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Association of maternal calprotectin plasma levels with abruption placenta

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Abstract

Objective The aim of this study was to investigate the relationship between placental abruption and maternal plasma calprotectin levels.

Materials and methods This prospective study included 3865 pregnant women aged 24 weeks' gestation and older who were admitted to Etlik Zübeyde Hanım Women's Health Training and Research Hospital between January 2021 and January 2024. Calprotectin levels were prospectively studied in 33 pregnant women with placental abruption and compared with 48 healthy pregnant women matched for age, parity, body mass index (BMI) and week of gestation. Pregnant women with preeclampsia, gestational diabetes, chorioamnionitis, premature rupture of membranes, preterm labor, corticosteroid use within 7 days, cholestasis, fetal growth restriction, fetal anomalies, systemic infections and multiple pregnancies were excluded from the study. The calprotectin concentration in serum was measured with a Rayto Microplate Reader RT 2100 C (Rayto) using an immunoassay method at 450 wavelengths.

Results There was no difference between the groups regarding maternal age, gravidity, parity and BMI. Calprotectin levels were significantly higher in the placental abruption group ($p < 0.001$). The optimal cutoff value for calprotectin was 91.95 ng/ml (sensitivity 66.67%, specificity 89.58%, the area under the curve (AUC) 0.763, 95% confidence interval 0.649–0.876, and $p < 0.001$).

Conclusion The results obtained in the present study revealed an association between placental abruption and higher maternal serum calprotectin levels.

Keywords Placental abruption, Calprotectin, Inflammation, Sensitivity, Specificity

Introduction

Placental abruption (PA) is the premature detachment of the placenta from the uterine wall before the birth of the fetus due to decidual hemorrhage. PA occurs in approximately 0.4–1% of pregnancies. However, the incidence of PA increases in conditions known as major obstetric syndromes, such as preeclampsia, intrauterine growth restriction, preterm labor, premature preterm rupture of membranes (PPROM), and pregnancy losses [1–3]. PA typically constitutes an urgent obstetric emergency and represents a significant cause of maternal, perinatal morbidity and mortality [3, 4]. It is often exacerbated

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progressively by the accumulation of maternal blood, resulting from the rupture of maternal decidual arteries, which collects between the decidua and the placental surface [5]. Uteroplacental ischemia, decreased perfusion, chronic hypoxia and infarctions play a role in the pathophysiology of PA [5]. All these events culminate in a systemic inflammatory response. Calprotectin, which is expressed by immune cells, modulates the inflammatory response by stimulating leukocyte production and the secretion of cytokines [6]. Calprotectin (S100 A8/A9) is a calcium-binding protein that belongs to the family of S100 proteins, also known as myeloid-related protein 8/14 and calgranulin A/B [7]. The aim of the present study was to compare maternal serum calprotectin levels between cases of PA and healthy pregnant women.

