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Correlation between Calprotectin and Oxidized LDL in Preeclampsia

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Aim: To evaluate the relationship between serum calprotectin and oxidized low density lipoprotein (LDL) in preeclamptic patients. Additionally, we aimed to measure serum malondialdehyde (MDA) levels as an oxidative stress marker.

Materials and Methods: Serum calprotectin, oxidized LDL, and MDA levels were measured in a cross-sectional study of 61 preeclamptic patients (39 severe preeclampsia and 22 mild preeclampsia cases) and 20 healthy pregnant subjects. Serum MDA concentration was measured with spectrophotometric methods, and oxidized LDL and calprotectin were measured via enzyme-linked immunoassay (ELISA).

Results: Serum calprotectin ($P < 0.0001$ and $P = 0.014$) and MDA ($P = 0.001$ and $P = 0.028$) levels were significantly higher in the severe and mild preeclampsia groups than in the healthy control group. Serum oxidized LDL concentration was slightly higher, but not did reach a statistically significant level, in the severe preeclampsia group than in the healthy pregnant group ($P = 0.2$). There was a significant negative correlation between the levels of calprotectin and oxidized LDL ($r = –0.34$, $P = 0.006$) in the mild and severe preeclampsia groups. The correlation between calprotectin and MDA was not statistically significant ($P = 0.08$).

Conclusions: The findings suggest that increased calprotectin is associated with preeclampsia. Further study is necessary in order to determine whether or not high calprotectin levels prevent oxidation of circulating LDL in preeclampsia cases.

Key Words: Calprotectin, oxidized LDL, preeclampsia

Preeklampside Kalprotektin ve Okside LDL Arasındaki İlişki

Amaç: Bu çalışmada preeklamptik hastalarda serum kalprotektin ve okside düşük dansiteli lipoprotein (LDL) arasındaki ilişki araştırıldı. Ayrıca oksidatif stress markeri olarak serum malondialdehit (MDA) düzeylerinin ölçümü yapıldı.

Yöntem ve Gereç: Bu amaçla 61 preeklamptik hasta (39 şiddetli preeklampsi ve 22 hafif preeklampsi hastası) ve 20 sağlıklı gebe kadında (kontrol) serum kalprotektin ve okside LDL konsantrasyonları ELISA metodu ile, MDA konsantrasyonu ise spektrofotometrik metodla ölçüldü.

Bulgular: Şiddetli ve hafif preeklampsi gruplarında serum kalprotektin ($P < 0,0001$ ve $P = 0,014$) ve MDA ($P = 0,001$ ve $P = 0,028$) konsantrasyonları kontrol grubuna gore anlamlı olarak yüksekti. Kontrol grubuna göre şiddetli preeklampsi grubunda, serum okside LDL konsantrasyonlarında hafif (fakat istatistiki olarak önemli olmayan) bir artış vardı. Total preeklampsi grubunda kalprotektin ile okside LDL arasında önemli ve negatif bir korelasyon gözlendi ($r = –0,34$, $P = 0,006$).

Sonuç: Çalışmadan elde edilen sonuçlar kalprotektin düzeylerinin artışının preeklampsi ile birlikte olabileceğini göstermektedir ve bu hastalarda kalprotektin artışının dolaşımda bulunan LDL moleküllerinin oksidasyonunu önleyip önlemediği araştırılmalıdır.

Anahtar Sözcükler: Kalprotektin, okside LDL, preeklampsi

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Introduction

Preeclampsia is a common disease of human pregnancy and a leading cause of both maternal morbidity and neonatal mortality (1). Inadequate or failed trophoblast invasion and consecutively deficient remodeling of spiral arteries are associated with preeclampsia. Numerous theories on the pathophysiological mechanisms are currently discussed, but the etiology and pathogenesis of preeclampsia are not fully understood. In the pathogenesis of preeclampsia, endothelial dysfunction and inflammation appear to play major roles. An increased level of pro-inflammatory cytokines in the circulation of

preeclamptic women, and maternal circulating activated leukocytes and monocytes may contribute to the processes (2,3).

Additionally, reduced invasion of the trophoblast into the uterus and its spiral arteries are characteristic of this disorder; thus, blood supply to the placenta is greatly reduced. This situation leads to placental hypoxia, followed by placental oxidative stress and dysfunction. If the level of reactive oxygen species (ROS) exceeds the antioxidant capacity or if antioxidant levels decline, which may be associated with preeclampsia (4,5), then oxidative stress will occur. ROS increase the levels of lipid peroxidation products, namely MDA. Lipid peroxidation may cause peroxidative damage to the vascular endothelium and this situation may be associated with clinical symptoms in preeclampsia (6). Increased oxidative stress may lead to LDL oxidation, and oxidized LDL contributes to endothelial injury and, in turn, dysfunction in preeclampsia. In preeclampsia, increased (7,8) normal (9), or low (10) serum oxidized LDL levels have been reported.

