Research Article [Araştırma Makalesi]

Serum Mast Cell Tryptase, Eosinophil Cationic Protein, Endothelin-1 and Cytokine Levels in Preeclampsia and Healthy Pregnancy

[Preeklampsi ve Sağlıklı Gebelikte Serum Mast Hücre Triptaz, Eozinofilik Katyonik Protein, Endotelin-1 ve Sitokin Seviyeleri]

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ABSTRACT

Aim: Preeclampsia is characterized by both local and systemic changes in cytokine balance and endothelial dysfunction. It was aimed to investigate the possible role of cytokines, endothelin and mast cell in the pathophysiology of preeclampsia in this study.

Material and Methods: Twenty five pregnant women with preeclampsia (group 1), 25 healthy pregnant women (group 2) and 25 healthy non-pregnant women as a control (group 3) were included in the study. The soluble interleukin-2 receptor, interleukin-10, tumor necrosis factor alpha and eosinophil cationic protein were measured by using a non-radioimmunoassay chemiluminescent method, endothelin-1 and myeloperoxidase by using enzyme-linked immunosorbent method and mast cell tryptase by using fluorescent enzyme immunoassay method.

Results: The myeloperoxidase activity was significantly higher in group 1 than in groups 2 and 3 ($p<0.05$). Mast cell tryptase level was significantly lower in groups 1 and 2 than in group 3 ($p<0.05$). Soluble interleukin-2 receptor level was significantly higher in group 2 than in groups 1 and 3 ($p<0.05$). Tumor necrosis factor alpha level was significantly higher in groups 1 and 2 than in group 3 ($p<0.05$). Endothelin-1, interleukin-10 and eosinophil cationic protein levels did not change significantly among the groups.

Conclusion: The result of this study indicated that mast cell was restrained in both preeclampsia and healthy pregnancy. The inflammatory changes might also contribute to the pathogenesis of preeclampsia.

Key Words: endothelin-1, eosinophil cationic protein, mast cell tryptase, preeclampsia

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ÖZET

Amaç: Preeklampsi endotel fonksiyon bozukluğu ve sitokin dengesinde hem bölgesel hem de sistemik değişikliklerle karakterizedir. Bu çalışmada, preeklampsinin patofizyolojisinde mast hücreleri, endotelin ve sitokinlerin muhtemel rolünün araştırılması amaçlanmıştır.

Gereç ve Yöntemler: 25 preeklamptik gebe (grup 1), 25 sağlıklı gebe (grup 2) ve kontrol grubu olarak ise 25 gebe olmayan sağlıklı kadın (grup 3) çalışmaya katılmıştır. Çözünebilir interlökin–2 reseptör, interlökin–10, tümör nekrozis faktör alfa ve eozinofil katyonik protein seviyeleri kemilüminesans yöntemle, endotelin–1 ve miyeloperoksidaz seviyeleri enzim-bağlı immun yöntemle ve mast hücre triptaz seviyesi ise floresan enzim immun ölçüm yöntemiyle belirlenmiştir.

Bulgular: Miyeloperoksidaz aktivitesi grup 1'de, grup 2 ve 3'e göre anlamlı olarak artmıştır ($p<0.05$). Mast hücre triptaz düzeyi ise grup 1 ve 2'de, grup 3'e göre anlamlı olarak azalmıştır ($p<0.05$). Grup 1 ve 3'le karşılaştırıldığında, çözünebilir interlökin–2 reseptör düzeyi grup 2'de anlamlı olarak yüksek bulunmuştur ($p<0.05$). Tümör nekrozis faktör alfa düzeyi ise grup 3'le karşılaştırıldığında, grup 1 ve 2'de anlamlı olarak yüksek bulunmuştur ($p<0.05$). Endotelin–1, interlökin–10 ve eozinofil katyonik protein seviyeleri gruplar arasında anlamlı değişiklik göstermemiştir.

