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PLASMA PRO-INFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINE LEVELS IN PREECLAMPSIA

PREEKLAMPSİDE PLAZMA PRO-İNFLAMATUAR VE ANTİ-İNFLAMATUAR SİTOKİN DÜZEYLERİ

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ABSTRACT

Objective: Cytokines appear to contribute to the maintenance of normal pregnancy and development of pathological conditions. The aim of this study was to evaluate plasma cytokine pattern in preeclampsia and whether there is any relationship with disease severity or not.

Materials and methods: Plasma samples of 65 women (25 healthy nonpregnant (NP), 20 normotensive pregnant (NTP) and 20 preeclamptic pregnant (PEP) women) were investigated by means of TNFα, IL-1β, IL-12, IL-4 and IL-10 concentrations. Statistical significance was analysed by Student's t-, Kruskal- Wallis, Mann-Whitney U- and Spearman correlation tests.

Results: In PEP in comparison with NP women, together with elevation of pro-inflammatory TNFα, IL-1β and IL-12 (Th1 cytokines), there is an increase of anti-inflammatory IL-4 and IL-10 (Th2 cytokines). In addition, there is an increase of anti-inflammatory IL-4 and IL-10 levels in PEP in comparison with NTP. Among the preeclamptic group there is a significant positive correlation between IL-1β/IL-10 and TNFα/IL-12; also, a significant negative correlation between IL-4/systolic blood pressure, IL-4/LDH.

Conclusions: The rise of anti-inflammatory cytokines probably is a compensatory mechanism by which IL-4 and IL-10 counteract to pro-inflammatory cytokines, and thus balance their endothelium destroying effects. The presence of negative correlation between anti-inflammatory cytokines and blood pressure or some liver enzymes suggests that there is probably a relationship between plasma cytokines and disease severity. On the other hand, elevated maternal plasma IL-4 and IL-10 concentrations in preeclampsia may be a response to improve altered Th1/Th2 balance and to protect from maternal immunorejection.

Key words: Preeclampsia, Th1/Th2 cytokines, endothelial damage

ÖZET

Amaç: Sitokinler normal gebeliğin sürdürülmesi ve patolojik durumların ortaya çıkmasında önemli rol oynar. Bu çalışmada preeklampside plazma sitokin paternini incelemek ve hastalığın seyri ile bir ilişkisi olup olmadığını değerlendirmek hedeflenmiştir.

Gereç ve yöntem: 25'i sağlıklı ve gebe olmayan (NP), 20'si normotensif gebe (NTP), ve 20'si preeklamptik gebe (PEP) olmak üzere toplam 65 kadının plazma örneklerinde TNFα, IL-1β, IL-12, IL-4 ve IL-10 konsantrasyonları tayin edildi. İstatistiksel değerlendirme Student's t-, Kruskal-Wallis, Mann-Whitney U- ve Spearman korelasyon testleri ile gerçekleştirildi.

Bulgular: NP kadınlara kıyasla, PEP'de pro-inflamatuar ve Th1 sitokinleri olan TNFα, IL-1β, IL-12 artışı ile birlikte anti-inflamatuar ve Th2 sitokinleri olan IL-4 ve IL-10 de artmaktadır. Bununla birlikte, NTP'ye kıyasla PEP'de anti-inflamatuar IL-4 ve IL-10 düzeyleri artmaktadır. Preeklampsi grubunda IL-1β/ IL-10; TNFα/IL-12 arasında pozitif; IL-4/sistolik kan basıncı ve IL-4/LDH arasında ise negatif korelasyon bulundu.

