Received: 13 July 2022 | Revised: 3 October 2022 | Accepted: 6 October 2022

DOI: 10.1111/jch.14601

ORIGINAL ARTICLE

WILEY

Serum estradiol to testosterone ratio as a novel predictor of severe preeclampsia in the first trimester

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 82001579; Natural Science Foundation of Tianjin, Grant/Award Number: 21JCQNJC00040; Tianjin Health Research Project, Grant/Award Number: TJWJ2021QN053; Tianjin Central Hospital of Gynecology Obstetrics/Tianjin Key Laboratory of human development and reproductive regulation, Grant/Award Number: 2022XH10

Abstract

Preeclampsia (PE) is the most common medical complication during pregnancy and the second leading cause of maternal death worldwide. However, a better predictive model of PE remains to be explored. A total of 15 severe preeclampsia (sPE) and 75 healthy control patients were included in this study. Patient data was obtained from September 2019 to September 2021. Nuchal translucency (NT) and crown-rump length (CRL) of the fetus were acquired by ultrasound. Maternal blood samples were collected at 11^{+0} to 13^{+6} weeks of gestation. Chemiluminescent immunoassays were used to detect serum testosterone (T) and estradiol (E2) levels. Time-resolved fluorescence analysis was used to examine the levels of serum pregnancy-associated plasma protein A (PAPPA) and β-human chorionic gonadotrophin (β-HCG) protein. The sPE group exhibited increased T levels, and decreased E2 levels and E2/T ratios from 11 to 14 weeks of gestation, compared with the control group. E2 and the E2/T ratio showed positive linear correlation with CRL in pregnant women. Body-mass-index (BMI), T, and E2 were determined to be the main factors that affected the occurrence of sPE at the 12-week gestation period time point. The receiver operating characteristic (ROC) curve revealed that the AUC of the E2/T ratio was .717. The imbalanced T and E2 levels in the patients had a specific intrinsic relevance with sPE, which suggests them as novel predictors of the sPE.

KEYWORDS
estradiol, predictor, preeclampsia, serum markers, testosterone

1 | INTRODUCTION

Preeclampsia (PE) is the most common medical complication during pregnancy and the second leading cause of maternal death worldwide after obstetric hemorrhage.[1] PE is characterized by newly onset hypertension, proteinuria, or dysfunctions in multiple maternal organs after the 20th week of gestation.[2] The most frequently used predictors for PE are medical history, body mass index (BMI), blood pressure, uterine artery pulsatility index (UtA-PI), and maternal age. However, these types of predictors were not clearly associated with model discrimination.[3] Although multiple and widely varying models for preeclampsia prediction have been developed, the high degree of between-study heterogeneity impedes selection of the best model, or an aggregated analysis of prognostic models. Hence, the purpose of

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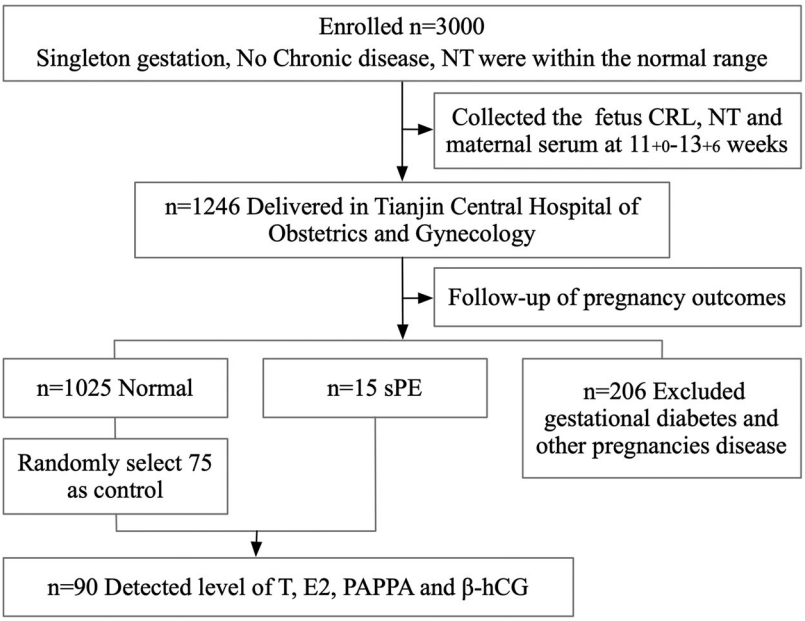

FIGURE 1 Distribution of patients in the control and sPE database.

this study was to establish a predictive model of PE in order to better predict its occurrence at an earlier timepoint.

