

Serum Interleukins (1 β , 6, 8, 10), Tumor Necrosis Factor Alpha and Interleukin-2 Receptor Levels in Patients with Severe Preeclampsia and Normotensive Pregnant Women

AĞIR PREEKLAMPTİK VE NORMOTENSİF GEBELİKLERDE SERUM İNTERLÖKİN (1 β , 6, 8, 10), TÜMÖR NEKROZİS FAKTÖR ALFA VE İNTERLÖKİN-2 RESEPTÖR DÜZEYLERİ

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Abstract

Objective: The aim of this study is to compare the concentrations of serum interleukins (ILs) (1 β , 6, 8, 10), tumor necrosis factor alpha (TNF- α) and interleukin-2 receptor (IL-2R) levels in patients with severe preeclampsia and normotensive pregnant women.

Material and Methods: The study was carried out at Dicle University University School of Medicine, Diyarbakir. One-hundred pregnant women were included in this prospective study. Pregnant women divided into two groups. Women with severe preeclampsia (n= 50) were compared with normotensive pregnant women (n= 50). Serum interleukin-1 β , interleukin-8 and TNF α levels were analyzed by immunometric assay using Immulite 2000 analyzer (Diagnostic Product Corporation, Los Angeles). Serum interleukin-6 and interleukin-10 levels were analyzed by solid-phase enzyme-labeled, chemiluminescent sequential immunometric assay using Immulite 2000 analyzer (Diagnostic Product Corporation, Los Angeles). Serum interleukin-2R (IL-2R) receptor levels were analyzed by chemiluminescent assay method using Immulite 2000 analyzer (Diagnostic Product Corporation, Los Angeles). The Student-t test was used for statistical analysis and a p value of < 0.05 was accepted as statistically significant.

Results: The mean \pm SD serum levels of Interleukin-1 β (pg/ml), Interleukin-6 (pg/ml), Interleukin-8 (pg/ml), Interleukin-10 (pg/ml), TNF- α (pg/ml) and IL-2R (U/ml) were 30.3 \pm 5.3, 16.9 \pm 4.3, 5.9 \pm 1.8, 3.8 \pm 1.1, 6.6 \pm 2.5 and 716.5 \pm 20.8 respectively in preeclampsia. The mean \pm SD serum levels of 1 β (pg/ml), Interleukin-6 (pg/ml), Interleukin-8 (pg/ml), Interleukin-10 (pg/ml), TNF- α (pg/ml) and IL-2R (U/ml) in the normotensive pregnancy group were 9.43 \pm 2.6, 9.5 \pm 3.3, 12.4 \pm 3.9, 2.7 \pm 0.7, 4.4 \pm 1.6 and 348.8 \pm 35.9 respectively. The mean serum levels of Interleukin-1 β , Interleukin-6, Interleukin-8, TNF- α and IL-2R were significantly higher (p< 0.001) in women with severe preeclampsia, and the mean serum level of interleukin-10 was significantly higher in women with normotensive pregnant women (p< 0.001).

Conclusions: Preeclampsia is associated with an imbalance between pro-inflammatory and anti-inflammatory cytokines in favor of pro-inflammatory cytokines and TNF- α , interleukins and IL-2R receptors may contribute to the pathophysiology of preeclampsia.

Key Words: Preeclampsia, interleukins, pregnancy

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Özet

Amaç: Ağır preeklampatik ve normotensif gebeliklerde serum interlökin (ILs) (1 β , 6, 8, 10), tümör nekrozis faktör alfa (TNF- α) ve interlökin-2 reseptör (IL-2R) seviyelerini karşılaştırmak.