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Sonuçlar: Anti-inflamatuar sitokin artışı pro-inflamatuar sitokinlerin neden olduğu endotel disfonksiyonuna karşı koyan bir kompensatuar mekanizma olabilir. Anti-inflamatuar sitokinler ile kan basıncı veya bazı karaciğer enzimleri arasında bulunan negatif korelasyon, plazma sitokin düzeyleri ve hastalığın seyri arasında bir ilişki olabileceğini ortaya koymaktadır. Bununla birlikte, preeklampside anne plazmasında IL-4 ve IL-10 konsantrasyonlarının artışının, Th1/Th2 sitokin dengesini etkileyerek, maternal imünorejeksiyondan korumaya çalışan bir cevap olduğunu söyleyebiliriz.
Anahtar kelimeler: Preeklampsi, Th1/Th2 sitokinleri, endotel hasarı

INTRODUCTION

Preeclampsia is a disorder peculiar to pregnancy and a major cause of maternal death and damage to the developing baby. Although the exact pathophysiologic mechanisms of preeclampsia remains elusive, studies to date have implicated multiple processes, including the following: abnormal trophoblastic invasion, vasospasm, platelet activation, changes in the relative amounts of prostaglandins, activation of lipid peroxides, and imbalance in the vasomotor-regulating factors (23,26,27,28). Besides the nitric oxide, endothelin-1 and prostaglandins, cytokines are important compounds implicated in diffuse endothelial cell damage and neutrophil activation (23,26). The fact that many gestational tissues such as deciduas, amnion and placenta, produce various cytokines, suggests that cytokines appear to contribute to the maintenance of normal pregnancy and development of pathological conditions (21). On the other hand, cytokines could trigger neutrophil activation and cell adhesion on the endothelium with resultant vascular damage. It was postulated that abnormal production of cytokines and disturbed balance between pro-inflammatory (TNFα, IL-1β, IL-6, IL-8, IL-12) and anti-inflammatory (IL-4, IL-10, IL-13) cytokines yield to vascular damage and preeclampsia (6,9,10). There are conflicting reports in the literature on serum/plasma concentrations of cytokines in preeclampsia. For example, pro-inflammatory TNFα and IL-1β were variously shown to be increased (6,10,17,22) or unchanged (9,15,20) in preeclampsia compared with normotensive pregnancies. However, increased (10), unchanged (6,9,17) or decreased (14) levels of anti-inflammatory IL-10 levels were reported. As a result of this discrepancy, we decided to measure plasma concentrations of pro-inflammatory TNFα, IL-1β and IL-12, and anti-inflammatory IL-4 and IL-10 in women with preeclampsia; and also to investigate whether there is any relationship between above mentioned cytokines and disease severity or not.

MATERIALS and METHODS

The study was approved by the Institutional Review Board at the Istanbul Medical Faculty and informed consent was obtained from each subject. Twenty proteinuric preeclamptic (PEP) women at gestational ages of > 32 weeks were admitted to the Gynecology and Obstetric Department of Istanbul Medical Faculty. Twenty-five healthy nonpregnant (NP) women (control I) and twenty normotensive pregnant (NTP) at similar gestational age (control II) volunteered as controls. Preeclampsia was defined as a blood pressure of > 140/90 mmHg after 30 min rest on two separate readings at least 6 h apart with proteinuria (>1+ on urine testing) and edema. PEP and NTP were primigravida, and the gestational age during the sampling was 35 ± 3 and 36 ± 3 weeks, respectively. Table 1 summarises the clinical characteristics of the NTP and PEP groups. Healthy NP women were 32 ± 3 (range 20 – 39) years old. NP women were not on oral contraceptives and were in the follicular phase of the menstrual cycle at the sampling. All women (control I, II and study groups) were not on any medication, were nonsmokers; were free from cardiovascular, hepatic, renal, endocrine and metabolic disorders. Also, none of the all women had evidence of any active infective process such as urinary tract infection or upper respiratory tract infection. Peripheral venous blood samples were collected in plain tubes for routine biochemical analysis, and in EDTA.K3 tubes for cytokine determination. Plasma and serum samples were placed in 0,5-1 ml portions into eppendorf tubes and kept at - 80^{0}C until used. For plasma cytokine determination commercial enzyme immunoassay kits obtained from Biosource International, Camarilo, California, USA (TNFα and IL-10) and Diaclone Research, Besançon, France (IL-1β, IL-4 and IL-12) were used. The manufacturer's instructions were followed. The sensitivity was: 1,7 pg/ml for TNFα, less than 5 pg/ml for IL-1β; less than 20 pg/ml for IL-12, less than 0,5 pg/ml for IL-4, and less than 1 pg/ml for IL-10. The intra-assay and interassay coefficients of variation (CV) were less than 10% for all. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH), were measured on a chemistry analyzer. For statistical evaluation Student's t-test (for normally distributed data), Kruskal-Wallis test (for not normally distributed data) plus post hoc Mann Whitney U tests were used. Spearman's correlation coefficient was used to test the significance of the relationship between variables.

