Plasma Calprotectin Levels in Preeclamptic Normotensive Pregnant and Nonpregnant Women

Sedat AKÇUM, Ebru ZÜLFİKAROĞLU, Süleyman ESERDAĞ, Utku ÖZCAN

Ankara, Turkey

OBJECTIVE: To compare the changes in plasma calprotectin levels in women with preeclampsia, late normal pregnancy and nonpregnant women.

STUDY DESIGN: A total of 30 preeclamptic patients were included in the study, 30 normotensive uncomplicated pregnant patients and 20 nonpregnant patients were taken as control groups. Plasma levels of calprotectin were analyzed using an enzyme-linked immuno-sorbent assay.

RESULTS: Significantly higher levels of maternal plasma calprotectin in preeclamptic group were discovered 783 (478- 928) µg/L, compared to normotensive pregnant control 618 (343- 887) µg/L and the nonpregnant women 574 (283- 797) µg/L (P=0.001). Further, plasma level of calprotectin was significantly higher in severe preeclampsia 954 (691- 985) µg/L than mild preeclampsia 589(492- 712) µg/L (p=0.037). Compared with pregnant control, the plasma level of mild or severe preeclampsia was significantly elevated (p= 0.047 and 0.012, respectively).

CONCLUSION: Elevated maternal plasma calprotectin level was demonstrated in preeclampsia suggesting that preeclampsia may represent an excessive maternal inflammatory response to pregnancy and calprotectin may contribute the pathogenesis of this disorder.

Key Words: Preeclampsia, Calprotectin, Maternal plasma

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Introduction

Preeclampsia is a worldwide leading cause of morbidity and mortality in pregnancy characterized clinically by new onset hypertension (HT), proteinuria and increased maternal and feto-maternal vascular resistance.[1] Endothelial dysfunction and insufficient trophoblast invasion play the major role in the pathogenesis of preeclampsia. In addition, preeclampsia appears to be associated with altered leukocyte activation and cytokine production.[2]

The etiology of preeclampsia relates to reduced invasion of the trophoblast into the uterus and its spiral arteries. This insufficient invasion may cause an inflammatory response in preeclampsia.[3] Redman et al proposed that normal pregnancy induces inflammatory changes in peripheral blood leukocytes, and that preeclampsia is associated with a further augmentation of these inflammatory responses.[4]

Calprotectin is a protein found in myelomonocytic cells. The antimicrobial activities of calprotectin against bacteria and fungi is mediated by zinc chelation.[5] In this way, it also inhibits the activity of zinc-dependent enzymes and induces apoptosis in both human and animal cells. An inverse correlation between neutrophil viability and calprotectin release was demonstrated by invitro experiments. Thus, human calprotectin released upon neutrophil disruption and reflects the cell death. During bacterial infections, autoimmune diseases and inflammatory processes such as active pulmonary tuberculosis, rheumatoid arthritis and systemic lupus erythematosus, plasma levels of calprotectin are also increased.

In a study of Espinoza, one of the antimicrobial peptide in the amniotic fluid related with intra-amniotic inflammation causing preterm labor and premature rupture of membranes has been found to be calprotectin.[6]

Besides antimicrobial and antiiflammatoary effects, calprotectin has cytokine-like effects, and is proposed to be an important mediator with regulatory functions in inflammatory reactions. In order to show the role of calprotectin in preeclampsia, we investigated the changes of calprotectin levels between preeclamptic, normotensive pregnant and also nonpregnant patients in this study.

Material and Method

We included 30 preeclamptic patient who received antena-

Department of Obstetrics and Gynecology Dr. Zekai Tahir Burak Women Health Teaching and Research Hospital, Ankara

Address of Correspondence: *Ebru Zülfikaroğlu*
7.Cad. 70 A /14 Bahçelievler, Ankara
zebru33@hotmail.com

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tal and obstetric care at Perinatalogy Clinic at Zekai Tahir Burak Women Health Hospital in Ankara between November 2006-April 2008. A total of 30 pregnant women with normal ongoing pregnancies and 20 non- pregnant women were taken as the control groups (Table 1).