Gereç ve Yöntemler: Bu çalışma Dicle Üniversitesi Tıp Fakültesinde toplam 100 gebe üzerinde yapıldı. Hastalar ciddi preeklampatik (n= 50) ve normotensif gebelik (n= 50) olmak üzere iki gruba ayrıldı. Serum interlökin-1 β , interlökin- 8 and TNF α seviyeleri immunometric assay yöntemi ile Immulite 2000 analyzer cihazı kullanılarak çalışıldı (Diagnostic Product Corporation, Los Angeles). Serum interlökin-6 ve interlökin-10 seviyeleri "solid-phase enzyme-labeled, chemiluminescent sequential immunometric assay" yöntemi kullanılarak Immulite 2000 cihazı ile çalışıldı (Diagnostic Product Corporation, Los Angeles). Serum IL-2R reseptör seviyeleri "chemiluminescent assay method ile Immulite" 2000 cihazı ile çalışıldı (Diagnostic Product Corporation, Los Angeles). İstatistiksel analizde student-t testi kullanıldı ve p< 0.05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: Ağır preeklampatik grupta ortalama (\pm SD) serum interlökin-1 β (pg/ml), interlökin-6 (pg/ml), interlökin-8 (pg/ml), interlökin-10 (pg/ml), TNF- α (pg/ml) and IL-2R (U/ml) seviyeleri sırasıyla 30.3 \pm 5.3, 16.9 \pm 4.3, 5.9 \pm 1.8, 3.8 \pm 1.1, 6.6 \pm 2.5 and 716.5 \pm 20.8 idi. Normotensif grupta ortalama (\pm SD) serum 1 β (pg/ml), interlökin-6 (pg/ml), interlökin-8 (pg/ml), TNF- α (pg/ml) and IL-2R (U/ml) seviyeleri 9.43 \pm 2.6, 9.5 \pm 3.3, 12.4 \pm 3.9, 2.7 \pm 0.7, 4.4 \pm 1.6 and 348.8 \pm 35.9 idi. Ortalama serum interlökin-1 β , interlökin-6, interlökin-8, TNF- α ve IL-2R seviyeleri ağır preeklampatik grupta normotensif gruba göre daha yüksekti (p< 0.001). Ortalama serum interlökin-10 seviyeleri normotensif grupta daha yüksekti (p< 0.001).

Sonuç: Preeklampside pro-inflamatuar ve anti-inflamatuar sitokinler arasında proinflamatuar sitokinler lehine bir dengesizlik söz konusudur, ayrıca TNF- α , interlökin ve IL-2R reseptörleri preeklampsinin patofizyolojisinde rol alır .

Anahtar Kelimeler: Preeklampsi, interlökinler, gebelik

Preeclampsia is a multisystem hypertensive disorder of pregnancy that affects 2-5% of pregnancies.^{1,2} Preeclampsia is characterized by hypertension and varying degrees of organ dysfunction including proteinuria, headache and

seizures which is responsible for a significant amount of maternal and perinatal morbidity and mortality.³ Although the etiology of preeclampsia remains unknown, poor placental invasion plays an important role in the etiology of preeclampsia.⁴ The local event of poor placentation also results in systemic maternal abnormalities such as vascular dysfunction and inflammatory response.⁵⁻⁷

Interleukins (ILs) and tumor necrosis factor alpha (TNF- α) are peptidic substances released in response to various inflammatory processes. Interleukins 1 β , 6, 8 and TNF- α are inflammatory cytokines produced by a variety of cell types, including monocytes-macrophages, amnion, chorion, and decidua cells.^{8,9,10} Soluble interleukin-2 receptor (sIL-2R) has been shown to be a sensitive and quantitative marker of T-cell activation and proliferation.¹¹

The preeclamptic maternal response is characterized by an enhanced inflammatory response and manifest by altered cytokine production. TNF- α and interleukins have been implicated in the pathogenesis of preeclampsia.^{7,12,13} Women who have preeclampsia have higher serum pro-inflammatory cytokines such as IL-6, 8 and TNF- α and reduced anti-inflammatory cytokines such as IL-10.^{7,14,15} Soluble interleukin-2 receptor, a marker for the activity of lymphocyte was found increased in pregnant women of first trimester, who late developed preeclampsia.¹¹

The purpose of this study was to measure IL-6, 8, 10, 1 β , IL-2R and TNF- α levels of preeclamptic women, and to assess the possible role of inflammatory cytokines and T-cell activation in the pathogenesis of preeclampsia.

Material and Methods

This case-control study was carried out at the Obstetric and Clinical Biochemistry Department of the University Hospital in Diyarbakir from September 1, 2004 to October 31, 2005. The patients were classified into two groups; group 1 (study group) included in severe preeclamptic women (n=50) and group 2 (control group) included in normotensive pregnant women (n=50). All subjects were recruited in a voluntary manner, giving their

written informed consent. The diagnosis of severe preeclampsia was established in accordance with the American College of Obstetrics and Gynecology definition.¹⁶ The healthy normotensive pregnancy was diagnosed on the basis of clinical, biochemical, and ultrasound findings and none of the patients had pre-existing hypertensive disorders or any renal, hepatic, or hematological diseases, and had received no medication.

