Serum Cytokines Profiles in Iranian Patients with Preeclampsia

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ABSTRACT

Background: Preeclampsia is a major cause of mortality and morbidity and is also a leading cause of preterm birth and intrauterine growth retardation. Several studies have reported abnormal levels of cytokines in women with preeclampsia. **Objectives:** To detect serum levels of various cytokines in pregnant women with and without preeclampsia in the third trimester of pregnancy. **Methods:** Thirty patients with preeclampsia and thirty normal pregnant women were enrolled in the study. Blood samples were taken and serum levels of IFN γ, IL-12p70, IL-18, IL-15, IL-4 and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA). **Results:** Preeclamptic women had significantly increased levels of circulating IL-12p70 ($p < 0.05$), IL-18 ($p < 0.001$), IL-4 ($p < 0.001$), IL-15 ($p < 0.05$) and IFN γ ($p < 0.001$). By contrast, circulating levels of IL-10 were not significantly different between the two groups. **Conclusions:** The present study supports the hypothesis of altered immune response in preeclampsia and suggests that dysregulation of cytokine expression occurs in preeclampsia with increased levels of IFN γ, IL-12p70, IL-15, IL-18 and IL-4.

Keywords: Preeclampsia, Cytokines, ELISA

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INTRODUCTION

Preeclampsia, a pregnancy specific disorder that occurs in 3-5% of pregnancies, is a major cause of mortality and morbidity and is also a leading cause of preterm birth and intrauterine growth retardation (1). The symptoms of this multisystem disorder, which appear during the second and third trimester of pregnancy, are caused by the increased vasoconstriction, which results in maternal hypertension, decreased uteroplacental blood flow, edema, proteinuria, abnormal clotting, and liver and renal dysfunction (2, 3). A major hurdle in clinical approaches to preeclampsia is the lack of understanding of the etiology and pathophysiology of the condition, thus hindering the development of effective prevention and treatment strategies (4).

A generalized dysfunction of maternal endothelial cells may underlie most of the clinical symptoms such as hypertension, fluid retention, and clotting abnormality. Interestingly, a dysregulation of the maternal immune response against the fetus has been suggested as a possible causal factor (5). Indeed, an inflammatory response has been shown to occur during preeclampsia (6, 7). The composition of the immunomodulatory milieu, specifically the presence and amounts of various cytokines in the serum of pregnant women may lend insight into the *in vivo* regulation of preeclampsia-associated conditions. Several studies have reported abnormal levels of cytokines in women with preeclampsia, but the pattern of cytokine expression (8) and a possible role in disease pathogenesis (9) remains controversial.

In the present study, we investigated the serum levels of various cytokines in preeclamptic women by enzyme-linked immunosorbent assay (ELISA). These cytokines included IFN γ, IL-12p70, IL-15 and IL-18 and the anti-inflammatory cytokines IL-4 and IL-10. We found that the levels of some cytokines were significantly enhanced in patients with preeclampsia. These data may be helpful for understanding the pathogenesis of, and developing treatments for preeclampsia.

MATERIALS AND METHODS

Subjects. Thirty women with preeclampsia and 30 control pregnant women were enrolled. Ten of preeclamptic women had severe and twenty had moderate preeclampsia. Subjects were selected at the Mirza Khoochak Khan hospital, Tehran, Iran between March and October 2006. The control group was selected from healthy pregnant women. The preeclamptic group consisted of women who were (a) normotensive before pregnancy and during the first 20 weeks of gestation, and (b) developed hypertension (blood pressure of 140 mm Hg or higher, or a diastolic blood pressure of 90 mmHg or higher on two or more occasions, 6hr apart) associated with new onset proteinuria of either greater than 100mg /dl by urine analysis (>1+ dipstick) or greater than 300mg/dl in a 24-h urine collection. Severe preeclampsia was defined as a systolic blood pressure of ≥160 mm Hg or a diastolic blood pressure of ≥ 110 mmHg with either a urine dipstick showing urinary protein of 3+ to 4+ or greater than 5.0 g of urinary protein in 24 h urine sample. Women who met the criteria of preeclampsia but did not have severe preeclampsia were diagnosed as moderate preeclampsia. Neither the subjects' age nor gestational age differed significantly between the two groups. No subject had co-morbid conditions such as diabetes, asthma, congenital heart disease, connective tissue disorders or an autoimmune disease. None of the subjects in the preeclamptic or normal con-

trol group were in labor at the time of sampling. None of the pregnancies were complicated by preterm rupture of membranes. Subjects with any symptom of infection were excluded from the study. All subjects submitted written informed consents. The study was authorized by the Ethical Investigation Committee of Tehran University of Medical Sciences.