Materials and methods

Ethical approval was obtained by the institutional review board from Ankara Etlik Zubeyde Hanım Women's Health Training and Research Hospital on 21.01. 2021 # 2021/16. The study complied with the ethical principles for medical research of the Declaration of Helsinki [8]. A total of 3865 pregnant women admitted to Etlik Zübeyde Hanım Women's Health Training and Research Hospital between January 2021 and January 2024 were included in the study. The study was conducted prospectively and observationally. Pregnant women with preeclampsia, gestational diabetes, chorioamnionitis, PPROM, preterm labor, corticosteroid administration within 7 days, cholestasis, fetal growth restriction and fetal anomalies, systemic infections (urinary tract infections, upper and lower respiratory tract infections, etc.) and multiple pregnancies were excluded from the study. Among 48 pregnant women who were hospitalized with the diagnosis of PA, 33 pregnant women were included in the study group after excluding pregnant women with PA occurring before 24 weeks of gestation (Fig. 1). The control group consisted of 48 healthy pregnant women who presented to our clinic as outpatients after each PA case (Fig. 1). The control group was matched with the PA group in terms of maternal age (± 2 years), gestational age (± 2 weeks), body mass index (BMI) (± 2 kg/m^2) and parity (0 or 1). During the follow-up of these pregnant women, no preeclampsia, gestational diabetes, chorioamnionitis, PPROM, preterm labor, antenatal corticosteroid therapy, cholestasis, fetal growth restriction and fetal anomalies or systemic infections (such as urinary tract infections, upper and lower respiratory tract infections, etc.) occurred. Informed consent was obtained from all pregnant women who participated in the study. Pregnant women with a PA at 24 weeks or more were selected. The reason for this was to definitively exclude the week of abortion and because it was related to the conditions and success of the neonatal intensive care unit of our hospital. Calprotectin was measured in maternal blood before PA. In the pregnant women diagnosed with PA, serum calprotectin levels, white blood cell (WBC) count, hemoglobin and hematocrit levels, platelet count, gestational week at delivery, neonatal birth weight, first and fifth minute APGAR scores were compared with healthy pregnant women. The final diagnosis of PA was made by postpartum inspection of the placenta.

For calprotectin analysis, maternal peripheral blood samples were collected in tubes with yellow or red lids. The samples were centrifuged at 3000 rpm for 10 min to obtain the serum, which was then transferred to Eppendorf tubes with closed lids. These tubes were delivered to the laboratory for analysis or stored at − 80 °C in an upright position before analysis. The collected serum samples were stored at room temperature and analyzed using a calprotectin (calpro) ELISA kit (E-EL-H2357, Elabscience). The calprotectin level in serum was measured using a Rayto Microplate Reader RT 2100 C (Rayto) using an immunoassay method at a wavelength of 450 nm. The minimum detectable calprotectin level was 0.94 ng/ml, the reference range was 1.56–100 ng/ml. The serum level of calprotectin in each sample was calculated using the Microstar statistical program (Microstar Laboratories).

There is no reference study on calprotectin levels in pregnant women diagnosed with placental abruption. Therefore, a preliminary study was conducted with 10 pregnant women from the study group and 10 pregnant women from the control group. According to the result of the preliminary study, in the power analysis, which was performed with 0.80 power, 0.05 alpha standard error, 0.737 effect size, it was determined that the sample size should be 64 (32 + 32). The pre-application data were included in the main application data. In our study, 81 (33 + 48) samples were obtained. According to the result of the post-hoc analysis, the power of the study was reported as 0.98.

Statistical analysis

The descriptive statistics were presented as mean, standart deviation, median, minimum and maximum values. The normality assumptions of the variables were tested using the Kolmogorov–Smirnov test. For continuous variables that were not normally distributed, the Mann–Whitney U test was used to compare differences between the two groups. In cases where the normality assumption was met, the t-test for independent samples was performed for pairwise comparisons between the groups. Logistic regression analysis was conducted to determine whether calprotectin level predicted PA. In addition, a receiver operating characteristic (ROC) analysis was performed to calculate sensitivity, specificity and positive and negative predictive values (Fig. 2). All analyzes