We obtained venous blood samples from all the participants within 24 hours of admission to assess the IL 1 β , 6, 8, 10, sIL-2R, and TNF- α assay. All blood samples were collected in sterile vacutainer tubes and allowed to clot for 10 minutes at room temperature and were centrifuged later for 5 minutes at 4000Xg. Sera were immediately stored in pyrogen-free tubes at -80°C until analysis.

Soluble IL-2R was analyzed by chemiluminescent assay method using Immulite 2000 analyzer (Diagnostic Product Corporation, Los Angeles) with an analytical sensitivity of 5 U/mL. The intraassay and interassay coefficient variation of this procedure was 2.9%-3.7% and 6.1%-8.1% respectively.

Interleukin-6 was analyzed by solid-phase enzyme-labeled, chemiluminescent sequential immunometric assay using Immulite 2000 analyzer (Diagnostic Product Corporation, Los Angeles) with a detection limit of 2 pg/mL. The intraassay and interassay coefficient variation of this procedure was 3.5%-6.2% and 5.1%-7.5% respectively. Interleukin-8 was analyzed by immunometric assay using Immulite 2000 analyzer (Diagnostic Product Corporation, Los Angeles) with an analytical sensitivity of 2 pg/mL. The intraassay and interassay coefficient variation of this procedure was 3.6%-3.8 and 5.2%-7.4% respectively.

TNF- α was analyzed by immunometric assay using Immulite analyzer (Diagnostic Product Corporation, Los Angeles) with an analytical sensitivity of 1.7 pg/mL. The intraassay and interassay coefficient variation of this procedure was 2.6%-3.6% and 4.0%-6.5% respectively. Interleukin-1 β was analyzed by immunometric assay using Immulite analyzer (Diagnostic Product Corporation, Los Angeles) with an analytical sensitivity of 1.5

pg/mL. The intraassay and interassay coefficient variation of this procedure was 2.8%-4.8% and 4.9%-7.7% respectively.

Interleukin-10 was analyzed by solid-phase enzyme-labeled, chemiluminescent immunometric assay using Immulite 2000 analyzer (Diagnostic Product Corporation, Los Angeles DPC) with an analytical sensitivity 1 pg/mL. The interassay and interassay coefficient variation of this procedure was 2.9%-3.4% and 4.2%-9.9% respectively.

The interleukin and tumor necrosis factor levels in two groups were statistically compared by the Student-t test. A value of $p < 0.05$ was considered statistically significant.

Results

A total of hundred women were recruited for the study; 50 were classified as having severe preeclampsia and 50 were control cases. The Demographic and obstetric features of the two groups (maternal age, gravida, parity, maternal weight, gestational week and mean systolic and diastolic arterial pressure at the time of sample collection are shown in Table 1. There was no significant difference in maternal age, gravida, parity, gestational week between the two groups ($p > 0.05$). The mean systolic and diastolic arterial blood pressure was 181 ± 10.9 and 121 ± 4.6 in group 1, and 113 ± 7.7 and 77.5 ± 6.2 mmHg in group 2 respectively ($p < 0.001$).

The mean \pm SD serum levels of Interleukin-6 (pg/ml), Interleukin-8 (pg/ml), Interleukin-10 (pg/ml), Interleukin-1 β (pg/ml) and TNF- α (pg/ml) in the severe preeclampsia group were 30.3 ± 5.3 , 16.9 ± 4.3 , 5.9 ± 1.8 , 3.8 ± 1.1 and 6.6 ± 2.5 respectively. The mean \pm SD serum levels of Interleukin-6 (pg/ml), Interleukin-8 (pg/ml), Interleukin-10 (pg/ml), Interleukin-1 β (pg/ml) and TNF- α (pg/ml) in the normotensive pregnancy group were 9.43 ± 2.6 , 9.5 ± 3.3 , 12.4 ± 3.9 , 2.7 ± 0.7 and 4.4 ± 1.6 respectively (Table 2).

