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Original Research

Maternal Serum Toll-Like Receptor 2 and Toll-Like Receptor 4 Concentrations in Early-Onset and Late-Onset Preeclampsia

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Abstract

Background: Preeclampsia (PE) is a clinically heterogeneous disorder, and early-onset PE (EO-PE) and late-onset PE (LO-PE) differ in their underlying pathophysiology. Innate immune activation, mediated in part by Toll-like receptors 2 and 4 (TLR2 and TLR4), is implicated predominantly in early disease, which is more strongly associated with placental dysfunction. Whether maternal serum concentrations of these receptors differ between the two phenotypes remains unknown. This study aimed to compare maternal serum TLR2 and TLR4 concentrations among patients with EO-PE, LO-PE, and normotensive pregnancies and to determine whether innate immune activation differs between the two PE subtypes. **Methods:** In this single-center, cross-sectional study, 87 pregnant women were enrolled: 30 with EO-PE (onset before 34 weeks), 27 with LO-PE (onset at or after 34 weeks), and 30 normotensive controls. PE was defined according to the 2020 criteria of the American College of Obstetricians and Gynecologists (ACOG). Serum TLR2 and TLR4 concentrations were measured and compared among the three groups. **Results:** Serum TLR2 differed significantly among the three groups (p = 0.001), with higher concentrations in EO-PE (0.367 ± 0.08 ng/mL) than in LO-PE (0.301 ± 0.08 ng/mL; p = 0.008) and normotensive controls (0.288 ± 0.06 ng/mL; p = 0.001). TLR2 concentrations did not differ between LO-PE and controls (p = 0.833). Serum TLR4 concentrations followed the same pattern (p = 0.002; EO-PE 0.570 ± 0.14, LO-PE 0.462 ± 0.15, controls 0.423 ± 0.17 ng/mL). TLR4 concentrations were higher in EO-PE than in LO-PE (p = 0.037) and controls (p = 0.002). Effect sizes for the EO-PE versus control contrast were large (Cohen's d = 1.01 for TLR2 and 0.91 for TLR4). Among patients with EO-PE, serum TLR2 concentrations were inversely correlated with gestational age at delivery (GAD) and birth weight. For discrimination of EO-PE from controls, the area under the receiver operating characteristic curve (ROC) was 0.759 for TLR2 and 0.745 for TLR4 (both $p < 0.001$). **Conclusions:** Serum TLR2 and TLR4 concentrations were significantly increased in EO-PE, whereas LO-PE did not differ significantly from normotensive pregnancy. This selective elevation suggests that circulating TLR2 and TLR4 levels reflect an innate immune contribution specific to the EO-PE phenotype and supports the view that EO-PE and LO-PE are pathophysiologically distinct. Confirmation in larger, prospective cohorts is warranted.

Keywords: preeclampsia; Toll-like receptor 2; Toll-like receptor 4; innate immunity

1. Introduction

Preeclampsia (PE) is defined by the development of hypertension after 20 weeks of gestation accompanied by proteinuria or other evidence of maternal end-organ dysfunction and remains a leading cause of maternal and perinatal morbidity and mortality worldwide [1,2]. The disorder affects an estimated 2–8% of pregnancies globally [1]. Although its etiology remains incompletely understood, abnormal placentation, excessive systemic inflammation, endothelial dysfunction, and immune maladaptation are considered central to its pathophysiology [2,3].

Dysregulation of the maternal innate immune system plays a critical role in the pathogenesis of PE [3,4,5]. Pattern-recognition receptors (PRRs), particularly the Toll-like receptor (TLR) family, link endogenous and microbial stimuli to downstream inflammatory signaling, predominantly through the MyD88-dependent activation of nuclear factor-κB (NF-κB) and subsequent transcription of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) [6,7]. TLRs recognize both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) released from the ischemic and stressed placenta [4,6]. Importantly, TLRs are expressed not only by maternal leukocytes but also by villous and extravillous trophoblasts, decidual cells, and endothelial cells, indicating their direct involvement at the maternal–fetal interface [4,8].

Among this family, TLR2 and TLR4 have received particular attention in the context of PE. Placental TLR4 expression is increased in preeclamptic pregnancies, with

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the strongest signal observed in early-onset PE (EO-PE), and is accompanied by amplified NF-κB-mediated inflammation [9,10,11]. Circulating TLR4 is similarly elevated in women with PE and has discriminatory value as a candidate biomarker [12]. TLR2 appears to have a distinct, rather than inconsistent, role compared with TLR4: in trophoblasts, TLR2 ligation preferentially induces apoptosis rather than cytokine release, consistent with distinct and complementary roles for TLR2 and TLR4 in placental dysfunction [8].