Methods. Blood samples were centrifuged within 30 minutes of venipuncture at 2500 × *g* for 15 min at 4 ° C and the serum was aliquoted and stored at –80 ° C until use to avoid possible interference with assay results due to repeated freeze-thaw cycles. Serum levels of IFN γ, IL-12p70, IL18, IL10 and IL-4 were assayed using an enzyme linked immunosorbent assay kit (Bender MedSystems, Austria). Serum levels of IL-15 were assayed by a different ELISA kit (R&D Systems, Minneapolis, MN). The manufacturers' protocols were followed for each assay. Standard curves were plotted for each of the cytokines using reference recombinant cytokines and the results were read from the curves. All samples were tested in duplicate. The sensitivity of each of the assays was as follows: 0.99 pg/ml for IFNγ, 0.99 pg/ml for IL-10, 9.2 pg/ml for IL-18, 3.2 pg/ml for IL-12p70, 2 pg/ml for IL-4, and 2 pg/ml for IL-15.

Statistical Analysis. All data were analysed using SPSS, version 13 for Windows software. The data were tested for normal distribution by Kolmogrov-Smirnov test. Student t-test or the Mann-Whitney U-test was used for comparisons between the two groups where appropriate. Differences at $p < 0.05$ were considered as statistically significant.

RESULTS

The clinical characteristics of the control and preeclamptic groups are presented in Table 1. Patients with preeclampsia displayed significantly increased systolic and diastolic blood pressure relative to controls ($p < 0.001$). None of the patients in the control group had proteinuria. All patients with preeclampsia had proteinuria (>1+ dipstick or greater than 300mg/dl in a 24-h urine collection). As expected, all other clinical and demographic variables were similar between the two study groups.

Table 1. Clinical characteristics of subjects

Parameter	Control group	Preeclampsia group	P value
Age (y)	28.3 ± 3.4	28.5 ± 4.5	NS
Gestational age at sampling(w)	36.7 ± 1.1	35.1 ± 3.5	NS
Systolic Blood Pressure(mmHg)	110 ± 8	152 ± 16	<0.001
Diastolic blood pressure(mmHg	75.0 ± 6	97 ± 10	<0.001

N=30 per group; NS, Non-significant

Table 2. Serum cytokine concentrations (pg/ml) of normal and preeclamptic pregnancies

Cytokine	Control Group		Preeclampsia		P value
	Median	Range	Median	Range	
IL-12	92	63-281	138	28-329	< 0.05
IL-18	180	39-338	250	16-500	< 0.05
IFN-γ	1.2	0-14.2	4.45	0-17	< 0.001
IL-4	10.24	0-41.8	22	0-185.2	< 0.001
IL-10	32.5	0.4-100.4	26.7	0.4-69.8	NS
IL-15	3.15	2.1-4.8	5.2	3.6-7.8	< 0.05

N=30 pergroup; Ns, Non-significant

Cytokine levels are presented in Table 2. Relative to controls, the women with preeclampsia exhibited significantly higher serum concentrations of the pro-inflammatory cytokines IL-12 ($p < 0.05$), IL-15 ($p < 0.05$), IL-18 ($p < 0.05$), and IFN γ ($p < 0.001$) (Figure.1). We also found elevated levels of IL-4 ($p < 0.001$) in women with preeclampsia compared with non-preeclamptic women. There was no significant difference in IL-10 levels between the two groups (Figure.2).