The mean serum levels of Interleukin-6, Interleukin-8, Interleukin-1 β and TNF- α were significantly higher ($p < 0.001$) in women with severe preeclampsia than normotensive pregnant women. Although, the mean serum level of interleukin-10

Table 1. Demographic and clinical characteristics of patients with severe preeclamptic and healthy pregnant women.

Characteristics	Group 1 (n= 50)	Group 2 (n= 50)	p
Maternal age (years)	32.4 \pm 4.8	31.7 \pm 3.9	NS*
Gestational age weeks)	35.2 \pm 1.8	37.5 \pm 1.1	NS*
Mean Systolic BP (mmHg)	181 \pm 10.9	113 \pm 7.7	< 0.001
Mean Diastolic BP (mmHg)	121 \pm 4.6	77.5 \pm 6.2	< 0.001
Gravida	2.5 \pm 0.6	2.8 \pm 0.4	NS*
Parity	1.0 \pm 0.6	1.0 \pm 0.5	NS*
Maternal weight (kg)	80.5 \pm 5.5	72.2 \pm 4.9	< 0.001

NS*, not significant according to Student's *t*-test.

Table 2. Serum interleukins and TNF- α levels of two groups.

	Group 1 (n= 50)	Group 2 (n= 50)	p value
IL-2R(U/ml)	716.5 \pm 20.8	348.8 \pm 35.9	< 0.001
IL-6 (pg/ml)	30.3 \pm 5.3	9.43 \pm 2.6	< 0.001
IL-8 (pg/ml)	16.9 \pm 4.3	9.5 \pm 3.3	< 0.001
IL-10 (pg/ml)	5.9 \pm 1.8	12.4 \pm 3.9	< 0.001
IL- 1 β (pg/ml)	3.8 \pm 1.1	2.7 \pm 0.7	< 0.001
TNF- α (pg/ml)	6.6 \pm 2.5	4.4 \pm 1.6	< 0.001

was significantly higher ($p < 0.001$) in normotensive pregnant women than severe preeclamptic women (Figure 1). The mean \pm SD serum levels of Interleukin-2 receptor (IL2R) (U/ml) was higher in group 1 (716.5 ± 20.8) than group 2 (348.8 ± 35.9) (Figure 2).

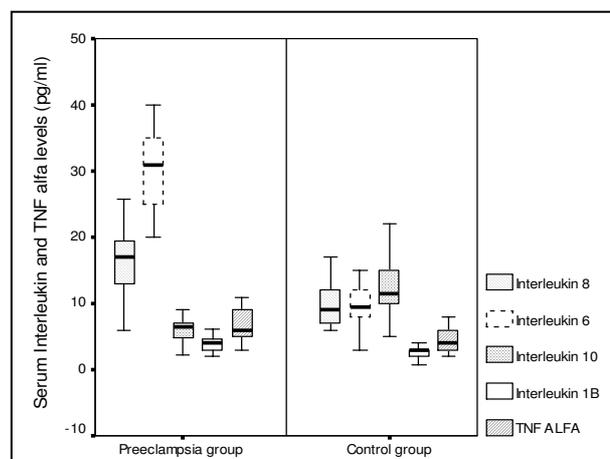


Figure 1. Serum levels of interleukins (6, 8, 10, 1 β) and TNF- α in two groups.

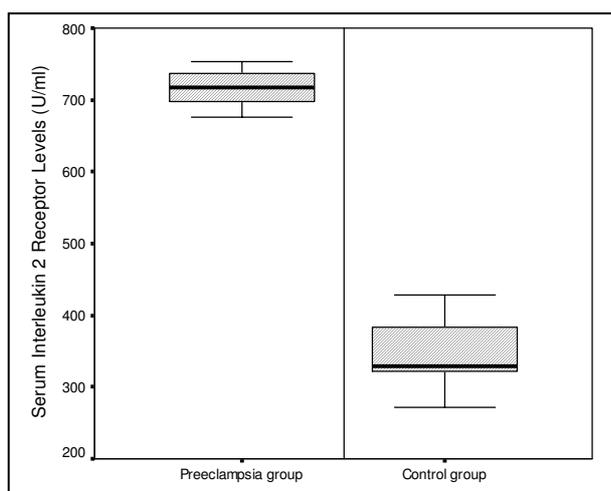


Figure 2. Serum interleukin-2 receptor levels in two groups

Discussion

Endothelial cell injury may play a central role in the pathogenesis of preeclampsia. Endothelial cell dysfunction associated with preeclampsia is characterized by an enhanced inflammatory response and altered cytokine production. Cytokines such as TNF- α and the interleukins may contribute to the pathophysiology of preeclampsia.^{7,17}

