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Maternal blood endometase level as a predictive biomarker for hypertensive disorders during pregnancy: a prospective cohort study

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Preeclampsia (PE) occurs due to inadequate spiral artery/trophoblast remodeling in early pregnancy. Endometases are involved in the remodeling of spiral arteries and placental trophoblasts. This study aimed to investigate differences in blood endometase levels between pregnant women with hypertensive disorders (PE and gestational hypertension [GHT]) and healthy pregnant women and to evaluate whether plasma endometase values could play a predictive role in PE or GHT diagnosis. A total of 90 pregnant women (n(PE) = 30, n(GHT) = 30, n(healthy pregnant) = 30) who presented at the hospital between December 2023 and May 2024 and were 26–32 years of age and > 20 weeks pregnant were included in the study. The endometase levels in all pregnant women were determined in maternal blood plasma via enzyme-linked immunosorbent assay. The endometase values were recalculated according to albumin values, and corrected endometase (cEndo) values were determined. No significant differences in blood endometase levels were observed between the groups ($p > 0.05$). The cEndo value was significantly lower in the PE and GHT groups than in the control group ($p < 0.05$). There was no statistically significant difference in the cEndo values between the PE and GHT groups ($p > 0.05$). A statistically significant negative linear relationship was detected between cEndo values and mean systolic blood pressure and mean diastolic blood pressure ($p < 0.05$). The cEndo values in the PE and GHT groups at early (≤ 32 weeks 3 days) and late pregnancy were compared, and no statistically significant difference was detected ($p > 0.05$). Maternal blood cEndo values may play a successful role in distinguishing hypertensive diseases of pregnancy (PE + GHT) from healthy pregnant women. cEndo does not play an effective role in the differential diagnosis between pregnant women with PE and those with GHT. Studies with larger patient populations are needed.

Keywords Corrected endometase, Gestational hypertension, Hemoconcentration, Matrix metalloproteinase 26, Preeclampsia

Abbreviations

PE	Preeclampsia
GHT	Gestational hypertension
cEndo	Corrected endometase
MMP	Matrix metalloproteinase
ELISA	Enzyme-linked immunosorbent assay
ACOG	American College of Obstetricians and Gynecologists
PLT	Platelet
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
LDH	Lactate dehydrogenase

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PI	Pulsatility index
IUGR	Intrauterine growth restriction
CV	Coefficient of variation
ROC	Receiver operating characteristic

Hypertension that occurs after the twentieth week of pregnancy, in which proteinuria or other systemic signs are not observed, is defined as gestational hypertension (GHT)[1]. It is diagnosed as preeclampsia (PE) when end-organ damage, HELLP syndrome or prodromal symptoms, such as occipital headache, visual findings, and epigastric pain, are present[2,3]. PE is characterized by poor remodeling of uteroplacental arteries and inadequate trophoblast invasion[4], leading to systemic hypertension and organ damage. This study investigated endometase, a matrix metalloproteinase (MMP) involved in tissue remodeling[5,6], as a potential biomarker for PE and GHT. Endometase/MMP-26 is expressed in the endometrium and placenta. Qiu et al. examined the relationship between endometase and trophoblastic remodeling for the first time and reported that endometase expression might play a role in remodeling the spiral arteries of the uterus and placental trophoblasts[7]. Considering that defective endovascular trophoblast invasion and inadequate remodeling of the uterine spiral arteries are associated with the development of hypertensive diseases during pregnancy, is there a deficiency in endometase expression in hypertensive pregnant women? Various studies have been conducted to examine the relationship between PE and the MMP family. These studies have generally examined the levels of different members of the MMP family in different tissues, such as placenta and umbilical cord blood, in PE patients[8–13]. Studies have also focused on the role of genotype in the production of some MMPs in PE[14,15]. There are no studies in the literature on the relationship between endometase levels obtained via enzyme-linked immunosorbent assay (ELISA) in maternal blood samples during pregnancy and hypertensive diseases during pregnancy. We hypothesize that blood endometase levels differ between women with hypertensive disorders and those with healthy pregnancies.

PE and GHT are significant causes of maternal and fetal morbidity and mortality[16,17]. Approximately 76,000 women and 500,000 infants die each year worldwide from these diseases[18]. Endometase levels may be important in ensuring early diagnosis of PE/GHT diseases during pregnancy, thus reducing mortality and morbidity rates with appropriate follow-up and treatments. In light of this information, the aim of this study was to compare the blood endometase levels of pregnant women with hypertensive disorders (PE/GHT) with those of healthy pregnant women and, in this way, evaluate whether plasma endometase values could play a predictive role in PE or GHT diagnosis.

Methods

The study was planned and completed as a prospective cohort study. This study was performed in accordance with the principles of the Declaration of Helsinki. Informed consent in accordance with national legislation and institutional requirements was obtained from all patients before each procedure. Approval was granted by the Ethics Committee of Basaksehir Cam and Sakura City Hospital (date: 13.12.2023, number: 650, protocol no: 2023 – 650).

