ORIGINAL ARTICLE

The Role of T Regulatory Cells in Pathogenesis of Preeclampsia

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ABSTRACT

Background: *Preeclampsia affects about 7-10% of pregnant females. It is a multisystem disorder characterized by the new onset hypertension (>140/ 90 mmHg) with coexisting proteinuria (>300 mg/24 h) and other maternal organ dysfunctions. Regulatory T cells function as strong inflammatory suppressors, preventing mother T cells from activating against fetal cells. IL-10 plays a vital role in the reduction of inflammation-mediated vascular dysfunction, hypertension, and hypoxia during gestation.* ***Objectives:*** *The study aims to compare the frequency of T regulatory cells and IL-10 in peripheral blood between PE patients and normal pregnant women during the third trimester.* ***Methodology****: This study was carried out on 25 pregnant women with PE at 30–40 weeks of gestation selected from the pregnant women hospitalized at El-Hussein Hospital of Al-Azhar University and Saied Galal hospital and 25 healthy gestational-matched pregnant women as a control group. Flow cytometric analysis of Tregs was carried out by the following antibodies: phycoerythrin-cy7-conjugated anti-CD127, phyco-erythrinconjugated CD25 and fluorescein isothiocyanate-conjugated anti-CD4. For accurate quantitative detection of human IL-10 in serum, we used the sandwich ELISA kit.* ***Results:*** *A highly significant difference (p<0.001**) was found as regard T regulatory cells% between preeclampsia patients and healthy control groups. As regard IL-10 serum level, there was a significant difference (p<0.001**) between the two studied groups.* ***Conclusions:*** *These data suggested that 3 Treg cells and IL-10 serum level are extremely critical determinants of safe pregnancy outcome.*

INTRODUCTION

Preeclampsia (PE), the most common hypertension disorder during pregnancy, affects around 5-10% of pregnant women [1]. It is defined by hypertension (>140/90 mmHg), proteinuria (>300 mg/24 h), and other maternal organ dysfunctions, such as renal dysfunction, weakened liver function, neurological or hematological complications, and uteroplacental dysfunction after 20 weeks of gestation[2].

Despite extensive researches, the precise etiology of this illness remains unclear. However, several factors have been connected to its development, including chronic inflammation, poor placentation, oxidative stress, hereditary factors, inadequate immune tolerance, placental ischemia, and hypoxia [3].

Changes in the immune system have a major role in the onset and course of preeclampsia. This syndrome may develop as a result of an overactive immune system and an increased maternal inflammatory response [4].

Regulatory T cells (Tregs) are a specific subgroup of T cells that act as powerful suppressors of inflammation to prevent autoimmune disorders and transplant rejection [5]. These T cells hinder the activation of maternal T cells against fetal cells, which is a well-established phenomenon in both mice and humans [6]. A reduction in maternal Treg populations can lead to fetal immunological intolerance and obstetrical problems such as miscarriage, PE, and preterm labor[7]. Tregs, which express CD4+ CD25+ and Foxp3, play an important role in immunological tolerance and homeostasis. Thus, its decreased level probably causes the maternal inflammatory response to become overactive, resulting in high levels IFN-γ, IL-1β, IL-6, and TNF-α, and low IL-10 [8,9].

Interleukin-10 is a vital anti-inflammatory cytokine involved in wound healing, cancer, and autoimmunity[10]. It is produced by CD8 T cells, CD4 T cells (Th1, Th2, and Th17 subsets), regulatory T cells (Treg), and B cells[11]. IL-10 is a pregnancy-compatible cytokine that regulates pro- and anti-inflammatory responses at the

maternal-fetal interface [12.] Observed that IL-10 reduces inflammation-induced vascular dysfunction, hypertension, and placental ischemia/hypoxia, also IL-10 production reduce NK-like cells and T cells in the uterus, resulting in immunological tolerance [13].

METHODOLOGY

Ethical considerations:

The study followed Helseinki's declaration and was accepted by the ethical committee at Al-Azhar University's Faculty of Medicine at Septamber 2020.