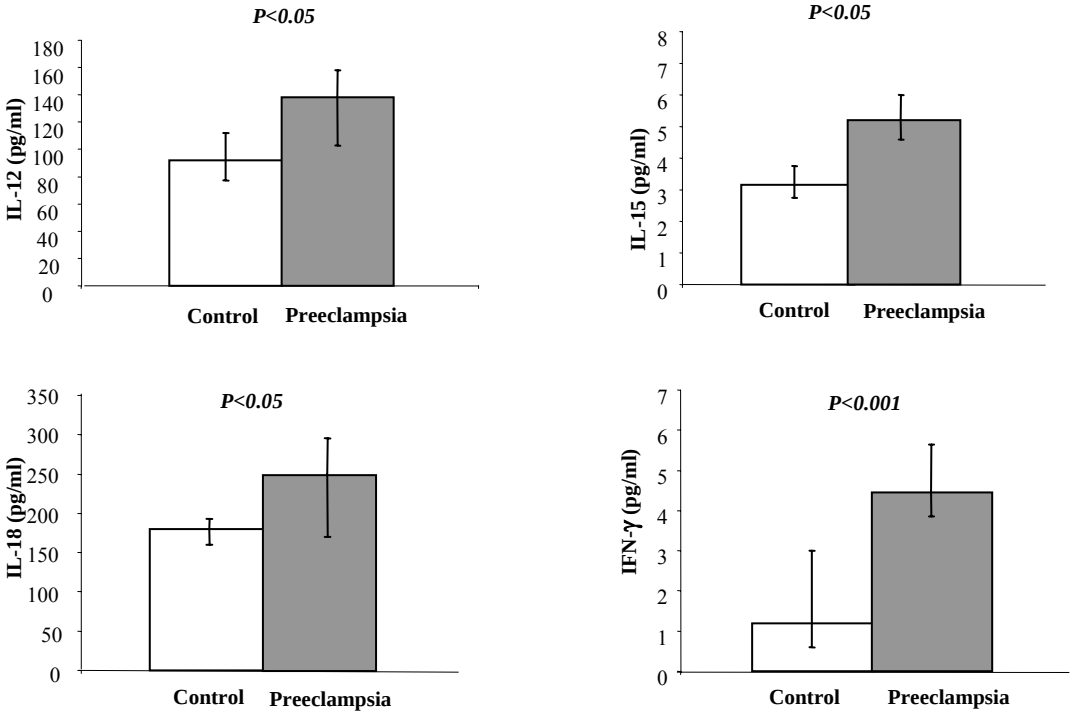

Figure 1. Serum levels of IL-12, IL-15, IL-18 and IFN-γ in normal pregnancy (control) and preeclampsia. Data are presented as median with quartiles.

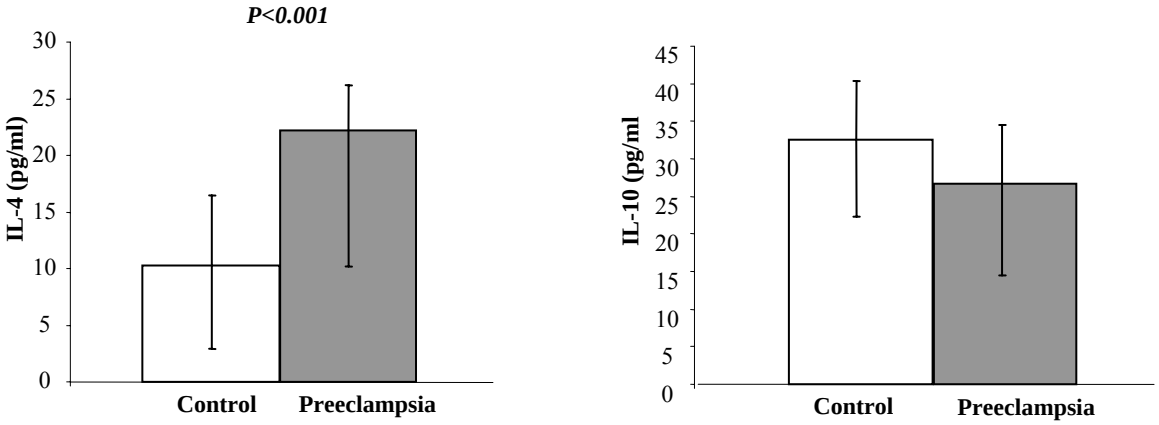

Figure 2. Serum levels of IL-4 and IL-10, in normal pregnancy (control) and preeclampsia. Data are presented as median with quartiles.

DISCUSSION

The present results report significantly increased levels of IFN γ, and the IFN γ- inducing cytokines IL-12p70, IL-15 and IL-18 in preeclamptic patients. In addition we observed markedly elevated levels of the anti-inflammatory cytokine IL-4 in such patients. No significant difference was seen in serum levels of IL-10 between the two groups.