Inclusion and exclusion criteria

Pregnant women who were 26–32 years of age, over 20 weeks gestation, and not in active labor were included. The exclusion criteria included multiple pregnancies, smoking, chronic hypertension, chronic diseases, autoimmune disorders, and fetal anomalies.

Study design

This study was conducted at Basaksehir Cam and Sakura City Hospital between December 2023 and May 2024. All cases were selected from women who were over 20 weeks pregnant after a complete physical and obstetric examination. Patients who met the study criteria and agreed to participate in the study between the specified dates were included in the study. Blood endometase levels were evaluated in 30 pregnant women who were diagnosed with GHT, 30 pregnant women who were diagnosed with PE, and 30 healthy pregnant women in the control group. While diagnosing GHT or PE, the American College of Obstetricians and Gynecologists (ACOG) preeclampsia diagnostic criteria were used as the basis. Hypertension ($\geq$140/90) that occurred after the 20th week of pregnancy and required medical treatment was classified as GHT. In GHT patients, there was no proteinuria or deterioration in laboratory findings. Patients with GHT were classified as having PE if they had proteinuria (> 300 mg/day), prodromal symptoms (headache, blurred vision, epigastric tenderness), end-organ damage, or HELLP syndrome findings[19,20]. Albumin, platelet (PLT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and uric acid levels were recorded from 4 mm venous blood samples taken routinely from the outpatient clinic controls of all pregnant women. Spot urine protein/creatinine ratios were recorded. Endometase values were studied from the plasma of the same blood samples. Endometase values were recalculated according to the albumin values of all patients, and corrected endometase (cEndo) values were obtained.

All pregnant women were scanned with Doppler ultrasonography. Doppler scanning was performed by the same researcher with color Doppler ultrasound (Hitachi, Tokyo, Japan) and a C253 transducer (curvilinear probe) at a frequency of 1–5 MHz. The uterine artery pulsatility index (PI) was recorded separately for the left and right sides. The bilateral uterine artery mean PI values were calculated. Umbilical artery PI values were obtained from the free-floating part of the umbilical cord. For all three groups, umbilical artery changes (normal, increased resistance, loss of diastolic flow, presence of reverse flow) and bilateral uterine artery changes (presence of notch, absence of notch) were recorded.

In addition, participants' gestational weeks, birth weeks, baby birth weights, 1st minute Apgar and 5th minute Apgar scores, and intrauterine growth restriction (IUGR) evaluations were recorded. For the differential

diagnosis of PE and GHT, the cutoff points for the albumin, endometase, cEndo, bilateral uterine artery mean PI and umbilical artery PI parameters were determined. The sensitivity and specificity values of these cutoff points were determined. According to the obtained values, how the abovementioned parameters could play a role in differential diagnosis was analyzed. By applying these parameters together, a demo calculation system that can be effective in the differential diagnosis of PE and GHT was formulated.

Laboratory methods and corrected endometase

Endometase levels were measured via the Human Matrix Metalloproteinase-26 (MMP26) ELISA test kit (BT Lab, Shanghai, PRC). The assay's reported coefficient of variation (CV) for both within-run and within-day measurements is less than 10%. Blood samples were collected into tubes containing EDTA K2 as an anticoagulant and subsequently centrifuged at 1500 × g for 15 min to separate the plasma. The plasma samples were then aliquoted into secondary tubes and stored at -80 °C with sealed caps until analysis.

Two important factors affect intravascular volume in PE patients. One of these factors is endothelial damage under the influence of trophoblastic mediators. The second is the decrease in the blood albumin level[21–24]. As a result, intravascular hypovolemia and hemoconcentration occur in PE patients. The change in blood parameters caused by hypovolemia due to a decrease in the albumin concentration can be corrected by referring to the albumin level. However, determining the severity of endothelial damage and how much fluid loss occurs due to endothelial damage is not possible. In our study, to minimize the effect of the hemoconcentration factor in PE patients, the blood endometase values of all participants were recalculated using albumin values as a reference. Corrected endometase values were obtained via the following formula: plasma endometase × albumin/100. There has been no reference study in the literature related to which albumin value would be considered normal on the basis of endothelial damage-induced hypovolemia, so a value of 100 was used in the denominator to ensure standardization. In this way, the endometase values of all patients were standardized to the same constant value. Analyses were performed according to both the plasma endometase and cEndo values.

Statistical analysis

A power value of 0.80, a margin of error of 0.05 and a sample size of 90 patients (3 groups) were used to determine the effect size. The sample size was determined on the basis of a reference study in the literature as 90 patients and 3 groups[8]. The effect size was determined to be 0.33 on the basis of the statistical tests required for multiple group comparisons via the gpower3.1 software program (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The Shapiro–Wilk test was used to evaluate whether the variables were normally distributed. Variables with a normal distribution are shown as the means ± standard deviations. Independent sample t tests were used for comparisons between two independent groups, and one-way analysis of variance (ANOVA) was used for comparisons between three or more independent groups. Variables that did not have a normal distribution are shown with median (minimum-maximum) values. The Mann–Whitney U test was used for comparisons between two independent groups, and the Kruskal–Wallis H test was used for comparisons between three or more independent groups. Categorical variables are presented as frequencies (n) and percentages (%), and Pearson's chi-square and Fisher's exact tests were used for comparisons. The relationships between variables were examined using the Spearman correlation coefficient. Receiver operating characteristic (ROC) curve analysis was performed to calculate the cutoff value. The optimal cutoff value, 95% confidence interval and area under the curve (AUC) were calculated using the Youden j index. Statistical analyses were performed using the IBM SPSS Statistics 22.0 program. The significance level was set at a p value of 0.05.