Study eligibility, design, and setting:

This comparative study included 50 egyptian women chosen from Gynecological and Obstetric Department at Al- Hussein University Hospital & Said Galal University Hospital from January 2021 to July 2022. Of these, 25 women who matched the criteria of preeclampsia served as the case group. The other 25 healthy gestational- matched pregnant women (as a control group).

Exclusion criteria:

PE patients with pre-existing clinical disorders, such as chronic hypertension or renal diseases, autoimmune diseases such as rheumatoid arthritis, Active labor or bleeding or maternal or fetal infection at the time of enrollment and blood sampling, history of immunosuppressive medicines were excluded from the study. All participants were assessed for clinical history and clinical examination during a hospital visit.

Study participants:

All patients with preeclampsia had systolic or diastolic blood pressure higher than 140/90 mmHg and proteinuria above 0.3 g/24 hr, according to internationally accepted criteria [14].

Laboratory tests:

From each study participant a venous blood sample (5 ml) was collected, divided into two portions. The first portion (2.5 ml) was collected in an EDTA tube for complete blood counts (CBC) and flow cytometry analysis. The remaining part was evacuated in a serum-separator tube and centrifuged at 3000 rpm for 10 min. Serum was separated and kept frozen at -20°C until used for analysis of IL10. They were assessed using commercial ELISA kits (Bioassay Technology laboratory, Cat. No E0102Hu) according to the manufacturer's instructions.

Flow cytometry analysis:

Phycoerythrin (PE)-cy7-conjugated anti-CD127, phyco-erythrin (PE)‑conjugated CD25 and fluorescein isothiocyanate(FITC)-conjugated anti-CD4 monoclonal antibodies were used (Beckton – Dickenson Immunocytometry system BDIS, San Jose, CA). PE Mouse IgG1, κ Isotype Control, FITC Mouse IgG1, κ Isotype Control and PE-Cy™7 Mouse IgG1 κ Isotype Control were used. Lymphocyte phenotyping was carried out by three-colour analysis using a FACSCanto flow cytometer and FACSCanto software (BectonDickinson, San Jose, CA, USA). For each sample, data from 10,000 cells were collected and analysed.

Gating of lymphocytes was done first by using FSC/ SSC characteristics. The gated populations into [FSC vs. SSC plot] were displayed on this plot which contains CD4 FITC vs. SSC. To include all CD4+ events. Then, CD 25 PE vs. CD127 PE-Cy7 was gated. Only double positive CD 25 and CD127low cells were included for calculation of the percentages of (CD4+, CD25+, CD127low) T regulatory cells.

ELISA for Interlukin-10:

Venous blood (2 mL) was taken prior from patients and control by venipuncture in gel & clot activator tubes (Vacu Test.00478567). The samples were centrifuged within 2 hrs of venipuncture for 10 min at 3000 rpm and the serum was aliquoted and stored at -80 C° until required. The concentration of IL-10 in the serum was assayed using a commercially available sandwich ELISA kit (Bioassay Technology laboratory, Cat.No E0102Hu) using a microplate reader (VARIOSKAN LUX, Thermo scientific).

Statistical analysis:

Recorded data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc, Chicago, Illinois, USA). The quantitative data were presented as mean± standard deviation and ranges. Also, qualitative variables were presented as number and percentages.

RESULTS

Demographic and clinical features of the study population:

A total of 50 subjects, including 25 preeclampsia patients (group I) and 25 normal pregnancy control individuals (group II) were enrolled in the present study. The demographic characteristic of the study subjects is summarized in table 1.

Table 1: Comparison between preeclampsia and control group according to demographic data

Demographic data	Preeclampsia Group (I)	Control Group (II)	t-test	p-value
Age (years) Mean±SD Range	 29.96±6.27 19-41	 28.24±7.20 18-41	0.900	0.372
Gestational Age (wks) Mean±SD Range	 36.36±1.50 33-39	 36.08±2.63 30-39	0.463	0.646

Using: t-Independent Sample *p-value >0.05 NS (non-significant)*

There was no statistically significant difference in age or gestational age between the two groups under study.

Higher significant difference (p<0.001**) was found in T regulatory cells% between preeclampsia patients and healthy control groups. In patients' group (group I) the mean level of T-reg cells % was 4.34±0.60 (range from 3.45 to 5.3) while in healthy pregnant women (group II) the mean level of T-reg cells % was 7.55±0.86 (range from 6.4 to 9.2) as showed in table 2.

