Assessment of T helper 17-associated cytokines in third trimester of pregnancy

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ABSTRACT

Background: Preeclampsia is a common pregnancy-specific disorder associated with significant maternal and fetal morbidity and mortality worldwide. It has been proposed that the imbalance between two CD4+ T cell subtypes, regulatory T cells (Treg) and T-helper 17 cells (Th17), is involved in the pathophysiology of preeclampsia. **Objectives:** To determine the serum levels of IL-17, IL-21, IL-23 and TGF-β in patients with preeclampsia. **Methods**: Blood samples were collected from 30 preeclampsia patients, 30 normotensive pregnant women and 30 healthy individuals with no history of malignancies or autoimmune disorders based on simple sampling. The serum levels of IL-17, IL-21, IL-23 and TGF-β were measured by the enzyme linked immunosorbent assay (ELISA). **Results:** The serum levels of IL-17 and TGF-β were significantly higher in preeclampsia patients compared to normal pregnant group and healthy individuals (p>0.0001) but interestingly, the opposite was the case for IL-23 (p=0.005). However, there were no significant differences in IL-21 between preeclampsia and normal pregnant group. **Conclusions:** Our results conclude that contrary to IL-21, serum levels of IL-17 and TGF-β significantly increased in preeclampsia compared to normal pregnant women, supporting an imbalance of cytokine profile in preeclamtic patients.

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INTRODUCTION

Preeclampsia is a pregnancy-specific syndrome that happens in the later half of the pregnancy. The symptoms of this disease include high blood pressure, proteinuria and general edema (1) and the syndrome is clinically injurious to both the mother and the fetus. The etiology and pathogenesis of this syndrome are not fully known.

Many studies explain alterations and inappropriate activation of the immune system such as cell-mediated immunity that may have an influence on the beginning of this disorder. Preeclampsia is represented by the excessive maternal inflammatory response with a predominance of the production of Th1 cytokines, such as IL-2, IL-6, IL-8, IFN-γ, TNF-α, as well as IL-12 (2-7).

Th17 lymphocytes that produce IL-17, are a recently discovered subset of T CD4+ lymphocytes. Detection of this cytokine has led to some researchers to speculate a predominance profile of Th1/Th17 over Th2/Treg in chronic inflammatory conditions such as cancers, autoimmune diseases and allergic disorders (8-13). The differentiation and growth of Th17 cells is directed by a combination of TGF-β1 and IL-6 or IL-21 (14-17). Also, IL-23 was initially recognized as an important cytokine to induce Th17 cell production (10). However, recent studies reported it is not necessary for the differentiation of Th17 cells but critical for expansion and survival of Th17 cells (18).

Darmochwal-Kolarz's results demonstrated up-regulation of the Th17 immune response in preeclampsia. They showed that decreased number and function of Treg cells may be responsible for activating the inflammatory response characteristic of this disorder. Therefore, the predominance of Th17 immunity could act through modulating the Th1/Th2 immune balance (20).

Thus, due to importance of changes of Th17-associated cytokines in pathogenesis of preeclampsia, the aim of the study was to examine IL-17, IL-21, IL-23 and TGF-β in peripheral blood of patients with preeclampsia, healthy pregnant and non-pregnant women.

MATERIALS AND METHODS

Subjects. The study group consisted of 30 patients of mean age, 27 ± 5 years (range, 19–40), diagnosed with preeclampsia who were recruited in Department of Obstetrics and Gynecology in hospitals of Jahrom University of Medical Sciences. Including criteria for the patients according American Congress of Obstetricians and Gynecologists (ACOG) was third trimester pregnancy complicated with preeclampsia: blood pressure ≥140/90 and preutoinuria ≥300 mg/24 hours (20). The second group was in 30 women of mean age, 26 ± 4 years in third trimester pregnancy, without preeclampsia. Third group were 30 healthy non-pregnant women. Patients with microvascular complications, as well as those with coexisting autoimmune, chronic, and acute inflammatory diseases were excluded from the study. Peripheral venous blood samples (5 mL) were collected by venipuncture before any clinical intervention.

The protocol for the present study was approved by the Ethics Committee of the Shiraz University of Medical Sciences (Shiraz, Iran). Informed consent was obtained from all subjects who participated in this study.

Enzyme linked immunosorbent assay (ELISA). The amounts of IL-17, IL-21, IL-23 and TGF-β in the patients' and controls'sera were measured at the same time by the

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same technician, using ELISA-kits (eBiosciences, San Diego, CA, USA). Briefly, the standard stocks were serially diluted in Reagent Diluent to generate 7 points for the standard curves. Diluted capture antibody was added to a microtiterplate. Plates were sealed and incubated overnight at room temperature, then washed with Wash Buffer. Premixed standards or samples were added to each well, and incubated for overnight at 4ºC. After incubation and washing, premixed Detection Antibody was added to each well and the plate was incubated for 2 h at room temperature. After incubation and washing, Streptavidin-HRP was added to each well. The incubation was terminated after 20 min at room temperature. Then, Stop Solution was added to each well, and the optical density of each well was immediately determined using a microplate reader set to 450 nm. The results were expressed in pg/mL.