Preeclampsia has been proposed to be a syndrome caused by an excessive systemic inflammatory response to pregnancy (6, 7, 10, 11). Because of this, we anticipated elevated serum levels of IFN γ and its inducer cytokines IL-12, IL-15, and IL-18 in preeclamptic patients relative to the control group. It is known that IFN γ enhances cytotoxic activation of T lymphocytes and NK cells, activates macrophages and phagocytosis, and induces pro-inflammatory cytokine expression. Therefore increased concentration of IFN-γ in pregnancy can be potentially harmful (12). There are not many reports about the levels of this cytokine in peripheral blood of preeclamptics. Our findings are consistent with the results of Pizano et al (3) who reported significantly higher concentrations of IFN-γ in maternal peripheral blood of preeclamptic women compared to normotensive ones. However these authors found no difference upon inspection of intracellular IFN-γ production in T lymphocytes between the two groups. As a result, higher concentrations of IFN-γ may be due to other sources of cytokines such as decidual or endothelial cells. On the other hand, another study found no difference in serum levels of IFN-γ between normal and preeclamptic pregnant women (9). The authors speculated that this could be explained by the known paracrine action of T-cell cytokines. Secreted cytokines are rapidly bound to receptors on neighbouring cells and excessive levels in preeclampsia or normal pregnancy may thus be captured at the site of secretion, resulting in similar serum levels in both groups.

Studies of serum or plasma IL-12 in preeclampsia have produced conflicting results (9, 13, 14). The cytokine IL-12p70 consists of two subunits, p35 and p40 and is a potent inducer of IFN γ (15, 16). Our observation of increased levels of IL-12p70 in preeclampsia confirms those reported by Daniel et al (13). Jonnson et al (9), observed no difference in serum IL-12p40 levels between preeclamptic patients and controls. To clarify the kinetics of this cytokine in preeclampsia, it may be better to assay IL12p70 and p40 subunits from the same sample.

IL-18 is also a pro-inflammatory cytokine that is primarily synthesized by macrophages and monocytes and is considered to have pleiotropic qualities (10). IL-18 and IL-12 synergistically exert their IFN-γ- inducing activities in T cells and NK cells (17). It has been reported that IL-18, without IL-12, regarded as a Th2- inducing cytokine (17, 18). Our finding of enhanced serum levels of IL-18 supports previous observations (10), but an opposite finding has also been reported (19). Elevated serum IL-18 may be due to the activation of monocytes and macrophages as a result of an enhanced maternal response to placental debris.

IL-15, a pro-inflammatory cytokine, was first described as a T-lymphocyte activating factor. It induces T cell proliferation and B cell maturation and is essential for NK cell development and cytotoxicity. IL-15 is expressed in numerous human tissues and cell types (6, 20). Its expression in the human endometrium and decidua (21) and trophoblasts (20) indicates a role for IL-15 in uterine function during pregnancy. Our data confirm the results of a previous study which demonstrated higher serum level of IL-15 in preeclamptic patients compared to normal pregnant women (6). However, two other studies failed to demonstrate any significant difference between the two groups (9, 22).

Unpublished data from our own laboratory showed that IL-15 gene expression in trophoblasts of preeclamptic women was significantly increased relative to normal pregnant women, indicating that the placenta may be the origin of IL-15 production. Our finding of increased levels of IFN γ, IL-12, IL-15 and IL-18 in preeclampsia is consistent with the suggestion that preeclampsia is associated with a greater inflammatory response than what is normally observed during pregnancy (9, 10). Pro-inflammatory cytokines might be among the mediators leading to endothelial dysfunction and thus may be associated with the pathogenesis of preeclampsia (10).

In this study, we did not detect a difference between IL-10 levels in preeclampsia and normal pregnancies. There are several lines of evidence indicating that IL-10 is an important cytokine in pregnancy (23). However, there is controversy regarding the serum levels of IL-10 in preeclamptic patients (1, 9, 24-26). The inconsistency in assaying circulating IL-10 has been raised as a limiting factor in the utilization of IL-10 as a disease marker (1). Some *in vitro* studies have demonstrated decreased production of IL10 by peripheral blood mononuclear cells from preeclamptic patients (12, 27). It should be noted that artificial *in vitro* conditions might affect cytokine responses.