The placenta is the primary endocrine organ for maintaining pregnancy and fetal growth. During pregnancy, the placenta releases hormones, including testosterone (T) and estradiol (E2). High concentrations of these hormones present in the maternal circulation. The T and E2 hormones are essential paracrine and autocrine regulators influencing the growth and differentiation of the placental trophoblast, the growth and maturation of the placental vascular tree, and uterine endovascular invasion by the extravillous cytotrophoblast.[4,5] As a modulator of uterine vessels, E2 reduces the resistance of the spiral uterine arteries and modulates the synthesis and release of angiogenic factors by placental cells. In contrast, T has the opposite effect.[6,7] Uterine spiral artery remodeling is an important factor in the onset of PE. Hence, we speculated that women with PE exhibited changes in concentrations of serum sex steroid hormones. However, different studies have reported differing levels of T and E2 in PE. Accordingly, changes in the levels of T and E2 in the serum or plasma of PE patients remains a topic of debate.[8–10] Moreover, there are few and inconsistent reports on whether T and E2 levels were abnormal in the first trimester of PE without clinical symptoms. Therefore, further studies that analyze the level of these hormones are required to underpin their significance in PE and severe PE (sPE) prediction.

It was reported that serum pregnancy-associated plasma protein A (PAPPA) levels in the first trimester and β-human chorionic gonadotrophin (β-HCG) in the second trimester were related to PE.[11,12] Whereas, another study demonstrated that serum PAPPA and β-HCG levels showed no obvious differences between PE and control groups at 15–20 weeks of gestation.[13] Crown-rump length (CRL) is associated with fetal gestational age, while the level of E2 and T change with increasing gestational weeks. Therefore, we also sought to analyze whether there was a correlation between E2 or T and CRL. In this study, we investigated predictive factors of sPE in the first trimester. The assessed factors were maternal serum level of T, E2, PAPPA, and β-HCG, and fetal CRL in the sPE patient and control groups. The correlative relationships between these factors and their potential as novel predictors of sPE was then determined. Overall, this study offers unique insights into the prediction and pathogenesis of sPE.

2 | MATERIALS AND METHODS

2.1 | Study patients

The study was conducted with patients that received consultation from the Tianjin Central Hospital of Obstetrics and Gynecology between September 2019 and September 2021. The clinical characteristics of control and sPE groups are displayed in Figure 1. Initially, based on the general PE incidence rate of 6% and the statistical power analysis of .80, the study involved 3000 patients. All women met the conditions of aged ≥18, gestational age (GA) at 11^{+0}–13^{+6} weeks, singleton pregnancy, no other chronic diseases before pregnancy, and normal nuchal translucency (NT). Pregnancy outcomes were obtained through medical records. A total of 206 patients with gestational diabetes and other pregnancies disease were excluded. A total of 15 patients with sPE were selected as the experiment group, and 75 healthy people were randomly selected as the control group. The women in the control group had normal pregnancies and at term deliveries without maternal pathology or complications. The women in the sPE group had no other pregnancy-related diseases. According to the guidelines of the American College of Obstetricians and Gynecologists, the sPE was defined as maternal systolic blood pressure (SBP) ≥160 mm Hg, diastolic blood pressure (DBP) ≥ 110 mm Hg, or both measured on two occasions separated by at least 6 h, and proteinuria > 300 mg/24 h or > 1+, or impaired liver function, or fetal growth restriction (FGR) after 20 weeks of gestation.[14]

TABLE 1 Characteristics of the pregnant women enrolled in this study

	Control ($n = 75$)	sPE ($n = 15$)	Statistics	*P*-value
Age (years)	30.12 ± 3.36	30.17 ± 3.53	$t = -.05$	.961
BMI[a] (Kg/m^2)	22.90 ± 3.39	23.76 ± 3.71	$t = -.91$	.372
GA at blood collection (weeks)	12.26 [11.96–12.74]	12.43 [12.14–12.71]	$Z = -1.15$	.25
SBP at diagnosis (mm Hg)	119.11 ± 5.19	159.44 ± 10.85	$t = -23.72$	<.001***
DBP at diagnosis (mm Hg)	73.91 ± 5.77	102.61 ± 10.96	$t = -15.85$	<.001***
GA at diagnosis (weeks)	—	32.89 [30–37.25]	—	—
GA at delivery (weeks)	39.08 [38–40]	35.94 [33.75–38.25]	$Z = -4.34$	<.001***
Fetal weight (g)	3326.71 ± 455.14	2631.17 ± 991.77	$t = 4.57$	<.001***
Fetal length (cm)	49.96 ± 1.64	47.50 ± 6.15	$t = 2.43$	.018*

Abbreviations: BMI, Body-mass-index; DPB, diastolic blood pressure; GA, gestational age; SBP, systolic blood pressure.
[a]BMI indicates the weight (kg) divided by the square of the height (m).
***$P < .001$; *$P < .05$.