Table 2: Comparison between preeclampsia patients and healthy control group according to T regulatory cell %

T reg cells %	Preeclampsia Group	Control Group	t-test	p-value
Mean±SD	4.34±0.60	7.55±0.86	-15.244	<0.001**
Range	3.45-5.3	6.4-9.2		

Using: t-Independent Sample
***p-value <0.001 HS (highly significant)*

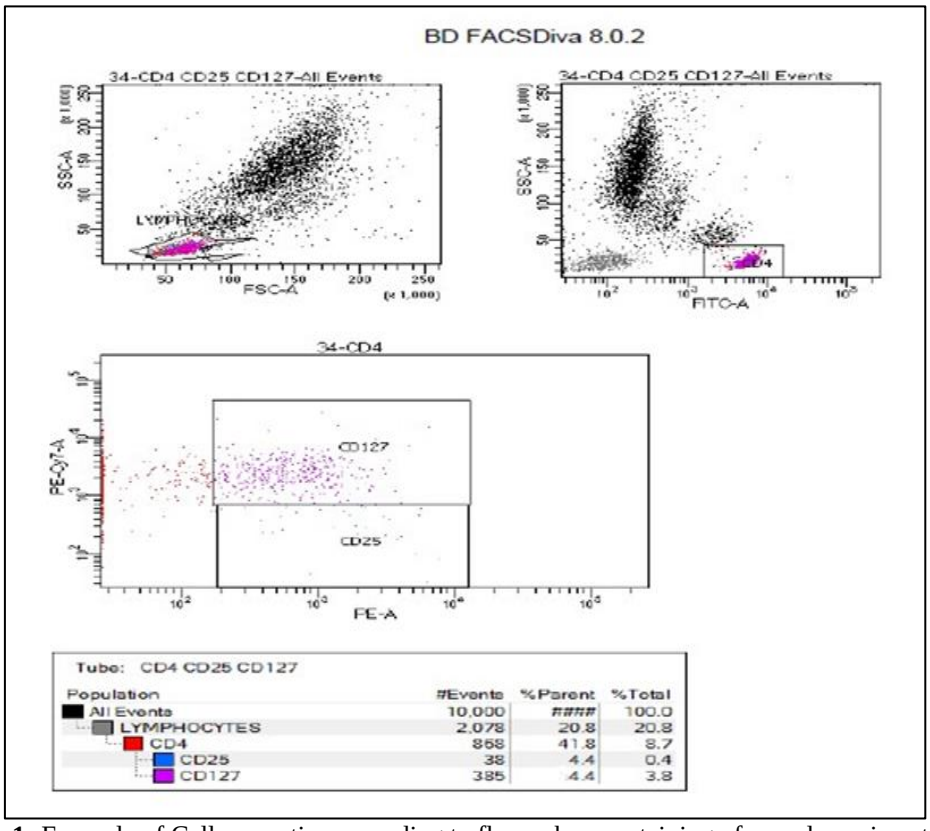

Fig. 1: Example of Cell separation according to fluorochrome staining of preeclampsia patient