Sonuç: Bu çalışmanın sonuçları, mast hücre aktivitesinin hem preeklampsi hem de sağlıklı gebelikte baskılandığını ortaya koymuştur. İnflamatuvar değişiklikler de preeklampsinin patogenezine katkı yapabilmektedir.

Anahtar Kelimeler: endotelin–1, eozinofilik katyonik protein, mast hücre triptaz, preeklampsi

Introduction

Preeclampsia is a common obstetric syndrome affecting about 4-5 % of pregnant women and causing significant morbidity and mortality in the fetus, newborn infant and mother in both developed and developing countries (1). Although the etiology of the disease is unknown, it has been suggested that a consequence of placental ischemia is the generation of cytotoxic factors that may act systemically to activate or injure the endothelium (2).

Cytokines may be thought as pre-inflammatory, immunosuppressive, or growth promoting factors (3). Altered levels of cytokines have been measured in the circulation of women with preeclampsia, although for reasons that are not always apparent much of the data are disturbingly inconsistent. While the placenta undoubtedly makes an important contribution to plasma cytokine levels, production by maternal peripheral blood mononuclear cells (PBMCs) and other tissues is also likely to be significant, although to what extent remains undetermined (4).

It remains unclear what the trigger is to activate monocytes in preeclampsia (5). Neutrophils are considered to be major effector cells in the tissue damage that occurs in inflammatory disease (6). One of the major granule proteins is the myeloperoxidase (MPO), a heme protein that accounts for 5 % of the total neutrophil protein (7).

Mast cells and eosinophils may also contribute to the inflammatory reaction, which may damage the surface epithelium and release vasoactive substances that allow leakage of serum antibodies into the uterine secretions (8). It was hypothesized that placental mast cells may also have an important role in normal and/or pathological processes during pregnancy. It is postulated that preeclampsia reflects an inflammatory-type reaction, in which mast cell-mediated events play a significant role. Mast cell tryptase (MCT), a neutral protease, is the major protein component of mast cell secretory granules. The secondary granules of human eosinophils contain several proteins; one of them is eosinophil cationic protein (ECP), and its value as a biomarker in patients with eosinophil-associated diseases (9). However, ECP is also present in human neutrophils (10). Therefore, in some instances the elevated levels of these proteins may be a consequence of their release from neutrophils (11).

Endothelins (ET) are a family of peptides produced by endothelial and vascular smooth muscle cells. They act locally to modulate vasomotor tonus, cell proliferation and hormone production. Of the three members identified, the only one produced by the endothelium is endothelin-1 (ET-1). Hypoxia, ischemia and hydrodynamic stress lead to induction of a variety cytokines, resulting in production of ET-1 (12). It has been shown that maternal plasma ET-1 levels increase in preeclampsia and correlate with the severity of the disease (13).

Understanding how and why endothelial cells become dysfunctional during preeclampsia is essential for the development of effective therapeutic strategies for this serious and prevalent disorder of human pregnancy. For this aim, this study was designed to test the possible role and association of cytokines, endothelin and inflammatory markers in the pathophysiology of preeclampsia by comparing plasma soluble interleukin-2 receptor (sIL-2R), interleukin-10 (IL-10), tumor necrosis factor alpha (TNF-α), ET-1, ECP and MCT levels, and activity of MPO in preeclamptic and normotensive pregnant women in the third trimester.

Material and Method

The investigation was performed over a period of 6 months from August 2007 to February of 2008. A total of 50 women in the third trimester of their pregnancy, 25 pregnant women with preeclampsia (group 1) and 25 normotensive pregnant women (group 2), attending the antenatal clinic or admitted to the maternity ward of Yuzuncu Yil University Medical Faculty Department of Gynecology and Obstetric, Van, were enrolled in this prospective study. Twenty-five non-pregnant healthy women were also selected as controls (group 3). The institutional ethics committee approved the study and all participants gave written informed consent.