Aberrant TLR activation contributes to several key pathological processes in PE, including impaired trophoblast invasion, defective spiral artery remodeling, and endothelial injury. Placental ischemia–reperfusion further amplifies DAMP release and sustains innate immune activation [3,9,13,14]. Beyond these correlative observations, animal studies demonstrate a direct causal link between TLR activation and the maternal vascular phenotype: experimental ligation of TLR3, TLR4, or TLR9 during gestation is sufficient to induce vascular inflammation, endothelium-dependent vasodilator dysfunction, and gestational hypertension in previously healthy pregnant animals, whereas immunomodulatory interventions that attenuate this response restore maternal vascular function. These findings position TLR signaling as a central mechanism in hypertensive disorders of pregnancy [13]. PE is also a clinically heterogeneous disorder, commonly stratified into EO-PE and late-onset PE (LO-PE) phenotypes that differ in their clinical presentation and underlying mechanisms. EO-PE is characterized by placental dysfunction and pronounced inflammatory activation, whereas LO-PE disease appears to reflect maternal cardiovascular maladaptation [15]. Supporting this distinction at the molecular level, circulating neutrophils from women with EO-PE exhibit increased expression of TLR2, TLR4, and NF-κB, while placental single-cell transcriptomic data reveal coordinated activation of pro-inflammatory pathways in maternal and fetal myeloid populations [11,16]. Both signatures are absent or minimal in LO-PE. Although TLR2 and TLR4 mRNA and surface expression have been examined in circulating immune cells, maternal serum levels of these proteins have not been compared between EO-PE and LO-PE. As such, the present study compared serum TLR2 and TLR4 concentrations across three groups: EO-PE (onset <34 weeks), LO-PE (onset ≥34 weeks), and normotensive pregnancies.

2. Materials and Methods

This single-center, cross-sectional study was conducted at the Department of Obstetrics and Gynecology of Başakşehir Çam and Sakura City Hospital, a tertiary referral center in Istanbul, Turkey, between September 2020 and February 2021. Written informed consent was obtained from all participants. The study protocol was approved by the İstanbul Medipol University Ethics Committee (Approval No.: 608) and complied with the Declaration of Helsinki.

A total of 108 pregnant women were initially enrolled (37 with EO-PE, 32 with LO-PE, and 39 normotensive controls). Of these, 21 were subsequently excluded: 14 because they delivered at another center and perinatal outcome data could not be obtained (5 with EO-PE, 3 with LO-PE, and 6 controls), 5 due to chronic hypertension or diabetes identified during follow-up (2 with EO-PE, 2 with LO-PE, and 1 control), and 2 because they withdrew consent (both controls). The final study population therefore comprised 87 women: 30 with EO-PE, 27 with LO-PE, and 30 normotensive pregnant controls. Women were eligible for inclusion if they were aged 18–40 years and were at or beyond 20 weeks of gestation. Controls were low-risk, normotensive pregnant women without coexisting medical conditions who were recruited during routine antenatal visits. PE was defined according to the 2020 criteria of the American College of Obstetricians and Gynecologists (ACOG) and stratified into EO-PE (disease onset before 34 weeks of gestation) and LO-PE (disease onset at or after 34 weeks of gestation) [1]. Exclusion criteria for all groups included multiple gestation, chronic hypertension, pregestational or gestational diabetes, chronic kidney disease, autoimmune disease, active infection, fetal anomalies, maternal smoking or substance use, and use of corticosteroids or other immunomodulatory medications during the index pregnancy.

Among women with PE, venous blood samples were collected immediately after diagnosis and before treatment initiation, whereas control samples were collected during routine antenatal visits. Each sample was collected into a serum separator tube and centrifuged at 2500 rpm for 10 minutes. The separated serum was stored at −80 °C until analysis.

Every serum sample was assayed in duplicate for TLR2 and TLR4 using commercial enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol, and kits obtained from Cloud-Clone Corp. (Katy, TX, USA; Catalog Nos.: SEA663Hu for TLR2 and SEA753Hu for TLR4). According to the manufacturer, intra-assay and inter-assay variability were below 10% and 12%, respectively, with detection limits of 0.112 ng/mL for TLR2 and 0.118 ng/mL for TLR4. Serum samples, standards, and biotin-conjugated detection antibodies were added to microplate wells pre-coated with antibodies specific for TLR2 or TLR4. Incubation and washing followed the manufacturer's protocol, after which absorbance at 450 nm was measured using a microplate spectrophotometer (Smart Microplate Reader; USCN Kit Inc., Wuhan, Hubei, China) to determine the optical density (OD). Separate standard curves were generated for TLR2 and TLR4 using the OD values corresponding to known standard concentrations. Serum TLR2 and TLR4 concentrations were calculated by comparing the OD values of the samples with the respective standard curves. Results were expressed in ng/mL.

All analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). Distributional assumptions were assessed with the Shapiro–Wilk test, and homogeneity of variance was assessed with Levene's test. Variables meeting the normality assumption were reported as mean ± standard deviation (SD) and compared using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test. The Kruskal–Wallis test, followed by Dunn's test for pairwise comparison, was used for variables that deviated from normality; these variables were reported as median (minimum–maximum). Effect sizes (Cohen's d) were calculated manually as the difference between group means divided by the pooled SD. Correlations between serum TLR2, TLR4, and clinical parameters were assessed using Spearman's rank correlation coefficient. Given the number of correlation analyses, p-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) within each subgroup. Correlations were considered robust if they remained statistically significant after FDR correction ($q < 0.05$), whereas other associations were regarded as exploratory. Receiver operating characteristic (ROC) curve analysis was used to assess the discriminatory performance of serum TLR2 and TLR4, and optimal cutoff values were determined from the ROC curves. To determine whether the between-group differences in serum TLR2 and TLR4 levels were independent of gestational age, multivariable linear regression models were performed for each receptor, with study group and gestational age at blood sampling entered as predictors. Additional models included individual clinical severity markers, including mean arterial pressure (MAP) and proteinuria. Collinearity was assessed using tolerance and the variance inflation factor (VIF). Statistical significance was set at a two-sided $p < 0.05$.

3. Results

A total of 87 pregnant women were enrolled: 30 with EO-PE, 27 with LO-PE, and 30 normotensive controls. Maternal demographic characteristics, including age, body mass index (BMI), gravidity, and parity, did not differ significantly among the groups. As expected, MAP and 24-hour proteinuria were markedly higher in both PE subgroups than in controls ($p < 0.001$). Gestational age at delivery (GAD) and birth weight were lowest in the EO-PE group (Table 1).

Serum TLR2 concentrations differed significantly between groups ($p = 0.001$), with mean values of 0.367 ± 0.08 ng/mL in EO-PE, 0.301 ± 0.08 ng/mL in LO-PE, and 0.288 ± 0.06 ng/mL in controls (Fig. 1). Post hoc analyses demonstrated that TLR2 concentrations were significantly higher in EO-PE than in LO-PE ($p = 0.008$) and controls ($p = 0.001$), whereas LO-PE did not differ from controls ($p = 0.833$) (Table 2). Cohen's d values indicated large effect sizes for the EO-PE versus control ($d = 1.01$) and EO-PE versus LO-PE ($d = 0.75$) comparisons.

Serum TLR4 concentrations followed a similar pattern ($p = 0.002$), with mean values of 0.570 ± 0.14 ng/mL in EO-PE, 0.462 ± 0.15 ng/mL in LO-PE, and 0.423 ± 0.17 ng/mL in controls (Fig. 2). TLR4 concentrations were significantly higher in EO-PE than in LO-PE ($p = 0.037$) and controls ($p = 0.002$), whereas LO-PE values did not differ from controls ($p = 0.639$) (Table 2). Cohen's d values indicated a large effect size for EO-PE versus control ($d = 0.91$) and a moderate effect size for EO-PE versus LO-PE ($d = 0.70$).

Within the EO-PE subgroup, serum TLR2 concentrations correlated positively with MAP ($r = 0.398$, $p = 0.030$) and inversely with GAD ($r = -0.560$, $p = 0.001$) and birth weight ($r = -0.618$, $p < 0.001$), whereas TLR4 showed only a weak negative correlation with GAD (Table 3a). Within the LO-PE subgroup, serum TLR2 concentrations correlated positively with proteinuria ($r = 0.494$, $p = 0.009$) and inversely with GAD ($r = -0.441$, $p = 0.021$); a moderate positive correlation between TLR2 and TLR4 concentrations was also observed ($r = 0.382$, $p = 0.049$) (Table 3b). Neither TLR2 nor TLR4 levels correlated significantly with gestational age at sampling within either subgroup. In the control group, no significant correlations were observed between serum TLR levels and GAD, birth weight, or other clinical parameters. After Benjamini–Hochberg FDR correction within each subgroup, only the correlations of serum TLR2 with birth weight ($q = 0.004$) and with GAD ($q = 0.007$) in the EO-PE group remained significant; the remaining correlations, including that with MAP and all correlations within the LO-PE subgroup, did not survive correction and were considered exploratory.

