

# Serum levels of tumor necrosis factor- $\alpha$ , interleukin-15 and interleukin-10 in patients with pre-eclampsia in comparison with normotensive pregnant women

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## ABSTRACT

**Background:** Pre-eclampsia (PE) is a pregnancy associated disorder characterized by hyper-tension and proteinuria. The first 2 stages of PE cause dysfunction in uteroplacental perfusion and oxidative stress while the third stage of PE is due to the release of inflammatory and angiogenic factors, which could lead to maternal endothelial damage and systemic inflammatory response. In this study, we compared the serum levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-15 (IL-15), and interleukin-10 (IL-10) in PE and normotensive women.

**Materials and Methods:** Serum samples of 84 pregnant women (44 PE and 40 normotensive) were evaluated for TNF- $\alpha$ , IL-15 and IL-10 by sandwich ELISA assay.

**Results:** The women with PE showed significantly higher serum levels of TNF- $\alpha$  and IL-15 ( $P = 0.001$  and  $0.01$  respectively) in comparison with normotensive pregnant women. Conversely, the serum levels of IL-10 in normotensive women were significantly higher compared to PE patients ( $P = 0.01$ ).

**Conclusion:** The results of this study demonstrated that inflammatory T helper 1-type responses are increased in PE women compared to normotensive pregnant women.

**Key words:** Interleukin-10, interleukin-15, preeclampsia, tumor necrosis factor- $\alpha$

## INTRODUCTION

Pre-eclampsia (PE) is a pregnancy associated disorder known by hypertension and proteinuria. It occurs in 3-5% of pregnancies after 20<sup>th</sup> week of gestational age. The disorder affects both mother and neonate.<sup>[1,2]</sup> The etiology of PE is not fully understood and a great number of mechanisms are proposed as the disease pathophysiology; however, the only definite cure of this syndrome is delivery.<sup>[3]</sup> Placenta plays a major role in the pathogenesis of PE. It is a general consensus on the role of disruption between vascular generation and anti-angiogenic responses in creating PE.<sup>[4]</sup> In addition, immune mal-adaptation hypothesis suggests that PE is the result of an inappropriate regulation of the normally

T helper 2 deviated response during the pregnancy.<sup>[5-7]</sup> In this way recent reports suggest that PE is associated with a Th1 predominant profile<sup>[8]</sup> while activation of the immune system leads to alteration in cytokine profile and defect in the acquisition of invasive phenotype by trophoblasts in PE patients.<sup>[6]</sup> PE develops in three stages. The first 2 stages of disease cause dysfunction in uteroplacental perfusion and oxidative stress while the 3<sup>rd</sup> stage of PE is due to the release of inflammatory and angiogenic factors, which could lead to maternal endothelial damage and systemic inflammatory response.<sup>[6,9]</sup>

Cytokines, growth factors, and adhesion molecules have been proposed as important mediators for successful placentation as well as endothelial function.<sup>[10,11]</sup> Over past decades, multiple analyses of cytokines provided substantial insight into the key players of the maternal immune system involved in PE.<sup>[5,12]</sup>

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is an inflammatory cytokine produced by human uterine and placental cells at the early and late stages of gestation and promotes the regulation of trophoblast growth and invasion. Furthermore, TNF- $\alpha$  could inhibit deeply integration and invasion of trophoblasts in maternal endometrium, which

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leads to PE.<sup>[11,13]</sup> Interleukin-15 (IL-15), which is also a proinflammatory cytokine, has profound effects on natural killer (NK) cells and their cytotoxicity activation. Regarding to the role of NK cells in the pathogenesis of PE, IL-15 could be a key player in development of disease.<sup>[14,15]</sup>

Interleukin-10 (IL-10) as an anti-inflammatory cytokine is produced by some subpopulations of T lymphocytes, monocytes, macrophages, and cytotrophoblasts.<sup>[16]</sup> Production of this cytokine is considered to play an important role in maternal immune tolerance of the fetus in normal pregnancy.<sup>[17]</sup>

In this study, we compared the serum levels of TNF- $\alpha$ , IL-15, and IL-10 in Iranian PE women with normotensive ones.

## MATERIALS AND METHODS

The study was a case-control study, in which serum samples of 84 pregnant women were studied. 44 PE (cases) and 40 normotensive (controls) pregnant women were enrolled in this study. These 2 groups were selected among the women admitted to a university associated hospital of Tehran University of Medical Sciences. This study was approved by ethics committee of Tehran University of Medical Sciences. All participants signed informed consent before enrolling to the study.

PE patients were diagnosed by hypertension (blood pressure  $\geq 140/90$  mm Hg) and proteinuria (at least 2 + on dips teak examination or  $\geq 300$  mg/24 h on at least 2 occasions 6 h apart) after the 20<sup>th</sup> week of gestation. These patients were normotensive before pregnancy and during the first 20 weeks of gestation. The PE women were under the supervision and treatment of obstetrics and gynecology specialist during pregnancy.