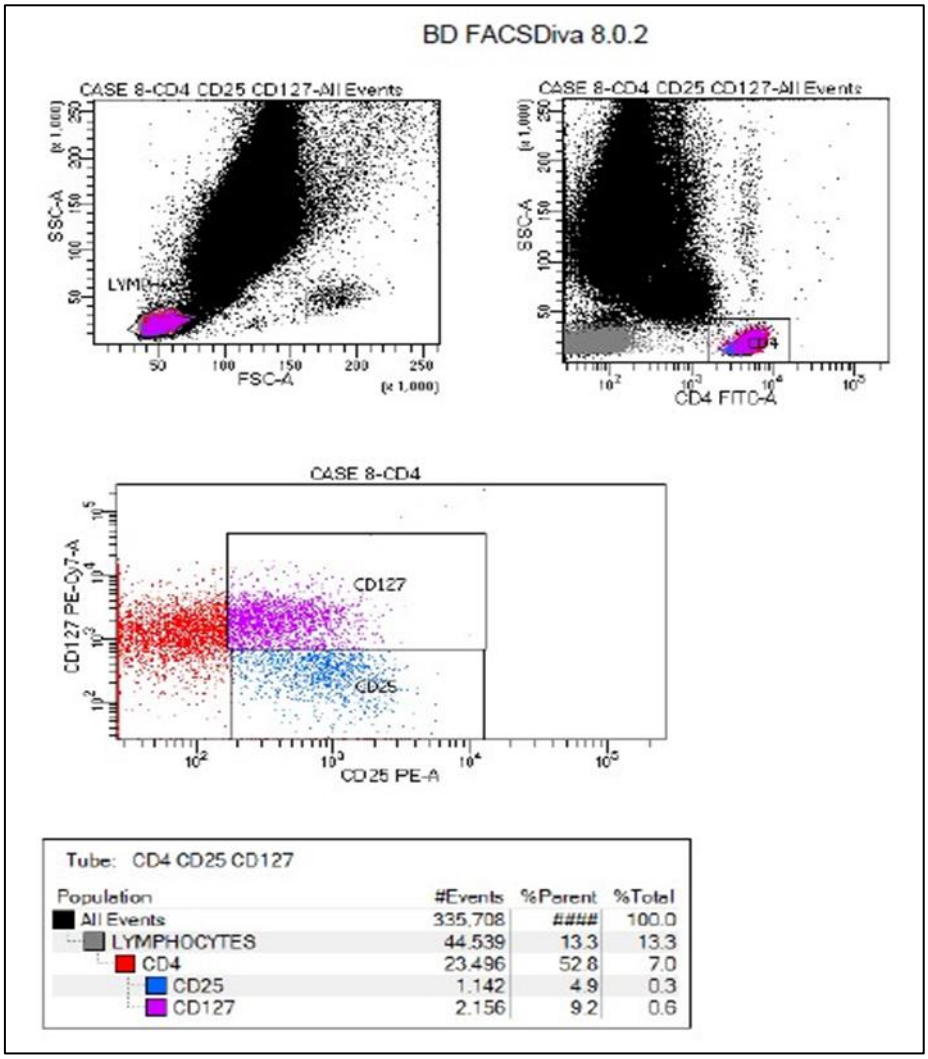

Fig. 1: Example of Cell separation according to fluorochrome staining of pregnant control woman

Significant differences were found between the two studied groups as regard IL-10 secretion where in preeclampsia patients IL-10 secretion (pg/ ml) the mean level was 99.17±7.37 (range from 87.45 to 115.43), while in healthy pregnant women the mean level was 485.19±36.61 (range from 432,65 to 542.7) as showed in table 3.

Table 3: Comparison between preeclampsia patients and healthy control groups according to IL-10 secretion

IL-10(pg/ml)	Preeclampsia Group	Control Group	t-test	p-value
Mean±SD	99.17±7.37	485.19±36.61	-51.678	<0.001**
Range	87.45-115.43	432.65-542.7		

Using: t-Independent Sample
***p-value <0.001 HS (highly significant)*

Significant differences were found between the two studied groups as regard IL-10 secretion where in preeclampsia patients IL-10 secretion (pg/ ml) the mean level was 99.17±7.37 (range from 87.45 to 115.43), while in healthy pregnant women the mean level was 485.19±36.61 (range from 432,65 to 542.7).

DISCUSSION

In the present study, gating on $CD4^+CD25^+CD127^{low}$ cells showed a significant decrease in Treg % in preeclampsia group (Mean±SD 4.34±0.60 % range from 3.45-5.3%) compared to healthy pregnant control group (Mean±SD 7.55±0.86% range from 7.55±0.86) (P<0.001).

These findings are consistent with the study done by Sasaki et al, [15] who found that population of $CD25^+CD4^+$ T cells in the Japanese and Polish pre-eclamptic cases (median 3.1%, range 1.9– 7·9%) was significantly lower compared to that in the normal pregnant subjects (P < 0·0001).