RESULTS

Median (minimum-maximum) plasma TNFα, IL-1β, IL-12, IL-4 and IL-10 concentrations in nonpregnant controls, normotensive pregnants and women with preeclampsia are presented in Table 2. Cytokine levels in preeclamptic women were significantly increased in comparison with nonpregnant controls. Plasma IL-4 and IL-10 levels in preeclamptic women were elevated also in comparison with normotensive pregnant controls ($p< 0,001$ and $p< 0,0001$, respectively). TNFα and IL-1β levels in normotensive pregnancies were higher than those in nonpregnant controls ($p< 0,0001$). Among the preeclamptic group there was a significant positive correlation between IL-1β/IL-10 ($r= 0.437$; $p< 0.05$),

Table 1. Clinical Characteristics of normotensive (NTP) and preeclamptic (PEP) pregnants. Values are given as mean ± SD (student's t-test).

	NTP	PEP	P VALUE
Maternal age (years)	29 ± 7	28 ± 5	NS
Gestational age (weeks)	36 ± 3	35 ± 3	NS
Systolic Blood Pressure (mmHg)	110 ± 10	150 ± 10	P< 0.0001
Diastolic Blood Pressure (mmHg)	70 ±9	100 ± 10	P< 0.0001
Birth weight (g)	2900 ± 800	2200 ± 600	P< 0.01
Placental weight (g)	515 ± 42	450 ± 30	NS
Platelet count (x103/mm3)	210 ± 80	159 ± 73	NS
AST (U/L)	20 ± 10	70 ± 14	P< 0.0001
ALT (U/L)	15 ± 5	57 ± 7	P< 0.0001
LDH (U/L)	276 ± 58	597 ± 74	P< 0.0001

NS= non significant

Table 2. Plasma TNFα, IL-1β, IL-12, IL-4 and IL-10 (pg/mL) in nonpregnant (NP) healthy women, normotensive pregnant (NTP) women and preeclamptic pregnant (PEP) women. Values are given as median (minimum-maximum) (Kruskal-Wallis and Mann Whitney U tests)

	NP	NTP	PEP
TNFα	22.4 (20.8 – 29.9)	229.3 (20.2 – 34.3)	30.6 (26.8 – 33.9)
		p^a< 0,0001	p^b< 0,0001; NSc
IL-1β	23.5 (20.6 – 24.7)	24.9 (22.0 – 28.00)	25.9 (22.6 – 27.9)
		p^a< 0,0001	p^b< 0.0001; NSc
IL-12	92.8 (85.3 – 138.0)	104.2 (91.1 – 130.3)	111,1 (89.9 – 141.8)
		p^a< 0.05)	p^b<0.01; NSc
Il-4	1.4 (0.5 – 3.5)	1,4 (0.7 – 5.5)	5.2 (1.0 – 17.7)
		NS[a]	p^b< 0.0001; p^c< 0.001
IL-10	14.2 (11.6 – 17.3)	13,6 (10.5 – 17.5)	15.1 (14.3 – 19.0)
		NS[a]	p^b< 0.001; p^c< 0.0001

NS= non significant

a) When NTP were compared with NP
b) When PEP were compared with NP
c) When PEP were compared with NTP

TNFα/IL-12 (r= 0.421; p< 0.05) also, a significant negative correlation between IL-4/systolic blood pressure(r= - 0, 611; p< 0,01), IL-4/LDH (r= -0, 0.522; p< 0,05).