Preeclampsia was determined by increased blood pressure (≥140/90 mmHg) that occured in a pregnant woman after 20 weeks of gestation, accompanied by proteinuria (≥0.3g/24 h), in the absence of urinary tract infection as defined by National High Pressure Education Program Working Group on High Blood Pressure in pregnancy.[7] Severe preeclampsia was diagnosed if one or more of the following were present: blood pressure of 160/110 mmHg or higher, excretion of 5 g or more of protein in a 24-h urine sample or a urine dipstick showing 3+ or 4+ in a random urine sample, oliguria, pulmonary edema, visual or cerebral disturbance, and pain in the epigastric area or right upper quadrant.

All preeclamptic and normotensive pregnant women were in the third trimester, none were in active labor or had rupture of membranes at the time of sampling and none of the patients were treated with corticosteroids or magnesium sulphate before blood sampling inorder to not to affect the results of the study. Pregnant patients gave birth either by vaginal or cesarean section. None of these patients had any signs of infection or any medical history of HT, renal disease, diabetes. Non- pregnant controls were healthy reproductive women. The patients included in the study were not smoking. Parity and gestation were matched for pregnant control and preeclampsia groups. Informed consent was given by all participants, and the study was approved by the regional medical ethics commitee.

An antecubital vein was used for vein puncture and blood was sampled into Vacutainer tubes with ethyl-enediaminetetraacetic acid (EDTA) (Vacutainer System; Becton-Dickinson Europe, Meyland, France). Plasma calprotectin levels were assessed by enzyme-linked immunosorbent assay (Phoenix Pharmaceuticals, Belmont, CA). Samples were kept on ice, and then centrifuged at room temperature for 10 minutes at 2000g. Haematological parameters were analyzed using Biotec ELX800, Biotec Instruments, Inc, USA at our hospital's Department of Clinical Chemistry.

Statistical analyses were performed using Statistical Package for the Social Sciences (version 11.0; SPSS; Inc, Chicago, III). The Wilcoxon test was used for matched paired data and the Mann- Whitney U test used for unpaired groups. Differences were compared significant for P values <0.05.

Results

The clinical characteristics of study patients are shown in Table 1. The preeclamptic group did not differ from the control groups with regard to maternal age, gestational age at sampling, gestational age at delivery or BMI. The babies of preeclamptic patients had a lower neonatal weight compared to the normotensive pregnant group. Compared with the control groups, the preeclamptic patients had significantly higher systolic and diastolic blood pressure.

In the two pregnancy groups, hemoglobin levels and the red cell counts were significantly lower compared with the nonpregnant group, whereas the white blood cell counts were significantly increased in the two pregnancy groups compared with the nonpregnant controls. In the preeclamptic group, platelet count was also lower than the other groups (Table 1).

Table 1: Characterictics of patients

	Preeclampsia (n=30)	Pregnant control (n=30)	Non-pregnant control (n=20)
Maternal age(years)	27.0 (25.7-28.4)	27.0 (25.9-28.7)	28.0(26.0-30.5)
Gestational age at sampling(days)	35.2 (33.8-36.4)	36.6 (33.7-36.7)	-
Gestational age at delivery(days)	35.7 (34.8-36.4)	40.3 (38.7-41.0)	-
Birth weight (g)	2150 (1950-2450)*	2750 (2500-2950)	-
Systolic blood pressure (mmHg)	158 (148-160)* **	110 (100-115)	108(110- 107)
Diastolic blood pressure (mmHg)	110 (100-110)* **	70 (60-80)	80(90- 70)
Maternal BMI (kg/m2)	29.9 (27.1-30.8)	28.9 (27.4-30.8)	-
Maternal hemoglobin (g/dL)	11.8 (10.7-12.6)	11.9 (11.3-12.4)	12.9 (12.5-13.8)
Maternal red cell count (x1012 /L)	3.7 (3.6-3.9)	3.9 (3.6-4.1)	4.7 (4.2-4.8)* ***
Maternal white cell count (x109/L)	8.9 (8.7-10.3)	8.3 (7.6-9.8)	6.8 (5.1-8.2)* ***
Maternal platelet count (x 109/L)	157 (143-180)	196 (180-228)	236 (190-248)* ***

Values are median with interquantile range
**p<0.001 compared with pregnant control*
***p<0.001compared with non- pregnant control*
**** p<0.001 compared with preeclampsia*

Significantly higher levels of maternal plasma calprotectin in preeclamptic group were discovered 783 (478-928) µg/L, compared to normotensive pregnant control 618 (343-887) µg/L and the nonpregnant women 574 (283-797) µg/L (P=0.001) (Figure 1). Further, serum level of calprotectin was significantly higher in severe preeclampsia 954 (691-985) µg/L than mild preeclampsia 589(492-712) µg/L (p=0.037) (Figure 2). Compared with pregnant control, the serum level of mild or severe preeclampsia was significantly elevated (p= 0.047 and 0.012, respectively).