In accordance with our findings, Santner-Nanan et al. [16] compared the population of Treg cells in healthy and PE pregnant women vs nonpregnant controls using combinations of three marker, including $CD4^+CD25^{high}$, $CD4^+CD127^{low}CD25^+$, and $CD4^+FOXP3^+$. Their results revealed that proportion of peripheral blood $CD4^+CD127^{low}CD25^+$, $CD4^+FOXP3^+$, and $CD4^+CD25^{high}$ cells is significantly higher (p < 0.001) in healthy pregnant women than PE patients and nonpregnant controls.

In a study done by Darmochwal-Kolarz et al.[17], patients with preeclampsia had significantly lower levels of CD4+CD25+FoxP3+ Treg cells in their peripheral blood compared to healthy normotensive pregnant women in the third trimester of normal pregnancy (4.01 ± 1.56% vs. 6.21 ± 2.14%, 5 p < 0.05), which is consistent with our findings.

In agreement with our study's results, Toldi et al.'s study [4] found that the percentage of CD4+ CD25hi FoxP3+ regulatory T cells was lower in pregnant women with PE than in healthy women [4.63 (4.22–5.56) % versus 3.69 (3.32–4.09) %, P = 0.0003].

In accordance with our findings, a lower level of Treg cells in preeclampsia patients than healthy pregnant controls (3.413 ± 1.652 vs. 4.347 ± 1.718, p = 0.006) was detected in the study of Eghbal-Fard et al.[3].

In Miko et al,[18] study, they reported that the percentage of $CD4^+CD25^+$T cells of gated lymphocytes was not statistically significant from healthy pregnant women (0.42 vs. 0.85).

The previous findings are not consistent with our results in which there is a significant difference (P < 0.001) between pregnant women with preeclampsia, the mean level of T-reg cells % was 4.34±0.60 (range from 3.45 to 5.3) while in healthy pregnant women, the mean level of T-reg cells % was 7.55±0.86 (range from 6.4 to 9.2). This may be explained by the smaller number of studied cases included in their study (19 patients only) which may interfere with the statistical results.

In the present work, significant differences (*p value*<0.001**) were found between the two studied groups as regard IL-10 secretion where in preeclampsia patients IL-10 secretion (pg/ ml) the mean level was 99.17±7.37 (range from 87.45 to 115.43), while in healthy pregnant women the mean level was 485.19±36.61 (range from 432,65 to 542.7).

In agreement with our findings, other studies also have demonstrated the diminished levels of IL-10 in women with PE. Therefore, dysregulation of IL-10 expression is probably associated with enhanced maternal anti fetal immune responses and abnormal placental development in PE [19].

Consistent with our work, a statistically significant diminution in IL-10 expression in the preeclampsia group compared to the normotensive group (101.36±16.40 pg/ml vs. 410.47±132.99 pg/ml, P < 0.05) [20].

Our study results are not consistent with the study done by Yu et al. [21],they reported non-significant differences between the IL-10 levels in the three groups (healthy pregnant, mild preeclampsia and severe preeclampsia P > 0.05). That may be explained by IL-10 was detected using Luminex® 200™ (Luminex Corporation, Austin, TX, USA) which is based on the principles of multiplex flowcytometry according to the manufacturer's protocol (Bio-Rad Laboratories, Hercules, CA,USA),while in our study it was detected using ELISA technique .

In Cui et al.[22] study, they found that level of IL-10 (pg/ml) in healthy pregnant controls (28.31±3.6) is higher than preeclampsia patients (either mild PE 21.54±0.89 or severe PE 14.84±2.73) that was significant difference (P < 0.001).

In agreement with our findings, the results demonstrated the decreased levels of IL-10 (27.15 ± 13.24 vs. 37.60 ± 20.39, p = 0.06) in preeclampsia patients than healthy pregnant controls detected in Eghbal-Fard et al.[3].

CONCLUSION

Treg cells and related cytokine reconfirmed a presumption that maternal systemic inflammation in PE can be associated with decreased Treg cell. The mechanism underlying Treg cells involved in pathogenesis of PE remains undefined, but we showed that there are significant relations between peripheral blood immune effectors Treg cells, cytokine profile (IL-10) and development of PE; thereby, immunological assessment of PE can be useful in the prediction of this disease and reduce the number of unsuccessful gestations.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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