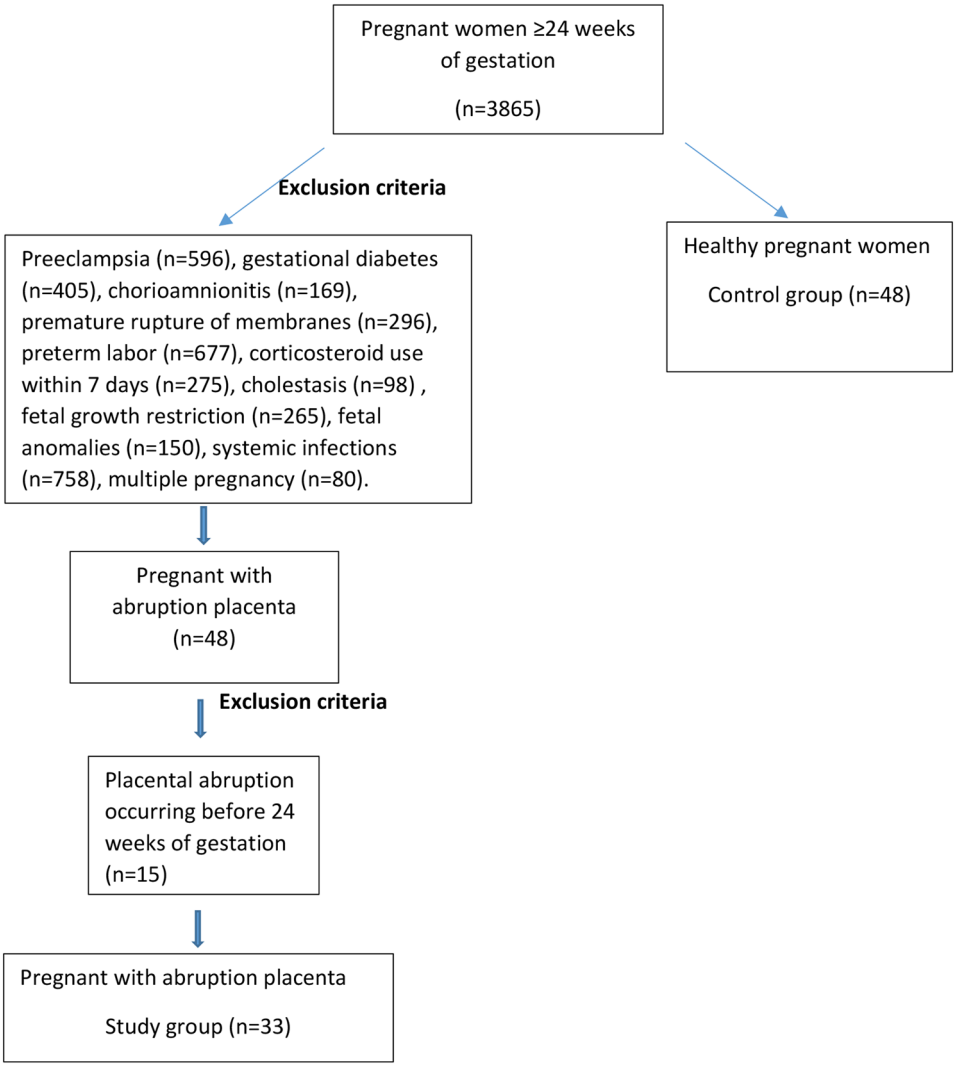

Fig. 1 Flowchart of study

were performed using IBM SPSS 25 and MedCalc software, and a p-value of <0.05 was considered statistically significant.

Results

A total of 81 patients were enrolled in the study, 33 (40.7%) in the PA group and 48 (59.3%) in the control group. As shown in Table 1, calprotectin levels were significantly higher in the PA group ($p<0.001$). In contrast, gestational week at delivery and neonatal birth weight were lower in the PA group ($p<0.001$ and $p<0.001$, respectively). Logistic regression analysis was performed to determine whether calprotectin levels predicted PA. According to this analysis, calprotectin level was identified as a significant predictor of PA ($p<0.001$). The diagnostic decision-making properties of the calprotectin level were investigated using ROC analysis. The ROC analysis yielded an area under the curve (AUC) of 0.763 (95% confidence interval: 0.649–0.876), indicating a statistically significant predictive value ($p<0.001$). Sensitivity and specificity were found to be 66.67% and 89.58% for the AUC cutoff value of >91.95 ng/ml (Table 2). The results of the ROC analysis are shown in Table 2; Fig. 2.