Inflammation plays a crucial role in the pathogenesis of preeclampsia. Inflammatory markers, such as C-reactive protein, are associated with preeclampsia (11). Calprotectin, a protein composed of 2 subunits of 8 and 14 kD, respectively, is released by neutrophils in the biological fluids under inflammatory states. Calprotectin appears to play a regulatory role in the inflammatory process and functions in both an antimicrobial and antiproliferative capacity (12). Studies related to serum calprotectin report that calprotectin levels were higher in preeclampsia patients than in normal pregnant women (13,14). The aim of the present study was to investigate the relationship between serum calprotectin and oxidized LDL in preeclamptic patients.

Materials and Methods

The case-control study included 61 preeclamptic patients (39 severe preeclampsia and 22 mild preeclampsia cases) and 20 healthy pregnant subjects. Healthy pregnant subjects were matched with the preeclamptic patients on the basis of maternal age and gestational age at the time blood was drawn. None of the women were current smokers. Preeclampsia was defined as the development of hypertension with proteinuria (15). Hypertension was defined as either systolic blood pressure greater than 140 mmHg or diastolic blood pressure greater than 90 mmHg on 2 consecutive measurements at least 6 h apart. Proteinuria was defined as excretion of greater than 300 mg of protein in a 24-h urine collection. Preeclampsia was classified as either mild or severe. Patients with preeclampsia were considered to have mild preeclampsia unless they developed any of the following (in which case they were classified as having severe disease): blood pressure greater than 160/110 mmHg on 2 consecutive measurements 6 h apart or greater than 5 g of protein excreted in a 24-h urine sampling. Written informed consent was obtained from all the participants, according to the criteria of the Atatürk University Medical Faculty Ethics Committee.

Venous blood samples were collected into tubes without anticoagulant. Serum was separated by centrifugation after clotting and stored at –80 °C until assayed. Serum MDA levels were determined spectrophotometrically, according to the method described by Ohkawa (16). A mixture of 8.1% sodium dodecyl sulfate, 20% acetic acid, and 0.9% thiobarbituric acid was added to 0.2 ml of serum, and then distilled water was added to the mixture to bring the total volume up to 4 ml. This mixture was incubated at 95 °C for 1 h. Following incubation, the tubes were left to cool under cold water and 1 ml of distilled water plus 5 ml of n-butanol/pyridine (15:1, v/v) were added, followed by mixing. The samples were centrifuged at 4000 g for 10 min. The supernatants were removed, and absorbance was measured with respect to a blank at 532 nm. Lipid peroxide levels were expressed as µmol/l. Calprotectin and oxidized LDL levels were measured with an Immunodiagnostik AG (Bensheim, Germany) ELISA kit, according to manufacturers' recommendations.

Statistical Analysis

As the variances were not homogeneous, nonparametric statistical methods were used. To compare nonparametric variables among the 3 study groups, Kruskal-Wallis analysis was performed. The Mann-Whitney U-test was used as a post hoc test. Spearman's rank order correlation testing was performed to calculate correlation coefficients. All statistical analyses were performed using SPSS v.10.0.

Results

The clinical characteristics of the 3 study groups are described in Table 1. The severe preeclampsia group had significantly higher systolic and diastolic blood pressure than the mild preeclampsia ($P < 0.0001$ for both) and healthy control groups ($P < 0.0001$ for both). Serum calprotectin ($P < 0.0001$ and $P = 0.014$) and MDA ($P = 0.001$ and $P = 0.028$) levels were significantly higher in the severe and mild preeclampsia groups than in the healthy control group (Table 2). Serum oxidized LDL concentrations were slightly higher in the severe preeclampsia group than in the healthy pregnant group, but the difference was not statistically significant ($P = 0.2$). There were no significant differences between the severe and mild preeclampsia groups with respect to oxidized LDL. There was a significant negative correlation between the levels of calprotectin and oxidized LDL ($r = –0.34$, $P = 0.006$) in combined preeclampsia group (mild and severe preeclampsia). The correlation between calprotectin and MDA was not statistically significant ($P = 0.08$).

Discussion

In the present study normal levels of oxidized LDL, and elevated calprotectin and MDA were observed in the severe and mild preeclamptic patients, as compared to the control subjects. Additionally, the results show a negative correlation between calprotectin and oxidized LDL in preeclamptic patients.

Some investigators have determined elevated levels of oxidative stress markers, such as MDA, in preeclamptic patients that are consistent with the present study' findings (7,17,18). The causes of increased oxidative stress in these patient groups are variable. One of the causes may be neutrophil activation. Neutrophils play an important role in the innate immune response to infection in mammals. Stimuli, such as infection and injury, induce the activation of neutrophils, which respond via phagocytosis and the release of antimicrobial peptides. Although mild neutrophil activation is known to occur during normal pregnancy, neutrophil activation is greater in preeclamptic pregnancies than in normal pregnancies (19,20). Mellembakken et al. (21) showed that

Table 1. Clinical characteristics of the study groups.