Results

Considering the inclusion and exclusion criteria, 90 pregnant women were evaluated. Thirty pregnant women who were diagnosed with preeclampsia, 30 pregnant women who were diagnosed with gestational hypertension and 30 healthy pregnant women were included in the study.

Evaluation of patients' clinical characteristics

When the PE, GHT and healthy control groups were compared according to their clinical characteristics, no statistically significant difference was found between the ages of the pregnant women ($p > 0.05$) (Table 1). The mean systolic and diastolic blood pressure values of patients diagnosed with PE and GHT were significantly greater ($p < 0.05$) (Table 2). The birth week and newborn weight values of the PE group were significantly lower ($p < 0.05$). Compared with those in the other two groups, the 1-minute Apgar score was lower and pathological (6/10) in the PE group ($p < 0.05$). However, the 5-minute Apgar scores were within normal limits (≥ 8/10) in all three groups. There was no statistically significant difference between the GHT group and the healthy control group in terms of birth week, newborn weight or 1st minute Apgar score ($p > 0.05$). Pregnant women in the GHT or healthy group generally reached term (Table 1).

Comparison of the endometase values

In the comparison between groups, no significant difference was observed in the blood endometase values ($p > 0.05$). The cEndo value was significantly lower in the PE and GHT groups than in the control group ($p < 0.05$). There was no statistically significant difference in the cEndo values between the PE and GHT groups ($p > 0.05$) (Table 2). On the basis of the gestational week at which blood samples were taken from 60 patients diagnosed with PE or GHT, the median week was determined to be 32 weeks and 3 days (20–40 weeks). When the cEndo values of the early-week (≤ 32 weeks 3 days) and late-week (> 32 weeks 3 days) groups were compared, no statistically significant difference was detected ($p > 0.05$) (Table 3). When the population was limited to participants whose gestational age was 32 weeks 3 days and earlier, a statistically significant difference was detected between the cEndo values of the PE/GHT groups and the control group ($p < 0.05$) (Table 4).

	Preeclampsia ($n=30$)	Gestational Hypertension ($n=30$)	Control ($n=30$)	p value	Difference
Albumin**	32(22–45)	36(32–39)	36(30–41)	< 0.001	2–3 > 1
Protein/Creatinine Ratio in Spot Urine**	2086(231-11623)	142(86–299)	129(64–350)	< 0.001	1 > 2–3
Platelet*	237.77 ± 73.58	232.43 ± 67.27	235.53 ± 62	0.954	
AST**	23.5(6-119)	16.5(9–41)	15(8–39)	< 0.001	1 > 2–3
ALT**	13.5(5-301)	11(6–53)	9.5(4–31)	0.065	
LDH**	296(140–818)	232.5(134–372)	199.5(129–357)	< 0.001	1 > 2–3
Uric acid**	5.9(2.4–9.9)	4.2(1.9–7.7)	3.1(2-4.5)	< 0.001	1 > 2 > 3
Age*	31.13 ± 6.36	30.37 ± 6.52	27.87 ± 5.33	0.100	
Birth Week**	31.5(24–37)	37(33–40)	38(25–40)	< 0.001	2–3 > 1
Newborn Weight*	1391.61 ± 568.26	2867.31 ± 605.72	2728.33 ± 704.05	< 0.001	2–3 > 1
1st Minute APGAR Score**	6(0–8)	7.5(3–9)	8(3–9)	< 0.001	2–3 > 1
5th Minute APGAR Score**	8(0–9)	8.5(7–10)	9(6–10)	< 0.001	3 > 1–2

Table 1. Comparison of blood parameters and neonatal parameters between the groups. $p < 0.05$, *One-way analysis of variance (ANOVA), **Kruskal Wallis H Test.

		Preeclampsia		Gestational Hypertension	Control	p value	Difference
Average Systolic Blood Pressure*		142(103–172)		136.5(108–161)	110(90–124)	< 0.001	1–2 > 3
Average Diastolic Blood Pressure*		87(60–110)		83.5(60–96)	69(57–83)	< 0.001	1–2 > 3
Endometase*		11.3(1.91–15.98)		10.56(0.47–21.81)	13.81(0.52–35.95)	0.299	
Corrected Endometase*		3.384(0.5921-5.7)		3.9018(0.1598–7.6335)	5.08(0.1976–13.661)	0.016	3 > 1–2
Umbilical Artery PI*		1.23(0.56–2.5)		0.95(0.63–1.42)	1(0.49–2.2)	< 0.001	1 > 2–3
Bilateral Uterine Artery Mean PI*		1.58(0.65–2.48)		1.01(0.59–2.64)	0.91(0.6–1.76)	< 0.001	1 > 2–3
Umbilical Artery Changes**	Normal	n	17	29	28	< 0.001	
		%	56.6%	96.7%	93.3%		
	Increased Resistance	n	5	1	2		
		%	16.7%	3.3%	6.7%		
	Diastolic Flow Loss	n	6	0	0		
		%	20.0%	0.0%	0.0%		
	Presence of Reverse Flow	n	2	0	0		
		%	6.7%	0.0%	0.0%		
Bilateral Uterine Artery Changes***	Notch Absent	n	15	25	27	0.001	
		%	50.0%	83.3%	90.0%		
	Notch Present	n	15	5	3		
		%	50.0%	16.7%	10.0%		