Preeclampsia was defined as systolic and diastolic blood pressure (BP) greater than 140 mm Hg and 90 mm Hg, respectively, with significant proteinuria (>300 mg per 24 h or a dipstick reading of ++ for 4 hours or longer) and no fundoscopic findings with hypertensive retinopathy after 20th gestational weeks (14). All women with hypertensive disease of pregnancy who hospitalized and delivered in the hospital were considered as candidates for this study. A detailed patient history was taken and a physical examination performed. Blood pressure was measured in the left arm with a sphygmomanometer. Previous renal disease, any infection diseases, secondary causes of hypertension and the use of any drug known to have an effect on blood pressure were taken as exclusion criteria. Subjects with hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome were also excluded from the study. For the normotensive pregnant women the exclusion criteria were the same as for the study group.

Fasting venous blood samples were taken from all subjects. All blood samples were drawn into two different test tubes; one of which includes no anticoagulant substance for measuring biochemical parameters and other one containing ethylene diamine tetra-acetic acid (EDTA) for measuring hematological parameters. The tubes were centrifuged for 10 min at 1800 x g. The serum samples were stored at –70 °C until they were analyzed. Urinalysis was performed to estimate the degree of proteinuria.

Serum sIL-2R, IL-10, TNF-α and ECP levels were measured by two-site chemiluminescent immunometric as-

say. MCT measurement was performed by fluorescent enzyme immunoassay. ET-1 and MPO levels were determined using enzyme-linked immunosorbent assay (ELISA) method. The other biochemical measurements were also performed by colorimetric methods.

Statistical Analysis

The results are expressed as mean ($\overline{X}$) ± Standard deviation (SD). Firstly, Kolmogorov-Smirnov normality test was used to test for normality assumption of the variables. And it was seen that some variables showed normal distribution. One-way ANOVA was employed to compare group means. In addition to ANOVA, Tukey test was used as multiple comparison tests. For the other variables, the suspicious of the violation of parametric methods was viewed. Thus Kruskal-Wallis test which is used for comparing k independent samples was used. In addition to mean and Standard deviation, Median was given for these variables. Then, Pearson correlation analysis was used to determine the relationships among the some variables.

Results

The descriptive statistics of the groups are given in Table 1. Increased body mass index (BMI) (p<0.01) and white blood cell (WBC) (p<0.05) and decreased levels of hemoglobin (p<0.05) were found in both groups 1 and 2 than group 3. The means of systolic and diastolic BP and urinary protein levels and activities of aspartate transaminase (AST), alanine transaminase (ALT), lactic dehydrogenase (LDH) in group 1 were found significantly higher than in groups 2 and 3 (p<0.01), but there was no significant difference between the means of groups 2 and 3. Decreased platelet count was found in group 1 as compared to group 3 (p<0.05). The eosinophil number was also similar among the groups.

Mean±SD age was 32.33 ± 5.85 years (range, 22-41 years) in the group 1 vs. 29.63 ± 6.00 years (range, 20-42 years) in the group 2, and 29.33 ± 5.15 years (range, 23-43 years) in the group 3 (p>0.05).

Gestational age was 34.66 ± 2.45 (range, 30-39 weeks) in the group 1 vs. 35.63 ± 2.10 weeks (range, 31-38 weeks) in the group 2 (p>0.05).