2.2 | Preparation of samples

Individual maternal blood samples were drawn into BD vacutainer® tubes, centrifuged at 4°C with a relative centrifugal force of 3000×g for 10 min, and then transferred to 1.5 ml polypropylene tubes. The serum samples were stored at −80°C until further analysis.

2.3 | Measurements

NT and CRL were measured by GE-Volusion E8 color Doppler ultrasound diagnostic apparatus at a frequency range of 2–5 MHz. Under sterile conditions, blood samples of pregnant women from 11^{+0} to 13^{+6} weeks of gestation were collected in plastic tubes with EDTA as anticoagulant, before centrifugation at 18 472×g for 10 min at 4 °C to separate the serum. Serum samples were stored at − 80 °C until further use. Serum T and E2 were detected using chemiluminescent immunoassays (USCN Inc., USA). Serum PAPPA and β-HCG protein levels were detected by time-resolved fluorescence analysis (Wallac Oy PerkinElmer, Turku, Finland).

2.4 | Statistical analysis

All statistical analyses were performed with SPSS 24.0 software package (IBM, Armonk, New York, USA) or R version 3.5.1 (R Foundation for Statistical Computing). Normal distribution data were calculated as mean ± SD, using the independent sample t-test. Non-normal distribution data were reported as medians (the 25th and 75th percentile), using the Mann-Whitney U test. Chi-square analysis was used to compare the rate of count data between groups (*n*%). The correlation between E2 or T and CRL, PAPPA, or β-HCG were analyzed using parametric Pearson correlation test. The logistics analysis was conducted to screen the factors predicting the incidence of sPE, and the model that incorporated the independent predictors was developed and is presented as the nomogram. The receiver operating curve (ROC curve) was drawn to calculate the predictive efficacy of the research indicators. $P < .05$ were considered as significant differences.

3 | RESULTS

3.1 | Characteristics and plasma profiling of the pregnant women enrolled in this study

The clinical characteristics of the enrolled pregnant women were summarized in Table 1. There was a significant difference in SBP, DBP, GA at delivery, fetal weight, and fetal length between sPE and control groups (Table 1). Differential hormone and protein levels were screened using serum samples obtained at 11^{+0}–13^{+6} gestational weeks from 15 sPE and 75 control pregnant women.

A combination of biochemical data (maternal serum PAPP-A and free β-HCG), CRL, and NT thickness at 11--13^{+6} weeks gestation were used to calculate the risk of fetal aneuploidy, and identified 85%–95% of trisomy 21 pregnancies with a 5% false-positive rate using a risk cut-off of 1/300.[15] As shown in Table 2 and Figure 1, there was no difference in CRL, NT, PAPPA, β-HCG between sPE and control groups with the same fetal sex (Table 2). Compared with the control group, the level of T was not significantly increased, but the level of E2 and the E2/T ratio decreased significantly in the sPE group from 11^{+0} to 13^{+6} weeks throughout gestation (Table 1 and Figure 2).

3.2 | Correlation between T or E2 levels and other factors

sPE patients exhibited different levels of circulating T and E2 at 11–14 gestational weeks. We further analyzed the positive linear correlation between T or E2 concentration and BMI, CRL, PAPPA, or β-HCG. As shown in Table 3, T had no significant correlation with other factors in any group; whereas E2 and E2/T ratio positively correlated with CRL ($r = .342$, $P = .001$; $r = .286$, $P = .006$, respectively). The results

TABLE 2 Plasma profiling in groups with control and sPE

	Control ($n = 75$)	sPE ($n = 15$)	Statistics	*P*-value
CRL (mm)	62.437 ± 8.031	61.767 ± 7.060	$t = .301$	.764
NT (mm)	1.624 ± .480	1.520 ± .441	$t = .776$	.440
PAPPA (mu/ml)	4.100 ± 2.306	3.705 ± 2.158	$t = .611$	.543
β-HCG (ng/ml)	57.625 ± 40.063	54.836 ± 34.213	$t = .252$	.802

Abbreviations: β-HCG, β-human chorionic gonadotrophin; CRL, crown-rump length; NT, Nuchal translucency; PAPPA, pregnancy-associated plasma protein A; sPE, severe preeclampsia.
$^{**}P < .01$; $^{*}P < .05$.

