with EPDS score. While positive correlation was found in IL-17A with EPDS score ($r_s = 0.259, p < 0.001$) (Figure 3F).

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**FIGURE 1** Study flow chart
3.5 | Comparison of T helper cells and their related cytokines in participants with and without PPD

Table 1 Characteristics of the participants

| Items                              | Participants (N = 226) |
|------------------------------------|------------------------|
| Age (years), mean ± SD             | 27.7 ± 3.6             |
| Smoke history, n (%)               |                        |
| No                                 | 194 (85.8)             |
| Yes                                | 32 (14.2)              |
| Drink history, n (%)               |                        |
| No                                 | 176 (77.9)             |
| Yes                                | 50 (22.1)              |
| Education level, n (%)             |                        |
| Primary school or less             | 7 (3.1)                |
| High school                        | 94 (41.6)              |
| Undergraduate                      | 114 (50.4)             |
| Graduate or above                  | 11 (4.9)               |
| Annual family income (CNY), n (%)  |                        |
| <100,000                           | 9 (4.0)                |
| 100,000–199,999                    | 86 (38.1)              |
| 200,000–299,999                    | 93 (41.2)              |
| ≥300,000                           | 38 (16.8)              |
| Previous gravidity number, mean ± SD | 1.4 ± 0.9          |
| Previous birth number, mean ± SD   | 0.3 ± 0.5              |
| Previous abortion number, mean ± SD| 1.1 ± 0.7             |
| Gestational week, mean ± SD        | 39.1 ± 1.9             |
| Unplanned pregnancy, n (%)         |                        |
| No                                 | 196 (86.7)             |
| Yes                                | 30 (13.3)              |
| Planned maternity leave time, n (%)|                        |
| ≤128 days                          | 162 (71.7)             |
| >128 days                          | 64 (28.3)              |
| Satisfaction of labor experience, n (%) |                 |
| Unsatisfied                        | 53 (23.5)              |
| Satisfied                          | 173 (76.5)             |
| Breastfeeding difficulty, n (%)    |                        |
| No                                 | 146 (64.6)             |
| Yes                                | 80 (35.4)              |
| Number of caregivers, n (%)        |                        |
| <2                                 | 73 (32.3)              |
| ≥2                                 | 153 (67.7)             |

Abbreviations: CNY, China Yuan; SD, standard deviation.

No difference of Th1 cells (p = 0.243) (Figure 4A) or Th2 cells (p = 0.749) (Figure 4B) was found between participants with and without PPD. However, Th17 cells (median [IQR]: 3.2 (1.5–4.5)% vs. 1.8 (1.2–2.9)%) were elevated in participants with PPD compared to those without PPD (p < 0.001) (Figure 4C). Moreover, no difference of IFN-γ (p = 0.425) (Figure 4D) or IL-4 (p = 0.918) (Figure 4E) was found between participants with and without PPD. While IL-17A (median [IQR]: 41.8 (33.1–54.3) pg/ml vs. 34.3 (27.8–45.1) pg/ml) was increased in participants with PPD compared to those without PPD (p = 0.002) (Figure 4F).

3.6 | Correlation of T helper cells and their related cytokines with STAI6 score

Th1 cells (r = 0.113, p = 0.090) (Figure 5A) and Th2 cells (r = −0.077, p = 0.252) (Figure 5B) were not correlated with STAI6 score. While Th17 cells were positively correlated with STAI6 score (r = 0.235, p < 0.001) (Figure 5C). Furthermore, no correlation was found in IFN-γ (r = 0.111, p = 0.095) (Figure 5D) or IL-4 (r = −0.090, p = 0.180) (Figure 5E) with STAI6 score, while positive correlation was found in IL-17A with STAI6 score (r = 0.240, p < 0.001) (Figure 5F).

3.7 | Comparison of T helper cells and their related cytokines in participants with and without PPA

No difference of Th1 cells (p = 0.190) (Figure 6A) or Th2 cells (p = 0.518) (Figure 6B) was found between participants with and without PPA. However, Th17 cells (median [IQR]: 2.8 (1.3–4.2)% vs. 1.9 (1.3–3.0)% were elevated in participants with PPA compared to those without PPA (p = 0.004) (Figure 6C). Moreover, no difference in IFN-γ (p = 0.292) (Figure 6D) or IL-4 (p = 0.316) (Figure 6E) was found between participants with and without PPA. While IL-17A (median [IQR]: 40.6 (33.7–52.6) pg/ml vs. 33.1 (27.6–44.2) pg/ml) was increased in participants with PPA compared to those without PPA (p = 0.001) (Figure 6F).

3.8 | Independent factors for PPD and PPA

Multivariate logistic regression model analysis presented that Th17 cells were independently correlated with an elevated risk of PPD (odds ratio [OR] = 1.600, p = 0.001), while annual family income (OR = 0.283, p < 0.001), the satisfaction of labor experience (vs. unsatisfaction) (OR = 0.239, p = 0.001), and caregiver ≥2 (vs. <2) (OR = 0.248, p = 0.001) were independently correlated with declined risk of PPD. Moreover, Th17 cells were independently correlated with an increased risk of PPA (OR = 1.371, p = 0.022), while annual family income (OR = 0.231, p < 0.001), previous gravidity number (OR = 0.644, p = 0.029), planned maternity leave time > 128 days (vs. ≤128 days) (OR = 0.332, p = 0.011), the satisfaction of labor experience (vs. unsatisfaction) (OR = 0.260, p = 0.001), and caregiver ≥2 (vs. <2) (OR = 0.151, p < 0.001) were independently correlated with declined risk of PPA (Table 2).