Table 2. Comparison of blood pressure, blood endometase, cEndo and doppler findings between the groups. $p < 0.05$, *Kruskal Wallis H Test, **Chi-square Fisher's exact test, ***Pearson's chi square test.

	Birth Week	n	Median (Min-Max)	Z	p value
cEndo	≤ 32 + 3	31	3.2973(0.1872–6.2208)	− 0.777	0.437
	> 32 + 3	29	3.7059(0.1598–7.6335)		

Table 3. Examination of cEndo values of PE and GHT groups in early/late pregnancy period. $p < 0.05$, Mann-Whitney U Test, cEndo: Corrected Endometase.

Group	*n*	Mean ± SD	t	*p* value
PE + GHT	31	3.0119 ± 1.7575	−3.365	0.002
Control	25	5.4818 ± 3.3132		

Table 4. Examination of cEndo values of participants with gestational age ≤ 32 weeks 3 days. $p < 0.05$, Independent Samples t-test.

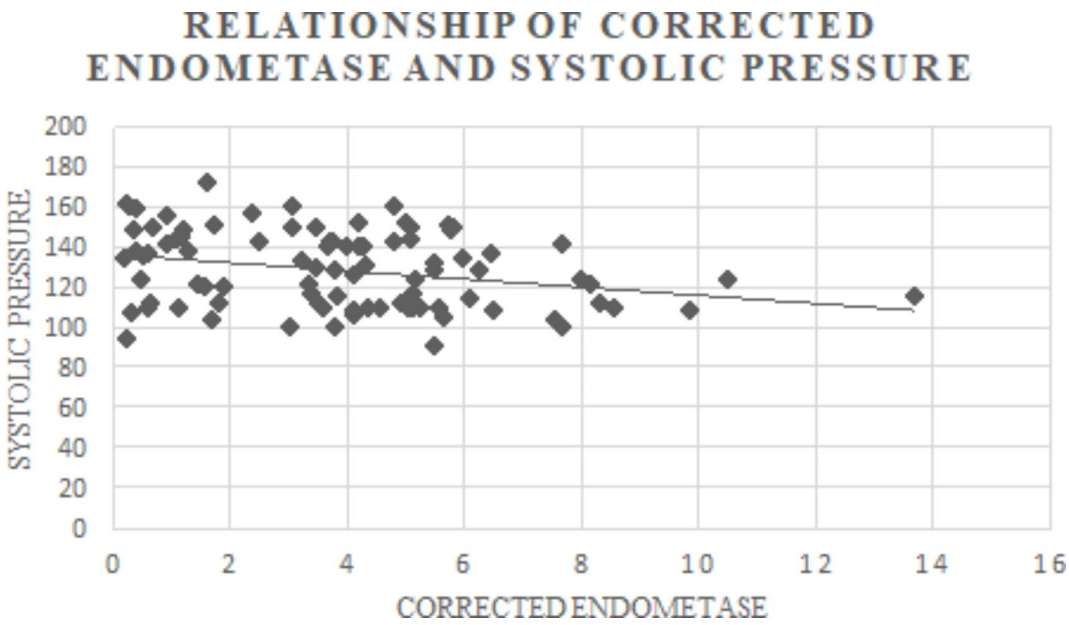

Fig. 1. Relationship of corrected endometase and systolic pressure.

Comparison of laboratory parameters between groups

Albumin was significantly lower in the PE group ($p < 0.05$). The albumin values of the GHT patients and healthy participants were similar. The protein/creatinine ratio in spot urine and the AST, LDH and uric acid values were significantly greater in the PE group ($p < 0.05$). In the comparison between groups, no significant difference was observed in terms of ALT or PLT values ($p > 0.05$) (Table 1).

Comparison of the Doppler findings between groups

The umbilical artery PI and bilateral uterine artery mean PI values were significantly greater in the PE group ($p < 0.05$). There was no significant difference in Doppler PI values between patients diagnosed with GHT and healthy participants ($p > 0.05$). In the PE group, umbilical artery Doppler changes were observed as increased resistance in 5 patients, diastolic flow loss in 6 patients, and reverse flow in 2 patients, with a significant difference ($p < 0.05$). Only an increase in resistance was observed in the GHT and control groups. No reverse flow or diastolic flow loss was observed. Notching was observed in the bilateral uterine arteries in 50% of the PE group. Notching was observed in 16.7% of the GHT group. The presence of notching was significantly greater in the PE group than in the other two groups ($p < 0.05$) (Table 2).