Statistical analysis. The serum levels of IL-17, IL-21, IL-23 and TGF-β in the peripheral blood were evaluated to the corresponding values from control samples using one-way ANOVA and T-test by SPSS software v. 15 (SPSS, Chicago, IL,173 USA). The variable levels were evaluated by means of Prism 4 software (Inc; San Diego CA, USA, 2003). $p < 0.05$ was regarded as significant in all statistical analysis.

RESULTS

Patient characteristics.

The clinical characteristics of the study participants are described in Table 1. There was no statistically significant difference in terms of age among the three study groups. Furthermore, no significant differences were observed in percentage of primiparas between preeclamptic patients and healthy pregnant women. However, all of the other clinical features presented in Table 1 differed significantly among our study groups.

Table 1. Clinical characteristics of preeclamptic patients, healthy pregnant and nonpregnant women.

| Variable	Preeclamptic patients ($n = 30$)	Pregnant women ($n = 30$)	Non-pregnant women ($n = 30$)
Age (years)	27 ± 5	26 ± 4	28 ±5
Pre-pregnancy BMI (kg/m^2)	25.5 ± 5.4[a]	21± 4.2	n.a.
BMI at blood draw (kg/m^2)	29.9 ± 5.1[a,b]	25.8± 5.1[b]	20.8 ± 3.3
Primiparas	60%	53.3%	n.a.
Systolic blood pressure (mmHg)	152 ± 15[a,b]	110 ± 10	115 ± 11
Diastolic blood pressure (mmHg)	91 ± 10[a,b]	75 ± 10	80 ± 10
Gestational age at delivery (weeks)	37 ± 3.2[a]	39 ± 1.3	n.a.
Fetal birth weight (grams)	3125[a]	3450	n.a.
White blood cell (×10^9/L)	9.7 ± 3.6[a,b]	9 ± 2.6[b]	8.2 ± 2.1
24 h urine protein (mg)	1973	None	n.a.

Date are presented as mean ± SD; n.a: not applicable; BMI: body mass index; a: p<0.05 versus healthy pregnant women; b: p<0.05 versus healthy non-pregnant women.

Cytokine assay in patient, pregnant and healthy groups.
The serum levels of IL-17, IL-21, IL-23 and TGF-β in patients with preeclampsia, pregnant and normal non-pregnant women were examined using ELISA method. It was shown that serum level of IL-17 in the preeclampsia group significantly increased compared to pregnant and normal non-pregnant groups ($p < 0.0001$) (Fig. 1A).

Comparison of the findings showed no significant difference in IL-21 serum level in patients compared to pregnant and healthy volunteers. However, healthy non-pregnant controls showed a significant difference compared to pregnant group ($p = 0.002$) (Fig. 1B). As shown in Fig. 1C, IL-23 serum level in patient and pregnant groups was less than normal controls ($p = 0.005$ and 0.002, respectively) (Fig. 1C).

Serum level of TGF-β in patients with preeclampsia and pregnant groups was substantially higher than non-pregnant group ($p < 0.0001$).
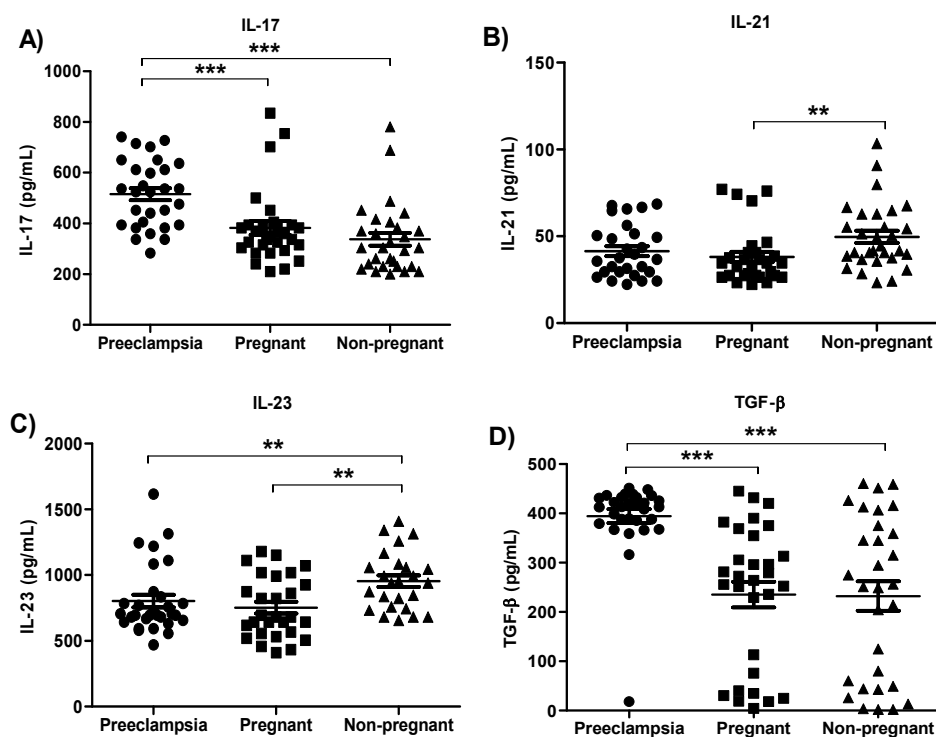
Figure 1. Serum level of IL-17, IL-21, IL-23 and TGF-β in the peripheral blood of patients with preeclampsia, pregnant and normal controls. **(A)** A significant difference was found in the serum level of IL-17 in peripheral blood of preeclampsia patients compared to pregnant women and non-pregnant controls. **(B)** IL-21 level was significantly higher in non-pregnant controls than patients. **(C)** Reduced IL-23 level was found in the patient and pregnant groups compared to control group. **(D)** TGF-β level was higher among preeclampsia patients compared to pregnant and non-pregnant samples Presented data were analyzed with the one-way ANOVA and T-test and the horizontal lines are showing the mean ± SEM of the groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$.