In multivariable linear regression, study group remained independently associated with both serum TLR2 ($\beta = -0.322$, $t = -3.08$, $p = 0.003$) and TLR4 ($\beta = -0.349$, $t = -3.27$, $p = 0.002$) after adjustment for gestational age at blood sampling, whereas gestational age at sampling was not independently associated with either receptor (TLR2, $\beta = -0.171$, $p = 0.107$; TLR4, $\beta = -0.035$, $p = 0.741$). Collinearity between predictors was negligible (tolerance = 0.90, VIF = 1.11 in both models). These findings indicate that the higher serum TLR2 and TLR4 concentrations observed in EO-PE were not explained by gestational age at sampling. In further regression models that included study group and individual severity markers, the association between study group effect and serum TLR2 and TLR4 concentrations remained significant after adjustment for MAP (TLR2, $p = 0.042$; TLR4, $p = 0.001$) and proteinuria (TLR2, $p = 0.013$; TLR4, $p = 0.004$).

The discriminatory performance of serum TLR2 and TLR4 was assessed by ROC curve analysis. For discrimination of EO-PE from normotensive controls, serum TLR2 yielded an area under the curve (AUC) of 0.759 (95% confidence interval [CI]: 0.635–0.884; $p < 0.001$). At an optimal

Table 1. Demographic characteristics and the perinatal outcomes of the study groups.

	Control (n = 30)	EO-PE (n = 30)	LO-PE (n = 27)
Age (years)	27.6 ± 6.0	27.9 ± 5.9	25.1 ± 6.0
BMI (kg/m^2)	28.0 ± 3.3	28.0 ± 3.8	28.8 ± 3.9
Gravida	2 (1–10)	3 (1–6)	2 (1–6)
Parity	1 (0–9)	1 (0–3)	1 (0–3)
GA at blood sampling (weeks)	31.1 ± 3.2	28.6 ± 2.2*†	34.7 ± 0.5*
Mean arterial blood pressure (mmHg)	82.3 ± 7.9	114.1 ± 14.3*	111.9 ± 8.9*
Proteinuria (mg/24 h)	122.5 (55–255)	919 (359–8398)*	495 (329–7545)*
GAD (weeks)	39.4 ± 1.0	30.5 ± 2.1*†	35.8 ± 0.8*
Birth weight (g)	3453.3 ± 351.5	1175.0 ± 416.8*†	2275.7 ± 498.6*
1-min Apgar	8 (6–9)	5 (2–7)*†	7 (5–9)*
5-min Apgar	10 (8–10)	6 (0–9)*†	8 (6–10)*
Cord pH	7.3 (7.1–7.3)	7.3 (7.2–7.3)	7.3 (7.2–7.4)

Data are expressed as mean ± SD (age, BMI, gestational age at blood sampling, mean arterial blood pressure, GAD, and birth weight) or as median (minimum–maximum) (gravida, parity, proteinuria, Apgar scores, and cord pH). *$p < 0.05$ versus controls, †$p < 0.05$ versus LO-PE; via Kruskal–Wallis followed by Dunn's multiple comparison test or ANOVA/Tukey HSD test. EO-PE, early-onset preeclampsia; LO-PE, late-onset preeclampsia; BMI, body mass index; GAD, gestational age at delivery; SDs, standard deviations; ANOVA, analysis of variance; HSD, honestly significant difference.