Discussion

As far as we know, this is the first study about relationship between PA and maternal plasma calprotectin levels. The results of the study revealed that maternal plasma calprotectin levels were significantly higher in the PA group than in the control group. Although the etiopathogenesis of PA is not fully elucidated, factors such as placental insufficiency, oxidative stress, hypoxia in intrauterine decidual vessels, thrombosis and necrosis, along with immunological reactions and acute or chronic inflammation in the decidua, have been implicated [9, 10]. Immune cells, maintain the balance of the silent

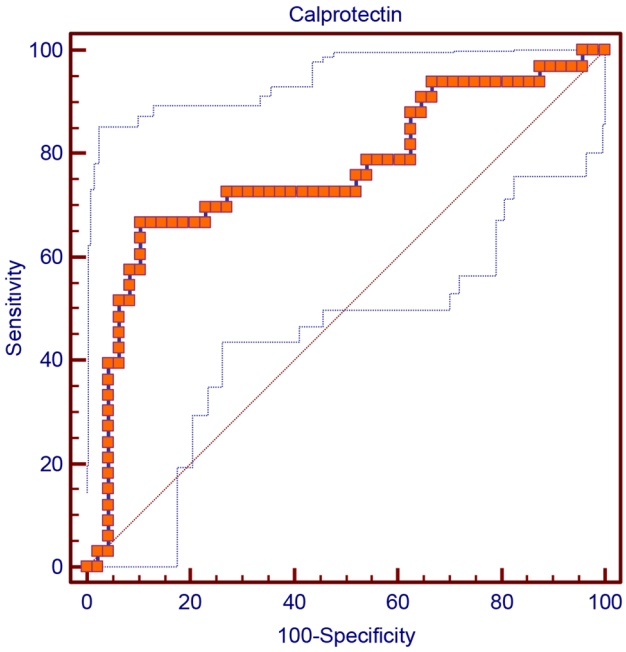

Fig. 2 Receiver Operating Characteristics (ROC) curve of calprotectin value, best cutoff value for calprotectin is > 91.95 ng/ml, which gives a sensitivity of 66.67% and specificity of 89.5%

Table 1 Comparison of demographic characteristics and laboratory parameters in patients in the control and study groups

	Study group (*n* = 33)	**Control group (*n* = 48)**	
	Mean ± SS or Median(Min-Max)	Mean ± SS or Median (Min-Max)	P- Value
Maternal age(years) [a]	28.18 ± 6.07	28.77 ± 5.51	0.652
Parity[b]	1.00 (0.00–7.00)	1.00 (0.00–6.00)	0.559
BMI (kg/m$^{2)a}$	27.97 ± 5.74	30.27 ± 5.19	0.065
GW at sampling [a]	31.99 ± 4.98	30.51 ± 3.98	0.143
GW at delivery[b]	32.40 (22.40–41.00)	39.00 (37.00–40.60)	0.000011
Neonatal weight (gram)[b]	1930.00 (410.00–3725.00)	3240.00 (2465.00–4520.00)	0.000054
APGAR 1.minute[b]	7.00 (0.00–9.00)	9.00 (8.00–9.00)	< 0.001
APGAR 5.minute[b]	8.00 (0.00–10.00)	10.00 (10.00–10.00)	< 0.001
Calprotectin (ng/ml)[b]	100.34 (4.74–119.03)	56.40 (0.65–119.13)	0.000064
Hemoglobin (g/dl)[b]	11.30 (6.40–16.00)	11.00 (8.40–14.00)	0.911
Hematocrit (%)[b]	34.00 (18.40–48.00)	35.00 (27.00–43.00)	0.463
WBC count (10^3/μL)[b]	11400.00 (1600.00–21000.00)	11000.00 (1092.00–15000.00)	0.200
PLT count (10^3/mm^3)[b]	222.00 (39.00–409.00)	232.50 (129.00–484.00)	0.124