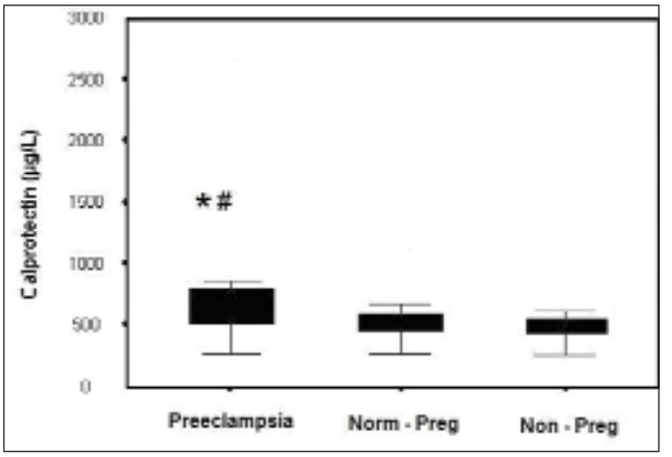

*Figure 1: Comparison of calprotectin levels in preeclamptic, normal pregnant, and non- pregnant women. Values are median with interquantile range. Women with preeclampsia had significantly higher calprotectin levels than non- pregnant control and (*p=0.004) and pregnant control (#p<0.001).*

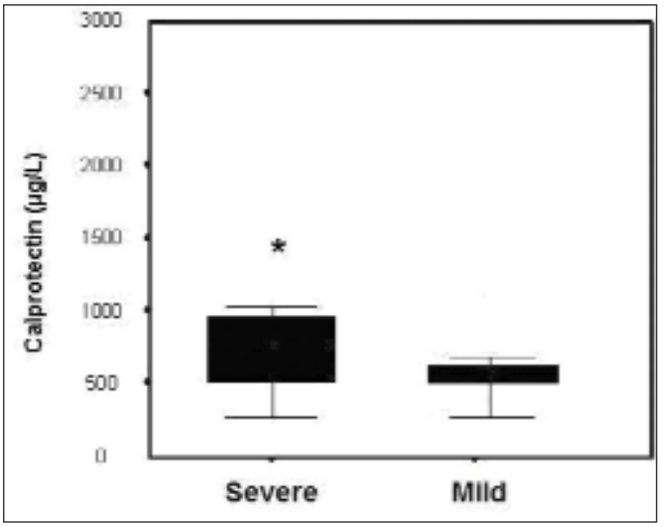

*Figure 2: Comparison of calprotectin levels in severe and mild preeclamptic patients. Values are median with interquantile range. Calprotectin levels were significantly higher in severe preeclampsia than mild (*p=0.037)*

Discussion

The main outcome of our study was that maternal plasma calprotectin level in preeclamptic patients is elevated compared with women in the third trimester of healthy pregnancy and nonpregnant patients. We found the serum calptotectin levels were comparable between non-pregnant women and women in the third trimester of normal pregnancy. Further we also noticed that calprotectin level was significantly different between severe and mild preeclampsia. The present findings suggest that a change in circulating calprotectin level is an important pathophysiology of preeclampsia and it may represent an excessive maternal inflammatory response to pregnancy by releasing from activated leukocytes.

It has been documented that in the early pregnancy, without sufficient changes in the uterine vasculature, the placenta is susceptible to the development of focal regions of ischemia and this leads to the endothelial dysfunction, production of cytotoxic and inflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin-1β (IL-β) and IL- 6 has been documented in the human placenta8. Benyo et al. showed that incubation of placental explants under reduced oxygen conditions results in the elevated production of TNFα, IL-α, and IL-β and they reported that also circulating levels of TNFα, IL-1α and IL-1 β are increased in women with preeclampsia11.