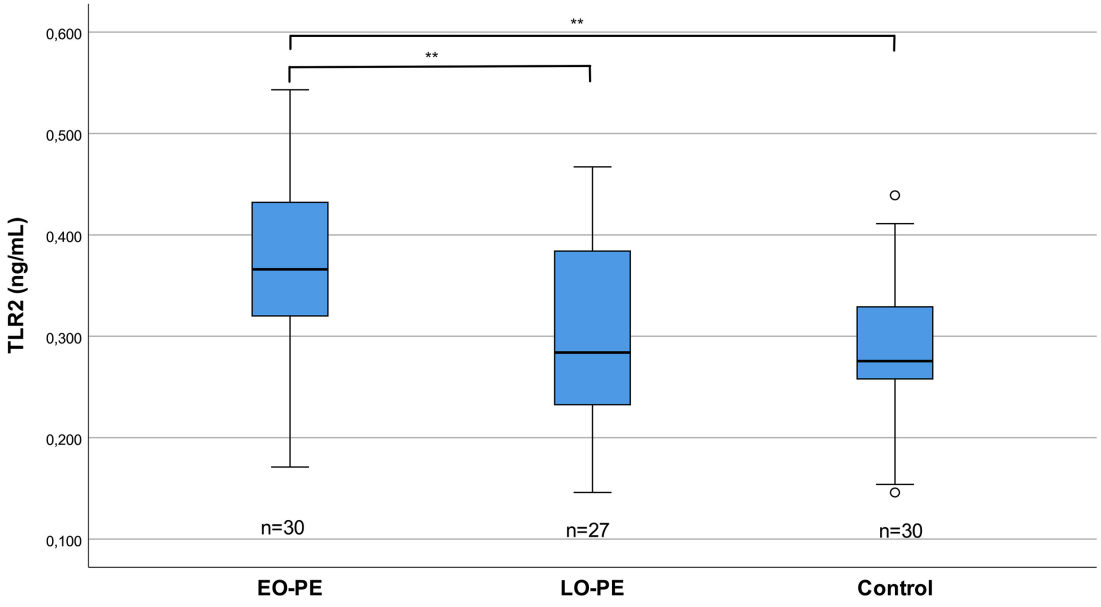


Fig. 1. Maternal serum TLR2 concentrations across study groups. Horizontal bars indicate statistically significant pairwise comparisons (ANOVA with Tukey's HSD test); **$p < 0.01$. Comparisons not marked were not statistically significant. TLR2, Toll-like receptor 2.

cutoff value of 0.330 ng/mL, the sensitivity and specificity were 73.3% and 76.7%, respectively. Serum TLR4 showed a comparable AUC of 0.745 (95% CI, 0.619–0.871; $p < 0.001$); at a cutoff value of 0.410 ng/mL, the sensitivity was 93.3% and the specificity 53.3% (Fig. 3A). For discrimination of EO-PE from LO-PE, serum TLR2 yielded an AUC of 0.685 (95% CI, 0.546–0.823; $p = 0.017$); at a cutoff value of 0.320 ng/mL, the sensitivity and specificity were 80.0% and 55.6%, respectively. Serum TLR4 yielded an AUC of 0.699 (95% CI, 0.560–0.838; $p = 0.010$); at a cutoff value of 0.443 ng/mL, the sensitivity was 86.7% and the specificity 55.6% (Fig. 3B).

4. Discussion

We hypothesized that maternal serum TLR2 and TLR4 concentrations would be elevated in EO-PE relative to LO-PE and normotensive controls, reflecting an EO-specific innate immune contribution. Our findings sup-

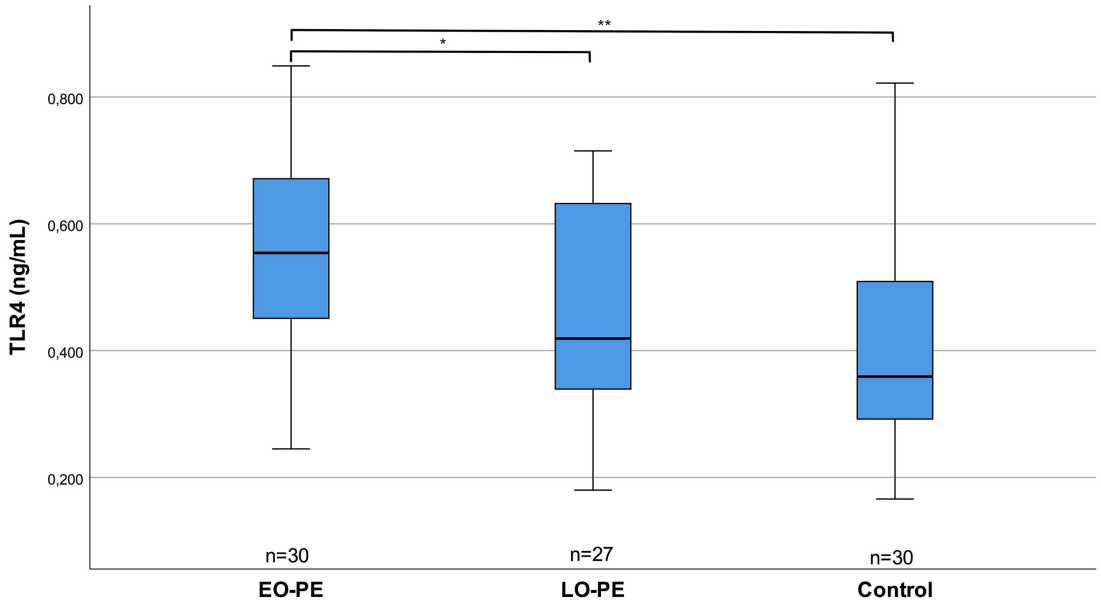


Fig. 2. Maternal serum TLR4 concentrations across study groups. Horizontal bars indicate statistically significant pairwise comparisons (ANOVA with Tukey's HSD test); *$p < 0.05$, **$p < 0.01$. Comparisons not marked were not statistically significant. TLR4, Toll-like receptor 4.