Abbreviations: BMI: Body mass index, GW: Gestational week, PLT: Platelet, WBC: White blood cell, [a]: Values are mean ± standard deviation, [b]: Values are median (minumun– maximum)

Table 2 Calprotectin breakpoints in receiver operating characteristics analysis

	Limit value
	> 91.95
Area Under Curve (AUC)	0.763
Standard Error	0.058
% 95 Confidence interval	0.649 – 0.876
Sensitivity (%)	66.67
Specifificity (%)	89.58
p	< 0.001

inflammatory process in the human uterine decidua [11, 12]. Disruption of this balance can lead to premature separation of the placenta [13, 14]. In approximately 10% of pregnancies, chronic placental inflammation can occur without infection [15]. The literature shows that an increased risk of PA is associated with an increased infiltration of macrophages and neutrophil cells in the uterus [16–18]. Although pregnant women with concomitant diseases that increase the risk of PA were excluded from the study, we believe that the high calprotectin levels in these patients may be due to the disruption of the silent inflammatory process in the uterine decidua and the discharge of calprotectin from activated neutrophils.

Calprotectin is actively released during inflammation and plays a crucial role in the modulation of inflammation, the stimulation of leukocytes and the release of cytokines [19]. In recent years, calprotectin has been used in conjunction with antineutrophil cytoplasmic antibodies as an important biomarker for the diagnosis, follow-up and assessment of response to treatment in diseases such as spondyloarthritis, rheumatoid arthritis, juvenile idiopathic arthritis and vasculitis [20–22]. Moreover, maternal plasma calprotectin levels have been shown to be higher in patients with preeclampsia compared to normotensive pregnant women [23]. It has been suggested that the high plasma calprotectin levels observed in preeclampsia are related to leukocyte activation [23]. Due to the role that inflammation plays in intrahepatic cholestasis in pregnancy, calprotectin levels have also been found to be significantly elevated in these patients [24]. It has been shown that detectable levels of calprotectin in body fluids, tissues, and feces are valuable markers for gastrointestinal pathologies [25]. It was found that high levels of calprotectin in amniotic fluid are associated with preterm birth and intraamniotic inflammation [26]. Studies have shown that markers indicating this systemic inflammatory response have different sensitivities and specificities [8, 20, 27]. In the present study, the sensitivity and specificity of calprotectin in PA were reported as 66.67% and 89.58%, respectively.

It is known that PA can increase fetal/neonatal complications such as hypoxia, asphyxia, low birth weight, prematurity and fetal growth restriction [27–29]. In the

present study, gestational week at delivery and neonatal birth weight also differed significantly between the PA and control groups. There was a significant difference between first and fifth minute APGAR scores between the PA and control groups. The strengths of this study include the prospective study design and the examination of maternal and perinatal outcomes. However, limitations include the lack of a postpartum pathologic examination of the placenta and the limited number of cases.

Conclusion

In conclusion, the high plasma calprotectin level found during placental abruption in this study is consistent with the findings related to leukocyte activation during abruption. Further studies on the role of calprotectin in pregnancy and pregnancy-related diseases are recommended.

Abbreviations

PA	Placental abruption
BMI	Body mass index
APGAR	Appearance, pulse, grimace, activity, respiration
PPROM	Premature pretem rupture of membranes
ROC	Receiver operating characteristic
AUC	Area under the curve

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None.

Author contributions
Concept for this article was conceived by NCK, ATC, YEU. Protocol drafted by NCK, with input from MO, OA, FG, ATC and YEU. Literature screening, data extraction and quality assessment performed by NCK, MO, OA, FG, ATC and YEU. All authors contributed to the analysis, interpretation and write up.

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Data availability
The data are available from the authors upon reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained by the institutional review board from Ankara Etlik Zubeyde Hanım Women's Health Training and Research Hospital on 21.01.2021 # 2021/16. The study complied with the ethical principles for medical research of the Declaration of Helsinki.

Informed consent
A verbal and written informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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