The normotensive control group consisted of healthy women with uncomplicated pregnancy who referred for normal routine check-up examinations to a university associated hospital. These women had blood pressure  $< 140/90$  mm Hg in all stages of pregnancy. Pregnant women with pre-gestational diabetes mellitus and chronic hypertension were excluded from the study. They did not receive any special drug during the pregnancy except routine supplements.

5 mL of venous blood sample were taken from both groups before beginning of the active phase of labor. Samples were centrifuged at 2500 G for 15 min at 4°C for serum separation. Serum specimens were aliquoted and stored at  $-70^{\circ}\text{C}$  until they were assayed.

Serum levels of TNF- $\alpha$  and IL-10 were measured using a sandwich ELISA test due to manufacturer's instruction (Bender MedSystems, Austria). The levels of IL-15 were assayed by using IL-15 sandwich ELISA kit (R&D Systems, Minneapolis, MN). In above mentioned ELISA kits routine procedures were done in this order; coating the plate with capture antibody, blocking the plate, adding standards, and unknown samples, washing, adding the biotin conjugated specific antibody, washing, applying enzyme-linked streptavidin, and finally adding chemical substrate. Optical density of color chemicals that produced in the wells of the plate were read and the concentrations of cytokines were measured according to plots. The serums that their cytokine levels were beyond the upper limit of the kit were diluted for assessment. For all mentioned cytokines, standard curves were plotted and the concentrations were calculated according the manufacturer's instruction.

Student's *t*-test was used for comparison between serum levels of two groups. *P* value  $< 0.05$  was considered statistically significant.

## RESULTS

The characteristics of women included in the study are presented in Table 1. There was no statistically significant difference related to age and gestational age at the time of sampling between two groups; however, there were higher systolic and diastolic pressures in PE group compared to normotensive one (*P* values: 0.01 and 0.03 respectively for systolic and diastolic blood pressures). The women with PE showed significantly higher serum concentrations of TNF- $\alpha$  and IL-15 (*P* values: 0.001 and 0.01 respectively) relative to the control group. In comparison, levels of IL-10 in the serum of normotensive pregnant women were significantly higher than PE patients (*P* value = 0.01). The results are demonstrated in Figures 1-3.

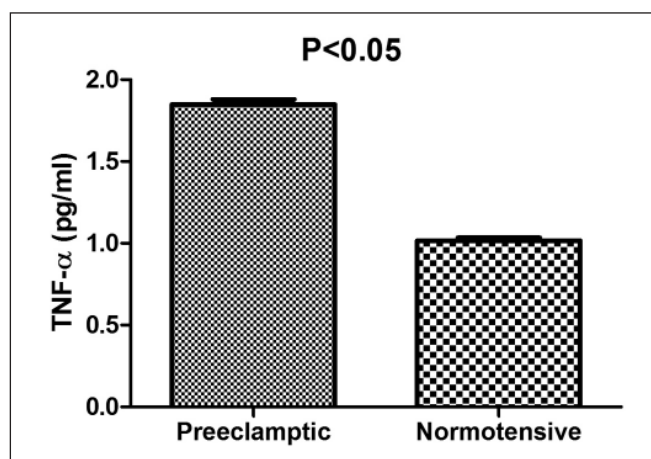
## DISCUSSION

Results of this study demonstrated that in women having PE, compared to women with a normal pregnancy,

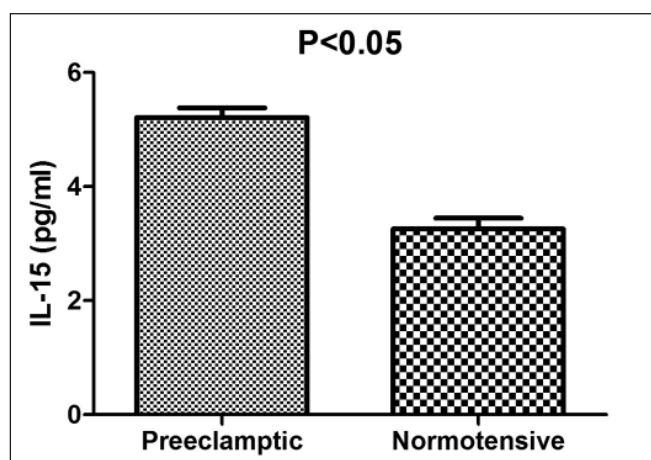
**Table 1: Clinical characteristics of the preeclamptic and normotensive women**

Characteristics	Preeclampsia group	Normotensive group
Age (years)	30.3 $\pm$ 3.1	28.6 $\pm$ 2.5
Gestational age at time of sampling	35.11 $\pm$ 2.1	36.19 $\pm$ 1.9
Systolic blood pressure (mmHg)*	15.47 $\pm$ 1.6	11.64 $\pm$ 1.3
Diastolic blood pressure (mmHg)*	10.73 $\pm$ 0.8	8.15 $\pm$ 0.95