DISCUSSION

Results demonstrate that preeclampsia is associated with significant increase in plasma pro-inflammatory (TNFα, IL-1β and IL-12) and anti-inflammatory (IL-4 and IL-10) cytokines in comparison with nonpregnant women; and increase in plasma IL-4 and IL-10 cytokines in comparison with normotensive pregnants.

TNFα and IL-1β are pro-inflammatory cytokines derived from monocytes, macrophages, epithelial cells and placental tissue (7,21). These cytokines regulate the inflammatory response, modulate cell growth and differentiation. There is evidence that TNFα plays a key role in early pregnancy events and placentation. More specifically, TNFα is expressed in endometrium and first trimester deciduas and is thought to be important in implantation and trophoblast invasion 16,25,30). Our results showing increased TNFα and IL-1β in normal pregnancy compared to nonpregnant state are in accordance with above mentioned postulations.

On the other hand, IL-1β secretion leads to a pro-inflammatory cascade, including production of TNFα, IFNγ, IL-2 and IL-12 (13). In preeclampsia, increased placental expression of cytokines such as IL-1β, are believed to cause elevated cytokine levels (22). Moreover, alteration of serum cytokine balance with predominance of Th1 immunity were observed in preeclampsia (2,8). Th1 cytokines have been shown to be detrimental to pregnancy; TNFα, IL-1β and INFγ bring about the killing of trophoblast cells; they inhibit the implantation of the mouse embrio and the proliferation of human trophoblast in

vitro (12,31). The administration of TNFα, IFNγ, or IL-2 to normal pregnant mice causes abortion (3), while low doses of anti- TNFα reduce resorption rates in a murine model of spontaneous abortion – another pregnancy disorder seemingly to have many common signs with preeclampsia. Recent data have suggested a role for TNFα in insulin resistance, obesity and hyperlipidemia – metabolic disturbances which are also common features associated with preeclampsia as well as cardiovascular disease (18). However, TNFα probably contribute to altered prostaglandin production (27,29) and impaired balance between vasomotor-regulating factors such as nitric oxide and endothelin-1 (28). Chen et al. reported an imbalance between prostacyclin and thromboxane production occurred in mononuclear cells from preeclamptic women (4). Furthermore, elevated levels of TNFα lead to a thromboxane predominance (5).

It is seen from the results, that in preeclamptic pregnants compared with nonpregnant women there is an elevation of pro-inflammatory TNFα, IL-1β and IL-12 (known as Th1 cytokines) as well as there is an increase of anti-inflammatory IL-4 and IL-10 (known as Th2 cytokines). In addition, there is increased IL-4 and IL-10 levels in preeclamptic women in comparison with normotensive pregnants. The rise of anti-inflammatory cytokines probably is a compensatory mechanism, by which IL-4 and IL-10 counteract to pro-inflammatory TNFα, IL-1β and IL-12, and thus balance their endothelium destroying effects. The fact that there is a significant correlation between IL-1β/ IL-10 supports our hypothesis. Moreover, the presense of a negative correlation between anti-inflammatory cytokines and blood pressure or some liver enzymes suggests that probably there is a relationship between plasma cytokines and disease severity. Indeed, it was postulated that IL-10 and IL-4 are potent immunosuppressive Th2 cytokines, which have several functions, including the inhibition of macrophage activity and function, in vivo suppression of cell-mediated immunity; inhibition of nitric oxide and prostaglandin production (1,11,19,24). IL-10 has been found in pregnant women and localized by immunohistologic methods to the interface area between maternal and fetal tissues and expressed at high concentration in placental tissues (19).

Although the role of IL-4 and IL-10 in the pathophiysiology of human gestation has not been properly elucidated, elevated maternal plasma IL-10 and especially IL-4 concentrations in preeclampsia may be a protective response to maternal immunorejection .

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