The cytokines IL-6 and IL-8 were significantly elevated in subjects with severe preeclampsia, but not mild, preeclampsia compared to controls.¹⁴ Takacs et al found 5 fold increased plasma interleukin-6 levels in preeclamptic women than normal pregnant women.¹⁸ Scott et al found 2.5 fold increased plasma interleukin-8 levels in severe preeclamptic women compared with normal pregnant women.¹⁹ Elevated IL-8 levels were in accordance with the work of some, but not all investigators. Greer et al found significantly elevated IL-6 levels but unaltered IL-8 levels in the plasma of preeclamptic subjects.²⁰ In our study, we found increased serum interleukin-6 and interleukin-8 levels in severe preeclamptic women compared with normotensive pregnant women ($p < 0.001$).

Interleukin-10 is an inhibitor of cytokine production. Interleukin-10 is produced by a subset of CD4+ cells, type 2 (TH 2) cells, and inhibits the

cytokine production by another CD4 cell subset, type 1 (TH 1) cells which suppresses the production of the so-called inflammatory cytokines IL 1, 6, 8 and TNF- α by activated monocytes.^{21,15} Preeclamptic placentas have been shown to have reduced IL-10 levels.²¹ In our study, we found decreased serum interleukin-10 levels in severe preeclamptic women compared with normotensive pregnant women ($p < 0.001$).

IL-1 has two defined forms interleukin-1 α and IL-1 β . IL-1 β promotes the synthesis of many other cytokines and mediators of inflammation and principal role of IL-1 β is in the initiation of early events in immune responses.^{23,24} We have demonstrated that serum interleukin-1 β levels were higher in severe preeclamptic women compared with normotensive pregnant women ($p < 0.001$).

Soluble interleukin-2 receptor (sIL-2R) is a sensitive and quantitative marker of T-cell activation and proliferation.¹¹ The activation of T helper cells leads to expression of IL-2, and receptors for IL-2. A soluble form of IL-2R appears in the serum at the same time as IL-2 expression occurs on the cells. Increased levels of IL-2R in serum correlate with increased T and B cell activation and also increased number of lymphokine activated killer cells that can kill primitive target cells.²⁵⁻²⁷ Eneroth et al had found increased serum concentrations of interleukin-2 receptor in the first trimester in ten women who later developed severe preeclampsia.¹¹ We have demonstrated that serum interleukin-2 receptor levels were significantly higher in severe preeclamptic women compared with normotensive pregnant women ($p < 0.001$).

TNF- α may have a role in the pathogenesis of preeclampsia because of endothelial effect.^{7,11,12} Conrad et al²⁸ hypothesized that inadequate trophoblast invasion and physiologic remodeling of spiral arteries may stimulate the overproduction of cytokines, such as TNF- α and elevated levels of TNF- α may contribute to endothelial dysfunction directly and indirectly. Hamai et al²⁹ reported that circulating TNF- α is elevated in those women at 11-13 weeks of gestation who later developed preeclampsia. TNF- α level was found higher in preeclamptic patients compared with normotensive

controls in the third trimester.^{30,31} We have found increased TNF- α levels in preeclamptic patients compared with normotensive controls in accord with previously reported results ($p < 0.001$).

In this study, we found that increased levels of IL 6, 8, 1 β , TNF- α and decreased levels of IL-10 in the serum of women with severe preeclampsia. These findings suggest that severe preeclamptic women have higher serum pro-inflammatory cytokines and reduced anti-inflammatory cytokines such as IL-10.

An increase in IL-2R activation in severe preeclamptic pregnant women in our study may indicate the presence of disturbance in immunological tolerance, disturbance in trophoblastic invasion and impaired placentation.

In conclusion, we hypothesized that preeclampsia is associated with an imbalance between pro-inflammatory and anti-inflammatory cytokines in favor of pro-inflammatory cytokines and TNF- α , interleukins and Interleukin-2R receptors may contribute to the pathophysiology of preeclampsia.

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