Comparison of the corrected endometase values with other parameters

A statistically significant negative linear relationship was detected between cEndo values and the mean systolic blood pressure, mean diastolic blood pressure and age parameters ($p < 0.05$) (Figs. 1 and 2). There was no significant difference between the cEndo values of all participants and birth week, birth weight, 1st minute Apgar or 5th minute Apgar scores ($p > 0.05$) (Table 5). No statistically significant differences were detected between the cEndo values of all participants and umbilical artery changes, bilateral uterine artery changes or IUGR parameters ($p > 0.05$) (Table 6). In the intragroup evaluations, the cEndo values did not have a predictive role in umbilical artery changes, bilateral uterine artery changes or IUGR parameters ($p > 0.05$). No statistically significant relationship was found between the cEndo value and gravida number in the PE group ($p > 0.05$).

ROC curve results

Since the cEndo values were similar in the PE and GHT groups, no distinguishing cutoff value was determined ($p > 0.05$). The best cutoff point for albumin values in distinguishing the PE and GHT groups was 33.5 g/l (sensitivity 80%, specificity 86.7%, confidence interval 95%), the best cutoff point for umbilical artery PI values was 1.063 (sensitivity 73.3%, specificity 73.3%, confidence interval 95%), and the best cutoff point for bilateral

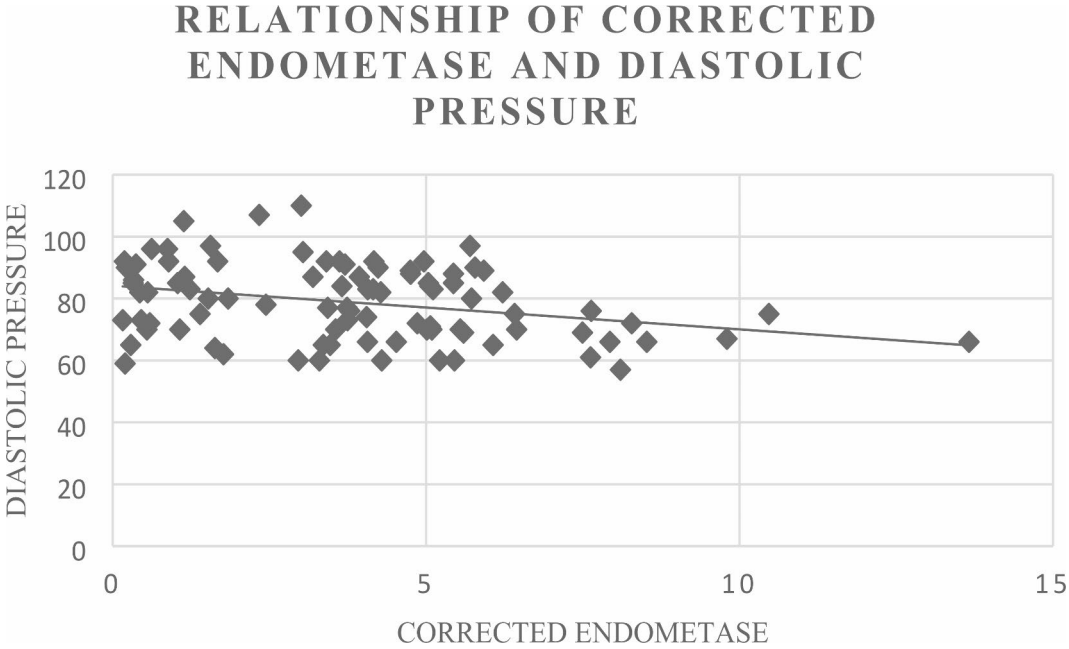

Fig. 2. Relationship of corrected endometase and diastolic pressure.

		Corrected Endometase
Umbilical Artery PI	r	0.027
	p	0.800
Bilateral Uterine Artery Mean PI	r	−0.043
	p	0.688
Average Systolic Blood Pressure	r	−0.269
	p	0.010
Average Diastolic Blood Pressure	r	−0.275
	p	0.009
Age	r	−0.264
	p	0.012
Birth Week	r	0.089
	p	0.437
Birth Weight	r	0.031
	p	0.784
1st Minute APGAR Score	r	0.125
	p	0.302
5th Minute APGAR Score	r	0.086
	p	0.477

Table 5. Relationship of corrected endometase values with Doppler PI and clinical parameters. $p < 0.05$, Spearman's correlation analysis.

uterine artery mean PI values was determined to be 1.36 (sensitivity 73.3%, specificity 80%, confidence interval 95%) (Table 7).

The best cutoff point for albumin values in distinguishing the PE and control groups was 34.5 g/l (sensitivity 90%, specificity 73.3%, confidence interval 95%), the best cutoff point for umbilical artery PI values was 1.14 (sensitivity 56.7%, specificity 83.3%, confidence interval 95%), and the best cutoff point for bilateral uterine artery mean PI values was determined to be 1.155 (sensitivity 83.3%, specificity 86.7%, confidence interval 95%) (Table 7).