Fig. 3. ROC curve analysis of maternal serum TLR2 and TLR4. (A) EO-PE versus control. (B) EO-PE versus LO-PE. AUC values are shown with 95% CIs; *$p < 0.05$, ***$p < 0.001$. AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic.

port this hypothesis. Maternal serum concentrations of both TLR2 and TLR4 were significantly higher in EO-PE than in normotensive controls, whereas LO-PE did not differ significantly from controls for either receptor. The two receptors showed a parallel pattern, with the highest concentrations in EO-PE, intermediate concentrations in LO-PE, and the lowest levels in controls. EO-PE concentrations exceeded both LO-PE and controls, whereas LO-PE did not differ significantly from controls. The magnitude of the difference was substantial for TLR2, with a large effect size for the EO-PE versus control (Cohen's $d \approx 1.0$). Within the EO-PE group, serum TLR2 was inversely correlated with GAD and birth weight, and these associations remained significant after correction for multiple comparisons. No such correlations were observed in the control group. These findings indicate a concurrent elevation of circulating TLR2 and TLR4 that is largely confined to the EO-PE phenotype and associated with adverse perinatal indices. Within the EO-PE group, neither serum TLR2 nor TLR4 levels correlated with the degree of 24-hour proteinuria, despite the wide range of proteinuria observed.

These observations are consistent with prevailing two-stage and phenotype-stratified models of PE, in which EO-PE is driven mainly by defective placentation, ischemia–

Table 2. Maternal serum TLR2 and TLR4 levels across groups.

	TLR2 (ng/mL)	TLR4 (ng/mL)
Control (n = 30)	0.288 ± 0.06	0.423 ± 0.17
EO-PE (n = 30)	0.367 ± 0.08	0.570 ± 0.14
LO-PE (n = 27)	0.301 ± 0.08	0.462 ± 0.15
Overall comparison	***p* = 0.001**	***p* = 0.002**
Post hoc pairwise comparisons		
EO-PE vs. control	***p* = 0.001** *d* = 1.01	***p* = 0.002** *d* = 0.91
EO-PE vs. LO-PE	***p* = 0.008** *d* = 0.75	***p* = 0.037** *d* = 0.70
LO-PE vs. control	*p* = 0.833	*p* = 0.639

Data are expressed as means (±SDs), via ANOVA followed by Tukey HSD multiple comparison test. Bold *p* values refer to statistically significant results. TLR, Toll-like receptor.

reperfusion injury, and an amplified placental inflammatory response. In contrast, LO-PE is thought to reflect predominantly maternal cardiovascular and metabolic maladaptation, with a comparatively modest placental contribution [2,3,15]. A circulating innate immune signal confined to EO-PE supports this model: the ischemic, stressed placenta releases DAMPs that engage TLR2 and TLR4 and drive NF-κB–dependent systemic inflammation, a process expected to be substantially more pronounced in the placentally driven early phenotype than in the maternally driven late phenotype [4,6,7].

Our serum findings extend to the circulating compartment, a pattern previously documented in tissue, with TLR upregulation in PE concentrated in EO-PE. In the placenta, TLR4 protein expression is increased in EO-PE but not in LO-PE relative to controls, and villous endothelial TLR4 expression is significantly higher in EO-PE than in LO-PE, accompanied by reduced expression of the negative regulator Tollip [10,17]. At the leukocyte level, circulating neutrophils exhibit increased TLR2, TLR4, and inflammasome expression in PE, most markedly in EO-PE, while single-cell transcriptomic profiling reveals gene expression dysregulation and activation of pro-inflammatory pathways almost exclusively in the EO-PE phenotype [11,16]. Placental TLR4–MyD88–NF-κB activation has further been shown to coexist with systemic inflammatory and oxidative markers [18]. Our finding that serum TLR2 and TLR4 are elevated specifically in EO-PE adds a circulating-protein layer to these tissue, leukocyte, and transcriptomic observations.

The behavior of TLR4 in our cohort is consistent with its established role in PE. Interstitial trophoblasts within the placental bed show increased TLR4 expression in PE. Through this receptor, these cells sense danger signals at the feto-maternal interface and contribute to a locally dysregulated cytokine environment, providing a mechanism

Table 3. Correlations between maternal serum TLR2 and TLR4 levels and other parameters in (a) the EO-PE group, and (b) the LO-PE group.