The comparison levels of sIL-2R, IL-10, TNF-α, ET-1, ECP, MCT levels and MPO activity of all groups are presented in Table 2. Significantly increased sIL-2R levels were obtained in the group 2 as compared to the groups 1 and 3 (p<0.05), but there was no significant difference between the levels in the groups 1 and 3. TNF-α levels significantly increased in the groups 1 and 2 as compared to group 3 (p<0.05), and there was a significantly difference between the groups 1 and 2 (p<0.05). MPO activity was significantly higher in group 1 than in groups 2 and 3 (p<0.05), and there was no significantly difference between the groups 2 and 3. MCT level was significantly decreased in the groups 1 and 2 as compared to group 3 (p<0.05). ET-1, IL-10 and ECP levels were similar among the groups. The correlations between the parameters as presented in Table 3. As seen in the Table 3, it was found significant correlations between the TNF-α and ECP (p<0.05, r=0.447) in group 1, sIL-2R and ET-1 (p<0.05, r= 0.478), IL-10 and TNF-α (p<0.05, r=0.598) and MCT and ET-1 (p<0.05, r=0.499) in the group 2, and also sIL-2R and IL-10 (p<0.05, r=0.473), TNF-α and ECP (p<0.05, r=0.426), and MCT and ECP (p<0.01, r=0.556) in the group 3.

Table 1. Clinical and biochemical characteristics of groups

	Group 1		Group 2		Group 3	
Parameters	Median	$\overline{X}$ ±SD	Median	$\overline{X}$ ±SD	Median	$\overline{X}$ ±SD
Age (y)	35.00	32.33 ± 5.85	28.00	29.63 ± 6.00	25.00	29.33 ± 5.15
Gestational age (wk)	34.00	34.66 ± 2.45	38.00	35.63 ± 2.10		
Systolic BP (mmHg)	160.00	160.0 ± 30.13[a**,b**]	120.00	120.45 ± 10.60	115.00	115.00 ± 11.85
Diastolic BP (mmHg)	90.00	96.67 ± 19.01 [a**,b**]	80.00	75.91 ± 5.90	70.00	69.09 ± 8.68
BMI (kg/m²)	28.13	28.86 ± 3.14 [a**]	27.55	27.52 ± 2.81 [a**]	22.55	22.45 ± 1.42
AST (U/L)	29.00	51.33 ± 48.34 [a**,b**]	17.00	17.24 ± 3.99	18.50	19.59 ± 4.19
ALT (U/L)	21.00	29.00 ± 23.61 [a**,b**]	10.00	14.95 ± 4.55	15.50	17.55 ± 8.02
LDH (U/L)	640.00	781.07 ± 434.10 [a**,b**]	405.00	439.62 ± 142.80	359.00	348.41 ± 58.10
Thrombocyte (x10⁹/L)	145.00	193.26 ± 111.80 [a*]	241.00	237.09 ± 41.20	252.50	263.73 ± 75.37
WBC (x10⁹/L)	10.80	11.26 ± 2.95 [a*]	10.40	11.85 ± 4.94 [a*]	7.25	7.55 ± 2.62
Eosinophil (%)	0.10	0.15 ± 0.20	0.09	0.10 ± 0.05	0.09	0.10 ± 0.05
Hemoglobin (g/dL)	12.80	12.30 ± 2.52 [a*]	12.70	12.31 ± 1.64 [a*]	13.90	13.62 ± 1.36
Proteinuria (mg/L)	303	353.70 ± 52.22 [a**,b**]	20.4	22.50 ± 10.85	5.7	7.95 ± 3.42.

Significantly different: [a]; as compared to group 3, [b]; as compared to group 2, *p<0.05, **p<0.01

Table 2. The levels of ET-1, sIL-2R, TNF-α, IL-10, ECP, MCT and activity of MPO in the groups. ET-1; endothelin-1, sIL2-R; soluble interleukin-2 receptor, TNF-α; tumor necrosis factor alpha, IL-10; interleukin-10, MPO; myeloperoxidase, MCT; mast cell tryptase, ECP; eosinophil cationic protein.