The best cutoff point for cEndo values in distinguishing the PE and control groups was 5.06345 (sensitivity 96.7%, specificity 53.3%, confidence interval 95%), and the best cutoff point for cEndo values in distinguishing the GHT and control groups was 1.0526 (sensitivity 43.3%, specificity 90%, confidence interval 95%). Since no

		n	Median (Min-Max)	*p* value
Umbilical Artery Changes*	Normal	74	3.9338(0.1598–13.661)	0.875
	Increased Resistance	8	3.8538(2.3374–6.0684)	
	Diastolic Flow Loss	6	3.35265(1.1488–4.964)	
	Presence of Reverse Flow	2	2.296(1.232–3.36)	
Bilateral Uterine Artery Changes**	Notch Absent	67	3.7059(0.1872–13.661)	0.307
	Notch Present	23	4.2339(0.1598–8.5206)	
Intrauterine growth restriction**	Present	21	1.6302(0.1598-5.70)	0.513
	Absent	69	4.2339(0.1872–13.661)	

Table 6. Examination of corrected endometase values in terms of Doppler changes and intrauterine growth restriction. $p < 0.05$, *Kruskal Wallis H Test, **Mann Whitney U Test.

	AUC (%95 Confidence Interval)	*p* value
PE + GHT		
Corrected Endometase	0.507(0.342–0.671)	0.929
Albumin	0.874(0.777–0.972)	< 0.001
Umbilical Artery PI	0.786(0.668–0.903)	< 0.001
Bilateral Uterine Artery PI	0.795(0.678–0.912)	< 0.001
PE + Control		
Corrected Endometase	0.701(0.559–0.843)	0.007
Albumin	0.871(0.777–0.964)	< 0.001
Umbilical Artery PI	0.742(0.614–0.870)	0.001
Bilateral uterine Artery PI	0.887(0.798–0.976)	< 0.001
GHT + Control		
Corrected Endometase	0.672(0.536–0.809)	0.022
Albumin	0.598(0.450–0.747)	0.191
Umbilical Artery PI	0.429(0.282–0.577)	0.348
Bilateral uterine Artery PI	0.591(0.445–0.737)	0.225

Table 7. ROC analysis results to distinguish PE and GHT groups.

significant differences were detected in the albumin, mean PI values of the umbilical artery or bilateral uterine artery in distinguishing the GHT and control groups, no cutoff value was determined ($p > 0.05$) (Table 7).

When the participants in the PE and control groups were classified according to the cutoff value of 5.06345 determined by the ROC analysis of cEndo, no statistically significant difference was observed in notch status ($p > 0.05$). When the participants in the GHT and control groups were classified according to the cutoff value of 1.0526 determined by the ROC analysis of cEndo, no statistically significant difference was observed in notch status ($p > 0.05$).

Discussion

According to our study results, the blood endometase value was not predictive of hypertensive disease during pregnancy. cEndo values obtained on the basis of albumin values were predictive of distinguishing pregnant women with hypertensive diseases from healthy pregnant women. cEndo values confirmed the hypertensive disorder of pregnancy but did not play a role in the differential diagnosis between PE and GHT. Similar cEndo values were observed between those who were < 32 weeks and those who were > 32 weeks pregnant. cEndo level was not affected by gestational week in early- or late-onset hypertensive disorders. As the mean systolic blood pressure and mean diastolic blood pressure increased, the cEndo value decreased linearly. Lower cEndo values were observed to be compatible with more severe clinical findings. The 33.5 g/l albumin cutoff value distinguished patients diagnosed with PE from those diagnosed with GHT with 80% sensitivity. A value of 1.063 for the umbilical artery PI and 1.360 for the bilateral uterine artery mean PI distinguished PE from GHT, with a sensitivity of 73.3%. In terms of Doppler changes, advanced Doppler abnormalities, such as reverse flow or end diastolic flow loss, were detected only in the PE group. There was no relationship between cEndo values and uterine artery Notch positivity (between groups or within groups).

The cEndo cutoff value of 5.06345 distinguished patients diagnosed with PE from the control group with 96.7% sensitivity. However, a low specificity of 53.3% was detected. The cEndo cutoff value of 1.0526 distinguished patients diagnosed with GHT from the control group with 90% specificity. However, a low sensitivity of 43.3% was observed. All the parameters were effective to some extent in the diagnosis of GHT or PE, but they could not reach sufficient sensitivity or specificity levels on their own. In addition, interpretations made by evaluating a single parameter may not be safe because different diseases other than PE/GHT may affect

that parameter. Therefore, a demo calculation system was created by our team to understand whether the cEndo value, Doppler measurements and albumin parameters together could play a role in the diagnosis of PE with increased sensitivity and specificity. In this calculation system, the albumin, umbilical artery PI, bilateral uterine artery mean PI and cEndo value parameters were used as the basis. In the formula, cube values were taken to increase the power of the Doppler and albumin parameters, which have higher sensitivity in the diagnosis of PE. Since cEndo values play a role in distinguishing between hypertensive disorders and healthy pregnant women, they play an important role in increasing the specificity of the formulation. By placing the parameters directly proportional to the diagnosis of PE in the numerator and inversely proportional to the diagnosis of PE in the denominator, the formula.