(a)	TLR2 (n = 30)	TLR4 (n = 30)
Age (years)	r = –0.237 *p* = 0.207	r = 0.319 *p* = 0.086
BMI (kg/m^2)	r = 0.179 *p* = 0.344	r = –0.088 *p* = 0.643
GA at blood sampling (weeks)	r = –0.395 *p* = 0.081	r = –0.233 *p* = 0.215
Mean arterial blood pressure (mmHg)	r = 0.398* ***p* = 0.030** *q* = 0.112	r = –0.040 *p* = 0.834
Proteinuria (mg/24 h)	r = 0.327 *p* = 0.078	r = 0.114 *p* = 0.547
GAD (weeks)	r = –0.560** ***p* = 0.001** *q* = 0.007	r = –0.397* ***p* = 0.030** *q* = 0.112
Birth weight (g)	r = –0.618*** ***p* < 0.001** *q* = 0.004	r = –0.284 *p* = 0.129
TLR2	-	r = 0.037 *p* = 0.845
TLR4	r = 0.037 *p* = 0.845	-

(b)	TLR2 (n = 27)	TLR4 (n = 27)
Age (years)	r = –0.424* ***p* = 0.027** *q* = 0.135	r = –0.079 *p* = 0.694
BMI (kg/m^2)	r = 0.220 *p* = 0.271	r = 0.213 *p* = 0.286
GA at blood sampling (weeks)	r = –0.379 *p* = 0.051	r = 0.035 *p* = 0.861
Mean arterial blood pressure (mmHg)	r = –0.181 *p* = 0.367	r = –0.160 *p* = 0.424
Proteinuria (mg/24 h)	r = 0.494** ***p* = 0.009** *q* = 0.135	r = 0.266 *p* = 0.179
GAD (weeks)	r = –0.441* ***p* = 0.021** *q* = 0.135	r = –0.226 *p* = 0.257
Birth weight (g)	r = –0.075 *p* = 0.709	r = 0.017 *p* = 0.931
TLR2	-	r = 0.382* ***p* = 0.049** *q* = 0.153
TLR4	r = 0.382* ***p* = 0.049** *q* = 0.153	-

r = Spearman's; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Bold *p*-values refer to statistically significant results. *q* = Benjamini–Hochberg FDR-adjusted *p*-value across the 15 correlations within each subgroup; $q < 0.05$ was considered significant after correction.

by which innate immune activation has been proposed to contribute to disease pathogenesis [9]. A systematic review of pattern-recognition receptor studies concluded that TLR4 is consistently upregulated in preeclamptic placentas and immune cells [19]. Elevated serum TLR4 has also been reported in PE and proposed as a candidate diagnostic biomarker [12]. At the molecular level, ligand engagement of TLR4 activates a predominantly MyD88-dependent cascade that converges on NF-κB to drive transcription of IL-6, TNF-α, and IL-1β, providing a plausible mechanism by which placental danger-signal release is translated into the systemic inflammatory phenotype of EO-PE [6,7].

The contribution of TLR2 has been more contested. Reports of placental and cellular TLR2 expression in PE are inconsistent: the original placental-bed study found that trophoblast TLR2 expression was unchanged, whereas immunolocalization studies have reported increased placental TLR2 expression in preeclamptic tissue, and a systematic review characterized the literature on TLR2 expression as contradictory [9,19,20]. This inconsistency may reflect the distinct functional biology of TLR2 rather than measurement differences alone. In trophoblasts, TLR2 ligation preferentially induces apoptosis rather than a cytokine response, and TLR2 signaling is strongly context- and coreceptor-dependent, with TLR1 and TLR6 heterodimerization directing the response toward either cell death or inflammation [8,21]. Our observation of elevated maternal serum TLR2 in EO-PE, which correlated inversely with gestational age and birth weight, provides circulating-compartment evidence that TLR2, alongside TLR4, contributes to the heightened innate immune state associated with early placental disease and suggests that serum measurement may capture a signal that is not consistently apparent in tissue expression studies.

The absence of a detectable serum elevation in LO-PE warrants careful interpretation. Some studies have reported increased TLR4 in PE cohorts that were not stratified by disease onset, including elevated serum TLR4 in an unselected cohort sampled at term gestation and increased placental TLR2, TLR3, and TLR4 in mild, term-gestation PE [12,20]. Several factors may explain this discrepancy. First, tissue expression and circulating protein represent distinct compartments and need not change in parallel. Second, the definitions of “late”, “mild”, and “term” PE are heterogeneous across studies. Third, placental TLR4 expression increases physiologically with advancing gestation; therefore, the consistent finding, observed here in serum and elsewhere in tissue, that early disease exceeds late disease runs counter to the normal gestational trajectory and supports the interpretation that the EO-PE elevation is disease- and phenotype-specific rather than an artifact of gestational age [4,17]. Finally, our LO-PE subgroup was modest in size, and a smaller or absent effect in late disease, as also observed for placental TLR4, where the LO-PE versus control difference did not reach significance, should not be interpreted as evidence of a categorical absence of TLR involvement. Rather, this finding is consistent with an attenuated innate immune contribution in the maternally driven late phenotype [10].

A preferential EO-PE signal is also apparent at the genetic level: although TLR4 coding polymorphisms show no overall association with PE, EO-PE–specific associations have been reported, consistent with, although not equivalent to, the phenotype-restricted protein elevation observed here [19,22,23,24].