In preeclampsia, elevated levels of calprotectin might constitute a part of the innate defense in myelomonocytic cells against microorganisms and this may predispose an abnormal trophoblast invasion to decidua. In the early stage of pregnancy, during placentation, enzymes such as matrix metalloproteinases (MMPs) play a major role in trophoblast invasion. Elevated levels of calprotectin can bind zinc and this may reduce the MMPs activity. Reduced MM-9 trophoblast expression at both the protein and the mRNA levels and also low levels of MMP-1 in decidual microvascular endothelial cells causes preeclampsia.[12]

As a marker, calprotectin may have a role in the early diagnosis of the disease if an elevation occured prior to the clinical development of the disease. In order to find out at what gestational age the calprotectin level rises in women who later develop preeclampsia, increase in calprotectin levels throughout pregnancy should be explored.

Braekke et al found maternal calprotectin, C-reactive protein (CRP) and plasminogen activator inhibitor type 1 (PAI- 1) to be elevated in preeclampsia compared with normotensive pregnancies.[13] But in the umblical vein, there were no differences between preeclampsia and controls regarding calprotectin and CRP levels suggesting that placental or maternal compounds triggering maternal inflammation are not transferred into the fetal circulation.

In a study of Sacks, the leukocytes of healty pregnant women differed from those of nonpregnant women (increased CD11b, CD14, and CD64 and increased intracellular reactive

oygen species).[14] In addition to these changes, in preeclampsia, there was reduced expression of L-selectin and further increases in intracellular reactive oxygen species. They reported that normal third trimester pregnancy is characterized by remarkable activation of peripheral blood leukocytes, which is further increased in preeclampsia. Holthe et al, found significantly elevated levels of maternal plasma calprotectin in preeclampsia compared to normotensive pregnant women.[15] But in contrast, the nonpregnant controls had a calprotectin level intermediate between the two pregnancy groups in their study. The pregnant controls had significantly elevated levels of calprotectin in our results in agreement with the findings of Sacks et al.

In the current research, serum calprotectin level is comparable in the third trimester pregnancy and pre- gravid state, suggesting that placenta does not seem to be the main origin of circulating calprotectin during pregnancy, altough this needs to be verified. Due to the limitation of this cross- sectional study, we could not clarify whether a causal relationship exists between the increase in calprotectin level and preeclampsia. Longitidinal estimations of calprotectin levels during normal and preeclampsia complicated pregnancies and studies on the antiiflammatoary and cytokine-like effects of calprotectin would enhance our understanding of the role of calprotectin in pregnancy and preeclampsia.

In summary, we demonstrated that calprotectin level is significantly elavated in the preeclampsia group suggesting that leukocytes are activated in preeclampsia and preeclampsia may represent an excessive maternal inflammatory response to pregnancy and may contribute the pathogenesis of this disorder.

Preeklamptik ve Normotansif Gebelikleri Olan ve Gebe Olmayan Kadınlarda Plazma Kalprotektin Düzeyleri

AMAÇ: Preeklamptik ve normal gebeliklerin geç dönemi ve gebe olmayan hastaların serum calprotectin düzeylerindeki değişiklikleri karşılaştırmak.

GEREÇ VE YÖNTEM: Otuz preeklamptik hasta çalışmaya dahil edilirken, 30 normotansif ve komlikasyonsuz gebe ve gebe olmayan 20 hasta kontrol grubu olarak seçilmiştir. Plazma kalprotektin düzeyleri ELİSA yöntemi ile tespit edilmiştir.

BULGULAR: Preeklamptik hasta grubunda plazma kalprotektin düzeyi belirgin şekilde yüksek bulunurken 783 (478-928) µg/L, normotensif gebelerde (343-887) µg/L ve gebe olmayanlarda ise 574 (283-797) µg/L bulunmuştur (P=0,001). Ayrıca, ağır preeklamptik hastalarda plazma kalprotektin düzeyi 954 (691-985) µg/L iken, hafif preeklamptik hastalarda ise 589(492-712) µg/L olup anlamlı şekilde farklı bulunmuştur (p=0,037). Kontrol gebe hasta grubuna göre ağır ve hafif preeklamptik gebelerde de anlamlı şekilde yüksek bulunmuştur(p=0,047 ve 0,012).

SONUÇ: Preeklampside plasma kalprotektin düzeyinin belirgin şelilde yüksek bulunması, preeklampsinin gebeliğe maternal inflamatuar cevabının abartılı bir göstergesi olabileceğini ve kalprotektinin de hastalığın patogenezinde rol alabileceğini göstermektedir.

Anahtar Kelimeler: Preeklampsi, Kalprotektin, Maternal plazma

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