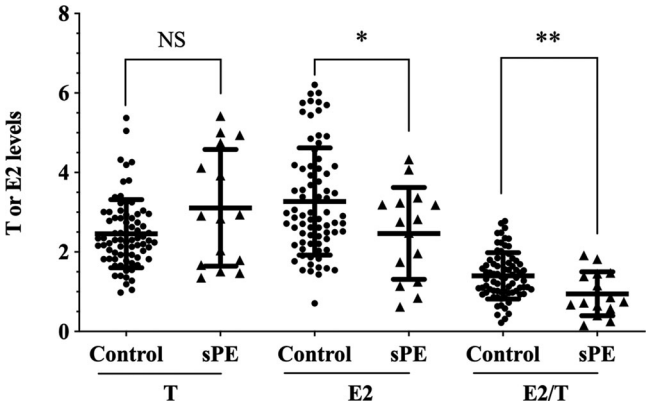

FIGURE 2 Serum T, E2 levels, and E2/T ratio in the first trimester. **, $P < .01$, *, $P < .05$

indicated that the E2 level and E2/T ratio had novel relevance to CRL in the first trimester of pregnancy.

3.3 | Construction of the predictive model for sPE

Logistics regression was applied to analyze the single factor with $P < .1$. The results showed that BMI, T, E2 and E2/T at 12 weeks were the main factors affected the occurrence of preeclampsia (Table 4). Then multi-factor analysis was performed. It is worth noting that the E2/T ratio had novel relevance to CRL in the first pregnancy, thus CRL was included in the multi-factor analysis. Logistics regression analysis showed that the impact of T levels on PE was statistically significant (OR = .251, 95%CI .070–.903, $P < .05$), and the impact of E2 levels on PE was statistically significant (OR = 8.369, 95%CI 1.139–61.499, $P < .05$) (Table 5).

Nomograms display an individual patient's value located on each variable axis, and a line is drawn upward to determine the number of points received for each variable value. The sum of these numbers is located on the total points axis, and a line is drawn downward to the risk axes to determine the risk of PE. The model that incorporated the above independent predictors was developed and is presented as the nomogram in (Figure 3A). We verified the specificity of the factors to predict sPE. As shown in (Figure 2B), the T, E2, E2/T predictor had an area under the ROC (AUROC) curve value of .605, .640, and .717, and sensitivity and specificity of 89.6/48.7, 46.3/86.7, and 86.6/60, respectively (Figure 3B). The results suggested that imbalanced T and E2 levels had a certain intrinsic relevance to sPE patients.

4 | DISCUSSION

While the exact cause of PE remains unknown, a large amount of evidence supported by preclinical PE models indicated that abnormal placentation in the early pregnancy stage was a crucial initial event in the PE onset.[16] This study's main objective was to detect T and E2 and explore factors associated with PE onset in the first trimester, by using maternal serum biochemistry and fetal ultrasound in singleton pregnancies. The results showed that the sPE patients exhibited decreased E2 levels and E2/T ratios, and there were no significant differences in the T, PAPPA, β-HCG levels, and CRL were shown between the sPE and the control groups at 11^{+0}–13^{+6} weeks gestation.

According to statistical analyses, E2 levels were different in preeclampsia. Some studies have reported that plasma or serum E2 concentrations were down-regulated, whereas other studies reported that there were no significant changes in preeclamptic compared to normotensive pregnant women during the first and second

TABLE 3 The correlation between the levels of T or E2 and other factors in pregnant women

	T(nmol/ml)		E2(ng/ml)		E2/T	
	r	*P*-value	R	*P*-value	r	*P*-value
BMI (Kg/m^2) [a]	.101	.345	.065	.545	−.109	.304
CRL (mm)[a]	−.03	.780	.342	.001**	.286	.006**
PAPPA (mu/ml)[a]	.171	.107	.120	.260	−.030	.780
β-HCG (ng/ml)[a]	.034	.749	.201	.058	.080	.410

Abbreviations: BMI, Body-mass-index; β-HC, β-human chorionic gonadotrophin; CRL, crown-rump length; PAPPA, pregnancy-associated plasma protein A.
[a]Pearson's correlation coefficient; **, $P < .01$.