These data are compatible with a model in which defective placentation in EO-PE generates ischemia–reperfusion and oxidative stress, releasing danger signals that activate TLR2 and TLR4 in trophoblasts, endothelial cells, and leukocytes, with the resulting NF-κB–driven inflammation reflected in elevated circulating receptor concentrations. Experimental work supports a causal role for this axis: gestational activation of TLR3, TLR4, or TLR9 is sufficient to elicit vascular inflammation, endothelial dysfunction, and hypertension in previously healthy pregnant animals [13]. The recurrent involvement of the TLR4–NF-κB pathway across human and experimental PE has also identified it as a potential therapeutic target. However, the present associative data do not permit causal or therapeutic inference in humans [18,19,25].

Although EO-PE and LO-PE differ in gestational age by definition, the elevated serum TLR2 and TLR4 concentrations in EO-PE are unlikely to represent a gestational-age artifact. In multivariable analysis, the association between study group and both receptors persisted after adjustment for gestational age at sampling; gestational age showed no independent association, and neither receptor correlated with gestational age within subgroups. These findings support a phenotype-specific rather than a gestational-age–related origin of the circulating signal.

Limitations

The strengths of this study include the combined assessment of serum TLR2 and TLR4 across clearly stratified EO-PE, LO-PE, and normotensive control groups, with large effect sizes in EO-PE. The main limitations are the single-center design and modest sample size, particularly in the LO-PE subgroup; the observational, single-time-point design, which supports associations rather than causal inference; and quantification by ELISA without parallel ligand, signaling, or expression data. Thus, serum levels need not reflect placental receptor expression or downstream signaling activity, and absolute values are assay-dependent [12,18,26]. Future studies incorporating placental tissue analysis or mechanistic investigation of downstream signaling would help determine whether these circulating changes reflect tissue-level receptor activation. The number of participants reflects the eligible women who presented during the recruitment period, without *a priori* sample size calculation or statistical power assessment. Nonetheless, the

primary comparison (EO-PE versus controls) yielded large effect sizes for both serum TLR2 (Cohen's d = 1.01) and TLR4 (Cohen's d = 0.91), indicating that the study was adequately powered to detect the principal between-group differences. Conversely, the smaller LO-PE subgroup (n = 27) may have been underpowered to detect more modest effects, and negative findings in this subgroup should therefore be interpreted with caution. Since EO-PE and LO-PE differ in gestational age by definition, and controls were not individually matched to each subgroup for gestational age, gestational age remains a potential confounder, although the multivariable analysis argues against gestational age as an explanation for these discrepancies. TLR2 and TLR4 are broadly expressed innate immune receptors that are activated across diverse inflammatory and infectious conditions rather than being specific to PE. Although the exclusion of women with infection, autoimmune disease, diabetes, and other inflammatory comorbidities reduces the likelihood that the observed elevations reflect such conditions, disease-specificity cannot be established from the present design [4,21,27]. Residual confounding cannot be excluded.

5. Conclusions

Maternal serum TLR2 and TLR4 were coordinately elevated in EO-PE and did not differ significantly from normotensive levels in LO-PE, with the EO-PE elevation correlating with adverse perinatal indices. These findings are consistent with phenotype-specific, danger-signal-driven involvement of innate immune signaling in early, placentally mediated PE and position circulating TLR2 and TLR4, particularly TLR2 given its large effect size, as candidate discriminatory markers of the EO-PE phenotype. Given their moderate discriminatory performance (AUC ≈ 0.75), these should be regarded as preliminary and exploratory rather than clinically applicable. Whether combining serum TLR2 and TLR4 in a multimarker panel improves discrimination beyond either receptor alone was not assessed in this study and warrants evaluation in larger cohorts. Prospective, multicenter studies with larger subgroups, serial sampling, and concurrent assessment of TLR ligands and downstream mediators are required to confirm these associations and clarify their mechanistic and clinical significance. We did not measure established angiogenic or inflammatory markers of PE, such as soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), C-reactive protein, and IL-6. Concurrent assessment of serum TLR2 and TLR4 alongside these markers would help define their specificity and incremental value and represents a valuable direction for future research [13,28].

Availability of Data and Materials

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

Author Contributions

SK: conception and design, drafting the manuscript, reviewing the manuscript. BK: conception and design, analysis of data, drafting the manuscript. UT: conception and design, acquisition of data, reviewing the manuscript. İD: acquisition of data, analysis of data. İP: conception and design, reviewing the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was approved by the İstanbul Medipol University Ethics Committee (Approval No.: 608) and conducted in accordance with the Declaration of Helsinki; written informed consent was obtained from all participants.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used Claude (Claude Opus 4.8, Anthropic) to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG55826.

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