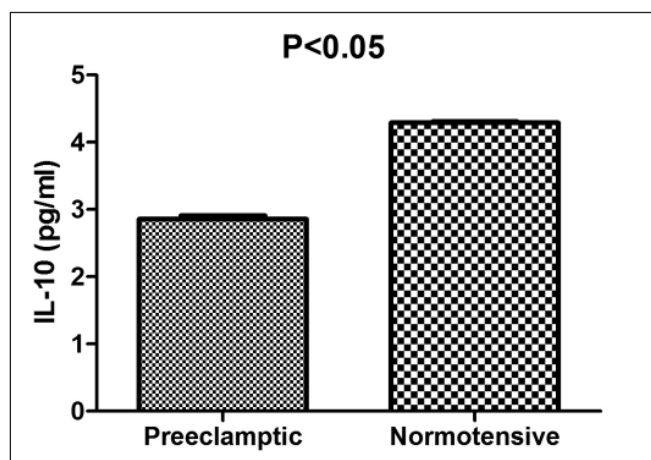
\**P* value $<0.05$



**Figure 1:** Serum levels of tumor necrosis factor- $\alpha$  in preeclamptic (PE) and normal subjects. Data are presented as mean  $\pm$  SEM. Each bar represented 44 PE and 40 normotensive pregnant women



**Figure 2:** Serum levels of interleukin-15 in preeclamptic (PE) and normal subjects. Data are presented as mean  $\pm$  SEM. Each bar represented 44 PE and 40 normotensive pregnant women



**Figure 3:** Serum levels of interleukin-10 in preeclamptic (PE) and normal subjects. Data are presented as mean  $\pm$  SEM. Each bar represented 44 PE and 40 normotensive pregnant women

inflammatory Th1-type responses are increased; while anti-inflammatory responses are decreased.

The higher levels of pro-inflammatory cytokines (TNF- $\alpha$  and IL-15) and lower levels of IL-10 in the PE group suggest an increased inflammatory status in PE during last months. Sharma *et al.* and Xie *et al.*, in agree with our results, found that the level of inflammatory cytokines such as TNF- $\alpha$  increased significantly in preeclamptic patients compared with health controls.<sup>[18-20]</sup> Furthermore, Laskowska *et al.* in consistent with us, indicated that TNF- $\alpha$  increased in PE patients and suggested this cytokine may be play a role in pathogenesis and consequences of PE.<sup>[21]</sup> In general, these studies demonstrated that TNF- $\alpha$  is a potent pro-inflammatory cytokine, its primary biological activity includes inflammation and it may contribute to the abnormal placental invasion.<sup>[11,13,22]</sup> However, Afshari *et al.*<sup>[23]</sup> and Jonsson *et al.*<sup>[5]</sup> observed no significant changes of TNF- $\alpha$  in PE women compared to normal pregnant women. About the other inflammatory cytokine we analyzed in this study, Hu *et al.* showed upraised serum levels of IL-15 in PE cases compared to normotensive ones.<sup>[14]</sup> In line with this finding, El-Baradie *et al.* in their study indicated that serum levels of IL-15 and IL-16 were elevated in PE relative to normotensive pregnant women.<sup>[15]</sup> In contrast Lu *et al.* represented not elevated levels of IL-15 in PE subjects compared to normal women.<sup>[24]</sup>

In normal pregnancy, the trophoblasts invade the endovascular tissue and replace in the uterine arterioles.<sup>[9,25]</sup> It seems that in PE Th1-type cytokines such as TNF- $\alpha$  and IFN- $\gamma$  induce trophoblastic apoptosis, inhibit differentiation and invasion of trophoblast, and finally, may be involved in the incomplete invasion of trophoblast to spiral arteries; pathologic processes that are integral parts of PE.<sup>[26]</sup>

Our findings showed higher levels of IL-10, which is an anti-inflammatory cytokine in sera of normal pregnant women. In concordance with our results, Sharma *et al.*<sup>[20]</sup> showed that IL-10 was decreased significantly in PE patients compared to normal pregnant women. These findings emphasize on the protective role of IL-10 in PE.<sup>[17]</sup> Inversely, Bakheit *et al.*<sup>[27]</sup> and Xie *et al.*<sup>[19]</sup> found higher serum levels of IL-10 in PE women and Jonsson *et al.*<sup>[5]</sup> observed no significant difference in IL-10 levels between normotensive and PE women.

These contradictory results of various studies could be related to sampling in different stages of pregnancy. PE as an inflammatory process starts from the first steps of placentation; however, this inflammatory grade lasts until parturition.

## CONCLUSION

Consequently, upraised levels of pro-inflammatory cytokines in our study may be the prerequisite of PE. Recognition of early detectable inflammatory markers could be a great help to predict and observe PE patients more accurately.

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