Parameters	Group 1 X̄ ± SD	Group 2 X̄ ± SD	Group 3 X̄ ± SD
ET-1 (pg/mL)	17.53 ± 2.54	12.15 ±1.17	14.65 ± 1.81
sIL-2R (U/mL)	363.91 ± 28.25 [b*]	469.00 ± 41.26 [a*]	384.63 ± 19.91
TNF-α (pg/mL)	14.17 ± 0.66 [a*,b*]	17.59 ± 1.56 [a*]	8.86 ± 0.51
IL10 (pg/mL)	2.26 ± 0.23	2.26 ± 0.29	1.76 ± 0.22
MPO (U/L)	75.96 ± 9.20 [a*,b*]	41.08 ± 5.85	28.92 ± 5.78
MCT (μg/L)	2.91 ± 0.27 [a*]	2.59 ± 0.30 [a*]	4.46 ± 0.41
ECP (ng/mL)	12.82 ± 1.05	13.08 ± 0.93	13.72 ± 1.41

Significantly different: [a]; as compared to group 3, [b]; as compared to group 2, *$p<0.05$, **$p<0.01$

Table 3. Pearson correlation coefficient (r) among the variables. ET-1; endothelin-1, ECP; eosinophil cationic protein, sIL2-R; soluble interleukin-2 receptor, TNF-α; tumor necrosis factor alpha, IL-10; interleukin-10, MCT; mast cell tryptase.

Parameters	Group 1 r	Group 2 r	Group 3 r
TNF-α-ECP	0.447*		0.426*
sIL2-R- ET-1		0.478*	
IL-10- TNF-α		0.598*	
MCT- ET-1		0.499*	
IL-10- sIL2-R			0.473*
MCT- ECP			0.556**

*$p<0.05$, **$p<0.01$

Discussion

The vascular endothelium plays an important role in the regulation of vascular tone, coagulation and fibrinolysis, and inflammatory response to internal and external stimuli (15). It was reported that significant differences in endothelial morphology between arteries from normal pregnant women and those from women with preeclampsia (16).

Generalized maternal endothelial cell dysfunction may explain the multisystemic nature of preeclampsia. There were controversial reports regarding to ET-1 levels in preeclampsia. Serum ET-1 levels were similar among the subjects in the present study. The contrast to our study, Slowinski et al. (17) have reported increased ET-1 levels in preeclampsia. Ariza et al. (18) have also reported that placental expression of ET-1 did not change in preeclampsia. It was concluded that the main source high plasma levels of ET-1 in preeclampsia is placenta (19). Hakkinen et al. (20) suggested ET-1 is mainly released from the placenta during delivery, because of much higher concentrations of ET-1 in the retroplacental blood in maternal plasma and cord blood. The different conditions such as measurement method, sample variety, pregnancy weeks might account for this discrepancy.

The present study demonstrates that preeclampsia and healthy pregnancy characterized to increased serum TNF-α level. However, serum sIL-2R level was decreased in preeclampsia. The main source for elevated cytokines might include leukocytes, which are at a heightened activation state during preeclampsia (21). It was also claimed that the other source of cytokines in preeclampsia is placenta, but it does not contribute significantly to the elevated circulating levels in the disease (22). The reports concerning cytokine levels are somewhat more confusing (23, 24). It should be noted, however, that several studies have failed to find elevated circulating TNF-α and IL-2 levels in preeclampsia (25, 26). In healthy pregnant women, TNF-α is thought to modulate the growth and invasion of trophoblasts in maternal spiral arteries. TNF-α may contributes to abnormal placental invasion, endothelial cell damage (27). However, sIL-2R mediates the action of IL-2, an immune system growth hormone. In opposition to our study, Kaleli et al. have reported (28) sIL-2R levels increased in women with severe preeclampsia. The serum IL-2 level was not measured in the present study. But, increased IL-2 levels were reported in previous studies (26, 29). The increased IL-2 levels also might be regulates the number of receptor on the cell membrane, and therefore, cellular uptake of IL-2.