	Severe preeclampsia (n = 39)	Mild preeclampsia (n = 22)	Normal pregnancy (n = 20)
Gestational age (weeks)	36.84 ± 1.61	36.77 ± 1.90	36.70 ± 2.00
Maternal age (years)	26.49 ± 4.81	26.59 ± 4.90	25.20 ± 4.06
Systolic blood pressure (mmHg)	172.95 ± 9.92[1]	145.00 ± 4.88[1]	121.44 ± 6.71
Diastolic blood pressure (mmHg)	112.44 ± 5.95[1]	95.91 ± 4.79[1]	65.50 ± 9.45

Results are given as mean ± SD. [1]$P < 0.0001$ compared to the normal pregnancy group.

Table 2. Serum calprotectin, oxidized LDL, and MDA concentrations in the study groups.

	Severe preeclampsia (n = 39)	Mild preeclampsia (n = 22)	Normal pregnancy (n = 20)
Calprotectin (ng/ml)	2006.00 ± 1177.60[1]	1677.49 ± 1222.09[2]	772.14 ± 451.42
Oxidized LDL (ng/ml)	197.08 ± 55.85	189.22 ± 52.46	178.61 ± 54.04
MDA (μmol/l)	3.99 ± 1.52[3]	3.91 ± 1.86[4]	2.67 ± 1.11

Results are given as mean ± SD. [1]$P < 0.0001$; [2]$P = 0.014$; [3]$P = 0.001$; [4]$P = 0.028$ compared to the normal pregnancy group.

expression of neutrophil activation markers was enhanced on cells from the uterus of preeclampsia patients. It is well known that various ROS and metabolites are generated during the respiratory burst of activated neutrophils. ROS produced by neutrophils may affect vascular tone directly by contracting smooth muscle tissue (22).

Calprotectin is present in high concentrations in neutrophils and can be released by activated cells at inflammatory sites (23-25). The heterocomplex consists of 2 proteins—calgranulin A and calgranulin B. Calprotectin plays a role in various physiological functions, such as inflammatory processes and antiproliferation of cells, as well as in the defense of neutrophils against bacterial infections. Elevated calprotectin levels have been observed at many sites of inflammation and in the extracellular fluid of patients with many types of inflammatory conditions, including rheumatoid arthritis (26) and ulcerative colitis (27). It may also be an important mediator, along with multiple regulatory functions, in inflammatory reactions such as preeclampsia. In the present study, while serum oxidized LDL levels were normal, calprotectin levels were higher in both preeclampsia groups than in the healthy pregnant group; in the combined preeclampsia group there was a negative correlation between calprotectin and oxidized LDL.

Furthermore, in a recent study on protein mapping in LDL, calgranulin A, a component of calprotectin, was detected in LDL and it was speculated that calgranulin A might play a role as a natural scavenger in LDL, preventing LDL oxidation (28). Here, we can now ask this question: is calprotectin also a circulatory antioxidant? The probable antioxidant role of this protein complex awaits further investigation.

In the present study, in contrast to MDA, no modifications were observed in the level of oxidized LDL in any of the study groups. Oxidized LDL cholesterol serves as a marker of both systemic and vascular oxidative stress, and inflammation. Importantly, modified LDL itself is able to induce inflammation, as it induces adhesion to endothelial cells and the oxidative potential of cells in the vascular wall, thus influencing early atherogenesis (29,30).

Studies of serum oxidized LDL levels in preeclampsia are inconsistent; some investigators reported significantly higher levels in preeclamptic women than in healthy pregnant women (7,8), whereas Belo et al. (9) did not observe any differences in plasma levels of oxidized LDL. Nonetheless, in the present study the oxidized LDL to LDL-cholesterol ratio was significantly higher in the preeclampsia group than in the normal pregnancy group, which suggests that the proportion of LDL that was oxidized was higher. On the other hand, Rajimekers et al. (10) reported that women with preeclampsia had lower oxidized LDL levels than healthy pregnant women that were matched according to gestational age. Additionally, they did not observe any differences in MDA concentration between the 2 groups. In contrast to some previous reports, in the present study serum oxidized LDL levels in the preeclamptic women were not different than those in the controls. Interestingly, calprotectin and oxidized LDL were negatively correlated, which could mean that normal oxidized LDL levels were due to an increase in calprotectin.

In conclusion, in preeclamptic patients serum calprotectin levels increased and oxidized LDL was negatively correlated with calprotectin. Further detailed studies are necessary to determine if calprotectin can prevent oxidation of circulating LDL molecules.

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