"(bilateral uterine artery mean PI × umbilical artery PI)^3/(albumin^3 × cEndo)".

was obtained. To obtain a constant coefficient value, the equation was equalized to 1.

"(bilateral uterine artery mean PI × umbilical artery PI)^3/(albumin^3 × cEndo) × constant coefficient = 1",

Constant coefficient = 1 × (albumin^3 × cEndo)/(bilateral uterine artery mean PI × umbilical artery PI)^3.

In order to all the results correspond to values below 1 for healthy pregnant women and pregnant women diagnosed with GHT and correspond to values above 1 for pregnant women diagnosed with PE; the following values were used in the constant coefficient formulation:

- Since the albumin value plays a role in distinguishing the PE group from both the healthy controls and GHT group, the mean value of the albumin values of the PE group was taken as the basis: 30.83.
- Since the cEndo value plays a role in distinguishing the hypertensive disease groups from the healthy group, the mean value of the cEndo values of the PE and GHT groups was taken as the basis: 3.174556667.
- Since the bilateral uterine artery mean PI value plays a role in distinguishing the PE group from both the healthy and GHT groups, the mean value of the bilateral uterine artery mean PI value of the PE group was taken as the basis: 1.602333333.
- Since the umbilical artery PI value plays a role in distinguishing the PE group from both the healthy and GHT groups, the mean value of the umbilical artery PI value of the PE group was taken as the basis: 1.318. Since the mean values of the PE group were used in the parameters, the exact value of 1 obtained as a result of the calculation was accepted for the diagnosis of PE. When the relevant values were calculated, the constant coefficient value was 9876.4035122572. The constant coefficient was subsequently added to the numerator value in the formula to make the formulation equal to 1. As a result, a demonstration test called McSH–PE was produced.

McSH–PE TEST = [(Bilateral Uterine Artery Mean PI × Umbilical Artery PI)^3/(Albumin^3 × cEndo)] × 9876.4035122572.

With the demo McSH-PE test, it was mathematically formulated that values of 1 and above 1 predict the diagnosis of PE. When all study groups (90 participants together) were re-evaluated with the McSH-PE test, PE patients could be detected with 76.66% sensitivity and 95% specificity, without the need for any additional clinical or laboratory parameters. The positive predictive value of the McSH-PE test (the probability that pregnant women diagnosed with PE by the test are actually sick) was 88.5%, and the negative predictive value was 89.1%. Considering its positive predictive and negative predictive power, the McSH-PE test has shown very high success rates (Table 8). In this way, the cEndo value, in addition to its role in the diagnosis of hypertensive disease (GHT + PE), can play an effective role in the differential diagnosis of PE and GHT with combined tests such as the McSH-PE test. Thus, undiagnosed pregnant patients who were not previously in the hypertensive patient group could be identified at an earlier stage. Appropriate patient follow-up can be performed, and preventive measures can be taken before a clinically life-threatening eclamptic or hypertensive crisis occurs.

The importance of MMPs in PE has recently begun to be recognized. In a study focused on the importance of genotype in PE disease, the MMP-8 TT genotype, one of the matrix metalloproteinases, was significantly greater in pregnant women with PE and was associated with a risk of developing severe PE during pregnancy[14]. In another study, the MMP-2, MMP-9, MMP-14, MMP-15 and MMP-26 gene expression levels in pregnant women diagnosed with severe PE and healthy pregnant women were compared via quantitative reverse transcription PCR from plasma. MMP-2, MMP-9, and MMP-15 gene expression increased significantly in the PE group. A decrease in MMP-14 gene expression and a relative increase in MMP-26 were detected[15]. The results of these studies show that different members of the MMP family follow different courses in the pathophysiology of PE. In a study comparing pregnant women diagnosed with early- or late-onset severe PE with healthy pregnant women, MMP-2 and MMP-9 levels were evaluated in maternal plasma. The MMP-2 level was found to be significantly high, and the MMP-9 level was found to be significantly low in pregnant women diagnosed with severe PE[8]. While the results of the last two studies regarding MMP-2 are compatible, their results regarding MMP-9 differ. In a different study that investigated MMP-2 in maternal plasma, the MMP-2 level increased significantly in PE disease[9].

McSH - PE	PE	GHT + C	TOTAL
≥ 1	23	3	26
< 1	7	57	64

Table 8. Patient distribution according to McSH – PE test formulation. PE: Preeclampsia, GHT: Gestational Hypertension, C: Control.

In a study on MMP-9 in the placenta, the protein expression of MMP-9 was found to be downregulated in the placentas of PE patients[10]. In a study that evaluated MMP-9 and MMP-2 levels in the placenta via real-time PCR, both MMP-9 and MMP-2 levels were found to be low in PE patients[11]. Similarly, in another study, MMP-9 expression was found to be downregulated in the placenta[12].