Because of the inflammatory nature of preeclampsia, our finding of increased levels of IL-4 in preeclamtic patients is somewhat surprising. Our results are consistent with those of Omu et al (4) who found that IL-4 serum levels were significantly increased in preeclampsia. It is tempting to speculate that the increased IL-4 levels observed in preeclamptic patients may act as a compensatory mechanism in response to elevated levels of IFN γ, IL-12, IL15 and IL-18. In contrast with the present findings, another study reported that serum concentrations of IL-4 in preeclamptic patients were significantly lower than the control group (3). Those authors sampled blood after delivery and assayed IL-4 in the plasma rather than the serum, which may explain the difference between the two studies. Jonsonn et al (9) reported that although IL-4 was not detectable in a majority of preeclamptic patients and normal pregnant women, sIL4-R levels were enhanced in the former group. They proposed sIL-4R as a surrogate marker of IL-4 because of differences in the proteolytic cleavage of these two molecules.

Clearly there is great variability in serum levels of cytokines in preeclamptic patients in different studies. It should be mentioned that differences in study design, number of subjects, assay sensitivity and gestational age at the time of sampling might all contribute to this variability. Some studies use serum and others use plasma for detecting blood levels of cytokines, this could be an additional source of variability. Because the present study was undertaken in women with established preeclampsia, it is not possible to determine whether the altered cytokine levels represent a cause or consequence of the disease. It would be interesting, therefore, to determine whether serum levels of cytokines are altered prior to the emergence of preeclamptic symptoms. Further studies are required to determine whether measurement of serum cytokines in the first and second trimesters could predict preeclampsia.

In summary, the present data suggest that dysregulation of cytokine expression occurs in preeclampsia with increased levels of IL-12p70, IL-15, IL-18, IFN γ and IL-4. Understanding of the quality and quantity of these cytokines in preeclampsia will have marked clinical impact on decreasing maternal and fetal morbidity and death.