This study has also shown that serum IL-10 levels were not different among the groups. IL-10 is an anti-inflammatory cytokine (4). It has been shown that circulating IL-10 is lower (30), higher (31) and not different (32) in women with preeclampsia compared with normal pregnant subjects. The relative contribution of fetoplacental and maternal sources to circulating cytokine levels in pregnancy remains an important but unanswered question. The difference results regarding to cytokine levels in pregnancy might be associated various reasons: The function of cytokine network at the maternal–fetal interface and pathogenesis of preeclampsia are far from being completely understood. In addition, sample handling might cause different results. Generally, measurements

of cytokine levels were performed in serum. The serum may not be ideal biologic compartment for evaluating cytokines since cytokines take effect in a paracrine manner and have short serum half life (26). The preeclamptic and healthy pregnant women had increased BMI index in the present study. This observation on BMI index might be another possible reason of difference on inflammatory marker levels.

Previous studies show that pregnancy was associated with a systemic inflammatory response and that pregnancy neutrophils exhibit markers of activation (33, 34). In the present study, significantly increased MPO activity and WBC count were found in both preeclampsia and normal pregnancy compared with non pregnant women and the highest MPO activity was observed in preeclamptic group. It was also claimed that expression of MPO may be an easy and useful indicator of the status of pregnancy (34). We think that increased MPO activity in healthy pregnant and preeclamptic women may represent a compromise to decrease the potentially dangerous levels of oxidants that could be produced by a fully activated leukocyte.

Mast cells play a key role in allergic reactions and increase in numbers under inflammatory conditions. Extracellular tryptase, when present, may be a marker of mast cell activation in disease (35). The serum MCT level was found significantly decreased both preeclampsia and normal pregnancy in the present study. The contrast to this finding, Fineschi et al. (36) showed increased tryptase-positive mast cells in pregnancy. Interestingly, increased MPO activity and decreased MCT level was observed in preeclamptic group in the present study. This observation could be explained to result of a study that reported by Cregar et al. (37). These authors reported that MPO inhibits human mast cell tryptase in a time-dependent manner. It is the native protein conformation of MPO and not its enzyme activity that is responsible for tryptase inhibition. Heparin, at high concentrations, can prevent the inhibition of tryptase by MPO. It was suggest that MPO inhibits tryptase by interfering with the heparin stabilization of tryptase tetramer (37). However, it is difficult to fully explain the discrepancy in serum MCT levels of that report and ours. There were found significant positive correlation between the MCT and ET-1 in the normal pregnant group and also MCT and ECP in control subjects in this study. The role of ET-1 in these mast cells remains unclear. ET-1 activity on the myometrium may be modulated by the proteases produced by the mast cells. Thus, Uchide et al. (38) have reported that ET-1-positive mast cells were found mainly in intact myometrium but not in or around inflammatory lesions caused by removal of the placenta at parturition. These authors suggested that ET-1 may participate via the mast cells in myometrial recovery rather than in inflammation.

Results from this study did not show statistically significant differences between the serum ECP levels in preeclamptic and normal pregnant subjects compared to normal nonpregnant subjects. As similar to our results, Borrego et al. (39) reported that serum ECP levels were not different between the nonpregnant and normal pregnant subjects. However, findings from a previous study of 20 sera obtained during normal pregnancy demonstrated that ECP level was decreased significantly compared with levels in normal nonpregnant sera (40). The reasons for the different results in the current study compared to the previous study are not known. There was no any finding that investigated serum ECP levels in preeclamptic subjects. Therefore, further studies comparing MCT activity and ECP levels in sera from preeclampsia and normal pregnancy are necessary to confirm these results and determine the significance of the observations.

In conclusion, this study showed the preeclamptic women had increased TNF-α level and MPO activity. However, sIL-2R level increased in alone healthy pregnant women. Accompanying these findings was the fact that serum MCT level decreased in both preeclampsia and healthy pregnancy. Serum IL-10, ET-1 and ECP levels were not also different between women with preeclampsia, normal pregnancy, and non-pregnant women. Our results also confirmed that mast cell and eosinophil activation were not contributed to the pathophysiology of preeclampsia and inflammation markers are also associated with preeclampsia.

Acknowledgments

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