4 | DISCUSSION

Regarding the incidence of PPD and PPA, it has been reported that 19.2% women suffer from PPD during the first 3 months...
after giving birth; furthermore, 19% Sweden women experience PPD after 2 months postpartum; additionally, another interesting study also illustrated that the incidence of PPD ranges from 9% to 12% among women during postpartum. In the current study, the incidence of PPD and PPA were 24.3% and 27.9%, respectively, which was numerically higher than in previous studies. The possible explanations might be that (1) different types of participants (such as those coming from different countries and different ages) might result in the different incidence of PPD and PPA; (2) different assessment criteria and different evaluation time points led to a different assessment result of the incidence of PPD and PPA. In the current study, we found that Th17 cells and IL-17A were positively correlated with PPD and PPA. The possible explanations might be that (1) Th17 cells might modulate the function of the brain through several methods, including regulating hippocampal neurogenesis, psychological stress resilience, and brain tissue homeostasis, which led to the development of PPD and PPA; (2) Th17 cells might take part in the regulation of brain inflammation through secreting proinflammatory cytokines such as IL-17A, which resulted in the development of PPD and PPA. Thus, Th17 cells and IL-17A were positively correlated with PPD and PPA.

As for factors to predict the risk of PPD and PPA, it has been reported that exposure of sulfur dioxide is correlated with an elevated risk of PPD; in addition, the mothers with worse sleep quality during pregnancy have more possibilities to experience PPD and PPA. In the current study, we found that Th17 cells are increased in depressed patients; meanwhile, it also has been presented that IL-17A is elevated but IFN-γ and IL-4 are normal in depressive patients. However, the data about the correlation of Th1, Th2, and Th17 cells and their related cytokines with PPD and PPA is limited. In the current study, we found that Th17 cells and IL-17A were positively correlated with PPD and PPA.
independently correlated with an elevated risk of PPD and PPA, which might be explained by that Th17 might take part in the modulation of the central nervous system, such as regulation of brain function and brain inflammation, which could result in the occurrence of PPD and PPA.11,25–30,33 In addition, we also found that annual family income, the satisfaction of labor experiments, previous gravidity number, caregivers number, and planned maternity leave time were independently correlated with the risk of PPD or PPA, indicating these factors should be paid more attention among pregnant women to potentially avoid PPD and PPA.

From the previous studies,5,20 PPA and PPD were commonly assessed at 6-week postpartum, thus we also used inclusion “within
In the current study, it was ensured that PPA and PPD could be assessed at 6-week postpartum. The planned maternity leave time in China was 128 days, so "128 days" was used as the cutoff time in the current study. However, the current study had several limitations: (1) the mechanism of Th17 cells in the development of PPD and PPA was not explored; (2) monitoring of Th1, Th2, and Th17 cells, as well as their related cytokines at multiple time points in women with PPD and PPA was needed; (3) the questionnaire or scale of EPDS and STAI6 assessment was completed by participants themselves, which might exist bias.

**Figure 5** Th17 cells and IL-17A were positively correlated with STAI6 score. Correlation of Th1 cells (A), Th2 cells (B), Th17 cells (C), IFN-γ (D), IL-4 (E), and IL-17A (F) with STAI6 score.

**Figure 6** Th17 cells and IL-17A were increased in PPA women vs. non-PPA women. Comparison of Th1 cells (A), Th2 cells (B), Th17 cells (C), IFN-γ (D), IL-4 (E), and IL-17A (F) in PPA women vs. non-PPA women.
To be conclusive, blood Th17 cells and IL-17A are positively linked with the risk of PPD and PPA, indicating which may be involved in the development of PPD and PPA.

**CONFLICT OF INTEREST**
The authors declare that they have no competing interests.

**DATA AVAILABILITY STATEMENT**
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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**TABLE 2** Factors related to PPD and PPA by multivariate logistic regression model analysis

| Items                                      | p Value | OR  | 95%CI Lower | 95%CI Upper |
|--------------------------------------------|---------|-----|-------------|-------------|
| PPD                                        |         |     |             |             |
| Th17 cells (%)                             | 0.001   | 1.600 | 1.216       | 2.104       |
| Annual family income                       | <0.001  | 0.283 | 0.163       | 0.490       |
| Satisfaction of labor experience (Satisfied vs. Unsatisfied) | 0.001   | 0.239 | 0.105       | 0.544       |
| Number of caregivers (≥2 vs. <2)           | 0.001   | 0.248 | 0.112       | 0.546       |
| PPA                                        |         |     |             |             |
| Th17 cells (%)                             | 0.022   | 1.371 | 1.047       | 1.796       |
| Annual family income                       | <0.001  | 0.231 | 0.131       | 0.407       |
| Previous gravidity number                  | 0.029   | 0.644 | 0.434       | 0.956       |
| Planned maternity leave time (>128 days vs. ≤128 days) | 0.011   | 0.332 | 0.142       | 0.775       |
| Satisfaction of labor experience (Satisfied vs. Unsatisfied) | 0.001   | 0.260 | 0.115       | 0.590       |
| Number of caregivers (≥2 vs. <2)           | <0.001  | 0.151 | 0.067       | 0.340       |

Abbreviations: CI, confidence interval; OR, odds ratio; PPA, postpartum anxiety; PPD, postpartum depression; Th17 cells, T-helper 17 cells.
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