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REFERENCES

1 Makris A, Xu B, Yu B, Thornton C, Hennessy A. Placental deficiency of interleukin-10 (IL-10) in preeclampsia and its relationship to an IL10 promoter polymorphism. Placenta. 2006; 27: 445-51.
2 Azizieh F, Raghupathy R, Makhseed M. Maternal cytokine production patterns in women with pre-eclampsia. Am J Reprod Immunol. 2005; 54: 30-7.
3 Arriaga-Pizano L, Jimenez-Zamudio L, Vadillo-Ortega F, Martinez-Flores A, Herrerias-Canedo T, Hernandez-Guerrero C. The predominant Th1 cytokine profile in maternal plasma of preeclamptic women is not reflected in the choriodecidual and fetal compartments. J Soc Gynecol Investig. 2005; 12: 335-42.
4 Omu AE, Al-Qattan F, Diejomaoh ME, Al-Yatama M. Differential levels of T helper cytokines in preeclampsia: pregnancy, labor and puerperium. Acta Obstet Gynecol Scand. 1999; 78: 675-80.
5 Jonsson Y, Matthiesen L, Berg G, Ernerudh J, Nieminen K, Ekerfelt C. Indications of an altered immune balance in preeclampsia: a decrease in in vitro secretion of IL-5 and IL-10 from blood mononuclear cells and in blood basophil counts compared with normal pregnancy. J Reprod Immunol. 2005; 66: 69-84.
6 Hu W, Wang H, Wang Z, Huang H, Dong M. Elevated serum levels of interleukin-15 and interleukin-16 in preeclampsia. J Reprod. Immunol. 2007; 73:166-71.
7 Wilczynski JR, Tchorzewski H, Banasik M, Glowacka E, Wieczorek A, Lewkowicz P et al. Lymphocyte subset distribution and cytokine secretion in third trimester decidua in normal pregnancy and preeclampsia. Eur J Obstet Gynecol Reprod Biol. 2003; 109: 8-15.
8 Keelan JA, Mitchell MD. Placental cytokines and preeclampsia. Front Biosci. 2007; 12: 2706-27.
9 Jonsson Y, Ruber M, Matthiesen L, Berg G, Nieminen K, Sharma S et Al. Cytokine mapping of sera from women with preeclampsia and normal pregnancies. J Reprod Immunol. 2006; 70: 83-91.
10 Huang X, Huang H, Dong M, Yao Q, Wang H. Serum and placental interleukin-18 are elevated in preeclampsia. J Reprod Immunol. 2005; 65: 77-87.
11 Freeman DJ, McManus F, Brown EA, Cherry L, Norrie J, Ramsay JE et al. Short- and long-term changes in plasma inflammatory markers associated with preeclampsia. Hypertension. 2004; 44: 708-14.
12 Darmochwal-Kolarz D, Leszczynska-Gorzelak B, Rolinski J, Oleszczuk J. T helper 1- and T helper 2-type cytokine imbalance in pregnant women with pre-eclampsia. Eur J Obstet Gynecol Reprod Biol. 1999; 86: 165-70.
13 Daniel Y, Kupferminc MJ, Baram A, Jaffa AJ, Fait G, Wolman I et al. Plasma interleukin-12 is elevated in patients with preeclampsia. Am J Reprod Immunol. 1998; 39: 376-80.
14 Dudley DJ, Hunter C, Mitchell MD, Varner MW, Gately M. Elevations of serum interleukin-12 concentrations in women with severe pre-eclampsia and HELLP syndrome. J Reprod Immunol. 1996; 31: 97-107.
15 Fieschi C, Casanova JL. The role of interleukin-12 in human infectious diseases: only a faintsignature. Eur J Immunol. 2003; 33: 1461-4.
16 Sakai M, Tsuda H, Tanebe K, Sasaki Y, Saito S. Interleukin-12 secretion by peripheral blood mononuclear cells is decreased in normal pregnant subjects and increased in preeclamptic patients. Am J Reprod Immunol. 2002; 47:91-7.
17 Sakai M, Shiozaki A, Sasaki Y, Yoneda S, Saito S. The ratio of interleukin (IL)-18 to IL-12 secreted by peripheral blood mononuclear cells is increased in normal pregnant subjects and decreased in pre-eclamptic patients. J Reprod Immunol. 2004; 61:133-43.
18 Aral M, Arican O, Gul M, Sasmaz S, Kocturk SA, Kastal U et al. The Relationship between Serum Levels of Total IgE, IL-18, IL-12, IFN-gamma and Disease Severity in Children with Atopic Dermatitis. Mediators Inflamm. 2006; 2006: 73098.
19 Adams KM, Mandel LS, Guthrie KA, Atkinson MW. Interleukin-18 in the plasma of women with preeclampsia. Am J Obstet Gynecol. 2003; 188: 1234-7.
20 Agarwal R, Loganath A, Roy AC, Wong YC, Ng SC. Expression profiles of interleukin-15 in early and late gestational human placenta and in pre-eclamptic placenta. Mol Hum Reprod. 2001; 7: 97-101.
21 Okada S, Okada H, Sanezumi M, Nakajima T, Yasuda K, Kanzaki H. Expression of interleukin-15 in human endometrium and decidua. Mol Hum Reprod. 2000; 6: 75-80.
22 Del Gobbo V, Giganti MG, Zenobi R, Villani V, Premrov MG. The immunosuppressive cytokines influence the fetal survival in patients with pregnancy-induced hypertension. Am J Reprod Immunol. 2000; 44: 214-21.
23 Kamali-Sarvestani E, Kiany S, Gharesi-Fard B, Robati M. Association study of IL-10 and IFN-gamma gene polymorphisms in Iranian women with preeclampsia. J Reprod Immunol. 2006; 72: 118-26.
24 Benian A, Madazli R, Aksu F, Uzun H, Aydin S. Plasma and placental levels of interleukin-10, transforming growth factor-beta1, and epithelial-cadherin in preeclampsia. Obstet Gynecol. 2002; 100: 327-31.
25 Madazli R, Aydin S, Uludag S, Vildan O, Tolun N. Maternal plasma levels of cytokines in normal and preeclamptic pregnancies and their relationship with diastolic blood pressure and fibronectin levels. Acta Obstet Gynecol Scand. 2003; 82: 797-802.
26 Hennessy A, Pilmore HL, Simmons LA, Painter DM. A deficiency of placental IL-10 in preeclampsia. J Immunol. 1999; 163: 3491-5.
27 Orange S, Horvath J, Hennessy A. Preeclampsia is associated with a reduced interleukin-10 production from peripheral blood mononuclear cells. Hypertens Pregnancy. 2003; 22: 1-8.