A study that investigated the levels of MMP-7 and MMP-26 in fetal umbilical cord blood revealed that the level of MMP-26 in umbilical cord blood increased in patients with PE, whereas the level of MMP-7 did not change. The high endometase value observed in the umbilical cord in this study may be due to the hemoconcentration factor we considered in our study. Unlike other studies, this study sampled fetal umbilical cord blood instead of maternal plasma or placenta[13]. In general, studies have been conducted on different tissues, such as maternal blood, umbilical cord blood or the placenta. Although different results have been reported in studies investigating the same MMP members in PE patients, studies on the same tissue sample have yielded similar results. These findings suggest that MMPs may have different effects on PE depending on the tissue in which they are located.

Endometases were investigated in maternal blood in our study. In the preliminary results, no increase or decrease in endometase values in maternal plasma was detected. However, when corrections were made according to the hemoconcentration factor, endometase was found to be significantly lower in the hypertensive disease groups. These results confirm the idea that "there may be a deficiency in the expression of endometase, which plays a role in spiral artery and placental trophoblast remodeling, in the early process of hypertensive diseases during pregnancy." Additionally, our study results support the results of study that have investigated the role of endometase in trophoblast remodeling. There is a need for further studies on the week of pregnancy in which maternal blood endometase values of participants with hypertension begin to be lower than those of normal pregnant women, particularly studies examining trophoblastic changes related to these periods.

Strengths of the study

This study is the first to analyze Endometase/MMP-26 levels in maternal blood samples from hypertensive patients via ELISA. It has been shown for the first time that the maternal blood endometase value during pregnancy may play a predictive role in hypertensive disorders during pregnancy. In addition, given the importance of intravascular volume loss, especially in PE patients, the necessity of correcting endometase values in accordance with the hemoconcentration factor is shared for the first time in the literature. The relationships between endometase values and Doppler findings were also analyzed for the first time.

Limitations of the study

The main purpose of our study was to reveal the relationships of endometase values with PE and GHT diagnoses and their role in differential diagnosis. The information we obtained from the analysis of the roles in the differential diagnosis of PE obtained through participant data was combined with the McSH-PE test formulation that we developed as a demonstration. With this combined test evaluation, patients whose albumin values were above the cutoff value and therefore for whom a diagnosis of PE could not be predicted were diagnosed with PE on the basis of Doppler findings. And it was confirmed that the patients were indeed in the PE study group. Healthy pregnant women were successfully excluded from the disease groups because of the effect of cEndo in the combined evaluation. The primary purpose of this study was not to develop a method such as the McSH-PE test for the diagnosis of PE. We present the McSH-PE test formulation, which we define as a demonstration, to provide ideas and guidance to new researchers. For this test to be used as a direct diagnostic tool, validation studies should be conducted in large patient populations and should be repeated with new studies in populations of different races and cultures. Although the specificity value of the test is quite high, we believe that the sensitivity should be improved with appropriate mathematical methods or additional parameters. For these reasons, our study does not present a diagnostic test tool for PE but offers the idea that a suitable test tool can be developed with relevant parameters. Although our study revealed that the cEndo values of PE or GHT patients were similar in the early or late gestational weeks of pregnancy, the predictive power of cEndo should be fully revealed by comparing the cEndo values of the patients before the clinical and laboratory findings appear with the cEndo values of the same patients after diagnosis. The changes in blood parameters as a result of hypovolemia caused by the decrease in the albumin level was taken into account and minimized in this study. However, the effect of endothelial damage, which is the second main pathophysiological process in PE, on hypovolemia was not calculated in this study. The power of the study will increase if the level of endothelial damage is clearly determined using an appropriate method for measuring hypovolemia. The participants in the study groups consisted of patients who applied to our hospital within a certain historical period, met the study criteria, and agreed to participate in the study. Studies based on large patient groups where randomized patient selection is performed will increase the power of the results by eliminating unknown factors that may affect the results regarding the patient population.

Conclusions

Maternal blood cEndo values may play a successful role in distinguishing hypertensive diseases of pregnancy (PE + GHT) from healthy pregnant women. The cEndo value does not play an effective role in the differential diagnosis of pregnant women diagnosed with PE and GHT. However, PE patients can be identified with high specificity rates by combined evaluations of albumin, umbilical artery PI and bilateral uterine artery mean PI values in addition to cEndo values.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available due to local ethical and legal requirements but are available from the corresponding author on reasonable request.

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Author contributions

Study concept and design were performed by MCS, HTK and SO. Blood samples were collected by SO. Data collection was performed by SO and HTK. Analysis of blood samples was done by AG. Data analysis was performed by MCS, HTK and SO. The first draft of the manuscript was written by HTK. The last draft of the manuscript was written by MCS. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

This study was performed in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Basaksehir Cam and Sakura City Hospital (date: 13.12.2023, number: 650, protocol no: 2023 – 650). All methods were performed following the relevant guidelines and regulations. Written and signed informed consent was obtained from all participants.

Consent for publication

Obtained from all the participants.

Competing interests

The authors declare no competing interests.

Additional information

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