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DISCUSSION

Over the last few decades, preeclampsia was known as a syndrome with the excessive activation of the maternal proinflammatory response. The local and generalized inflammation is thought to be associated with an imbalance of maternal Th1/Th2 response (2,7,21).

In this study, we measured serum levels of IL-17 along with those of IL-21, IL-23 and TGF-β in a large number of healthy non-pregnant and pregnant women and preeclamptic patients. According to our findings, serum levels of IL-17 are significantly higher in preeclamptic patients than in pregnant and healthy non-pregnant women. Previous results about circulating IL-17 levels in preeclampsia are controversial. For instance, Jonsson *et al.* demonstrated no significant difference between serum levels of IL-17 of preeclamptic women and normal pregnant women (22). In another study, serum levels of IL-17 in preeclamptic women were lower than healthy pregnant women (22). In our study, serum IL-17 levels were assessed in number of healthy pregnant women and preeclamptic patients (matched for gestational age and age) with a high sensitivity ELISA. In addition, we also engaged healthy no pregnant women in our study. Results of our study are consistent with those of previous studies have indicated a higher prevalence of peripheral blood Th17 cells and increased expression of RORc mRNA in the deciduas and peripheral blood mononuclear cells in women with preeclampsia (19,24-26). In addition, Gharesi-Fard *et al.* demonstrated that gene expression of ROR-γt increase in the preeclampsia placentas compared to the healthy ones (27). Increased circulating levels of IL-17 detected in preeclampsia may relate to the progression of generalized intravascular inflammatory reaction that is a distinguishing marker of the maternal syndrome of the disease (28). IL-17 by inducing the expression of other cytokines, chemokines, inflammatory effectors and antimicrobial proteins, leads to pro-inflammatory responses (29). It seems IL-17 has been associated with the pathogenesis of several inflammatory and autoimmune disorders, such as systemic lupus erythematosus, rheumatoid arthritis, diabetes, inflammatory bowel disease and multiple sclerosis (29-31). Actually, preeclampsia has previously been indicated to be connected to increased levels of pro-inflammatory cytokines, chemokines, acute phase proteins, and products of complement activation in maternal circulation (32-34).

In our study, TGF-β level in preeclamtic women was significantly higher than those of healthy pregnant women and control groups. Ozkan *et al.* demonstrated that TGF-β presented positive correlation with blood pressure (23). Since differentiation of Th17 cells require TGF-β, it appears that the elevation in the serum level of TGF-β in patients with preeclampsia is associated with an increase in IL-17 serum level in these patients. Also, in preeclamptic women, TGF-β level was significantly higher than those of controls. The results of our study are consistent with the results of the study of Ozkan and colleagues (23).

In this study, we showed that there is no significant difference between the serum levels of the IL-21 in the three groups. So far, no study has been done on the role of IL-21 in preeclampsia. However, in accordance with our study, Saifi *et al.* showed IL-21 in patients with unexplained recurrent spontaneous abortion had no significant difference compared to normal non-pregnant women (35). Although comparison of the findings showed no significant difference in IL-23 serum level in patients compared to pregnant women, IL-23 serum level in patient and pregnant groups was less than normal controls.

It seems decreased IL-23 level in pregnant and preeclamptic women is due to Th2-dominant situation in pregnancy. In accordance with our study, Gharesi-Fard *et al.* also showed that there were no difference in expression of IL-23 mRNA in the decidual layers of the placentas from the preeclamptic and healthy pregnant women (27).

In conclusion results obtained suggest the up-regulation of Th17 immune response in preeclampsia. Moreover, they suggest that not only the IL-17, but also TGF-β as a necessary cytokine for differentiation of Th17 cells are increased in preeclampsia. Increased serum level of IL-17 and TGF-β may be responsible for the activation of inflammatory response in preeclampsia. Although in present study there is not disease following, it seems IL17 has a potential effect to maternal endothelium-associated inflammation may provide a biomarker for rapid preeclampsia diagnosis before progression of the disease to the late-onset stage.

ACKNOWLEDGEMENTS

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