TABLE 4 Logistics regression analysis of single factor affecting sPE

	B	Standard error	Wald	*P*-value	OR	95%CI of OR
BMI	−1.099	.596	3.395	.065	.033	.104–1.073
CRL	.011	.036	.092	.761	1.011	.941–1.086
PAPPA	.036	.411	.007	.931	1.036	.463–2.320
β-HCG	.002	.008	.064	.799	1.002	.987–1.017
T	−.579	.263	4.868	.027*	.560	.335–.937
E2	.542	.265	4.185	.041*	1.720	1.023–2.893
E2/T	1.562	.612	6.513	.011*	4.768	1.4367–15.821

Abbreviations: BMI, Body-mass-index; β-HCG, β-human chorionic gonadotrophin; CRL, crown-rump length; PAPPA, pregnancy-associated plasma protein A.
*$P < .05$.

TABLE 5 Logistics regression analysis of multi-factors affecting sPE

	B	Standard Error	Wald	*P*-value	OR	95%CI of OR
BMI	−.131	.097	−1.34	.180	.547	.227–1.320
CRL	−.035	.043	−.82	.412	.656	.240–1.794
T	−1.235	.584	−2.12	.034*	.251	.070–.903
E2	1.157	.554	2.09	.037*	8.369	1.139–61.499
E2/T	−1.537	1.408	−1.09	.275	.318	.041–2.486

Abbreviations: BMI, Body-mass-index; β-HC, β-human chorionic gonadotrophin; CRL, crown-rump length.
*$P < .05$.

trimesters.[17–19] The majority of published studies have shown the level of T in PE patients at different gestational weeks was generally high, but some reports indicated that there were no changes in the level of T in PE patients.[20–23] Aromatase (CYP19A) is a rate-limiting enzyme for estrogen biosynthesis, which converts T into E2 in the placenta. It was reported that there was no significant difference in CYP19A1 between the plasma of patients with preeclampsia and that of normal people by GC–MS.[24] Compared with the control group, the protein and mRNA content of placental aromatase in the placenta of patients with preeclampsia was shown to be significantly reduced,[9,25,26] but one report showed that the aromatase in placenta of PE was up-regulated at the mRNA level, but there was no significant change at the protein level.[23] At present, few published studies have reported the ratio of E2/T for use in PE prediction. The abnormal changes of aromatase could partially explain the important role of E2/T ratio's balance in PE patients. However, the real reason for the imbalance of E2/T ratio remains to be explored, and whether it could be used to strongly predict the occurrence of PE still needs to be studied with larger sample sets.

It was reported that T and E2 were related to the occurrence of PE, but whether T or E2 was related to PAPPA, β-HCG, or CRL remained to be studied. It was also reported that serum PAPPA levels in the first trimester and β-HCG in the second trimester were associated with PE.[11,12] Furthermore, there was a correlation between maternal serum β-HCG, T levels, and PE previously reported.[27,28] Both β-HCG itself and percentage of predicted β-HCG were shown to have a negative regression on estriol, but not on 17β-estradiol.[29] Nevertheless, another study reported that serum PAPPA and β-HCG levels were not significantly different between the PE and control groups at 15–20 weeks of gestation.[13] Another reported that there was no association between peak E2 and low PAPPA levels or adverse pregnancy outcomes.[30] In the present study, we found that E2 or T had no significant relevance to β-HCG and PAPPA, but E2 and E2/T ratio positively correlated with CRL. A possible reason was that T and E2 change with increasing number of gestational weeks, and CRL size would typically also increase with gestational weeks. However, CRL showed no significant differences between sPE and the control group, which further indicated that the abnormality of T or E2 levels in pregnant women in the early stage of pregnancy may be related to the occurrence of PE. According to reports, BMI was negatively correlated with hormone E2, and a high BMI was strongly associated with PE.[31] However, in our study, there was no linear correlation between BMI and T or E2 in early pregnancy. The E2/T ratio combined with other indicators such as CRL and BMI may help predict PE better, but requires further studies to corroborate this speculation.

Many factors are suggested to affect the onset of PE, such as BMI, age and pre-gestational diabetes.[32] In our study, the logistics regression showed that sPE was related to BMI, T, and E2 at 12 weeks. The ROC curve revealed that the AUC of the E2/T ratio to predict sPE was .717. These results indicated that E2/T imbalance could be a predominant factor in predicting PE, but understandably, other factors may also affect occurrence of PE. A previous study showed that

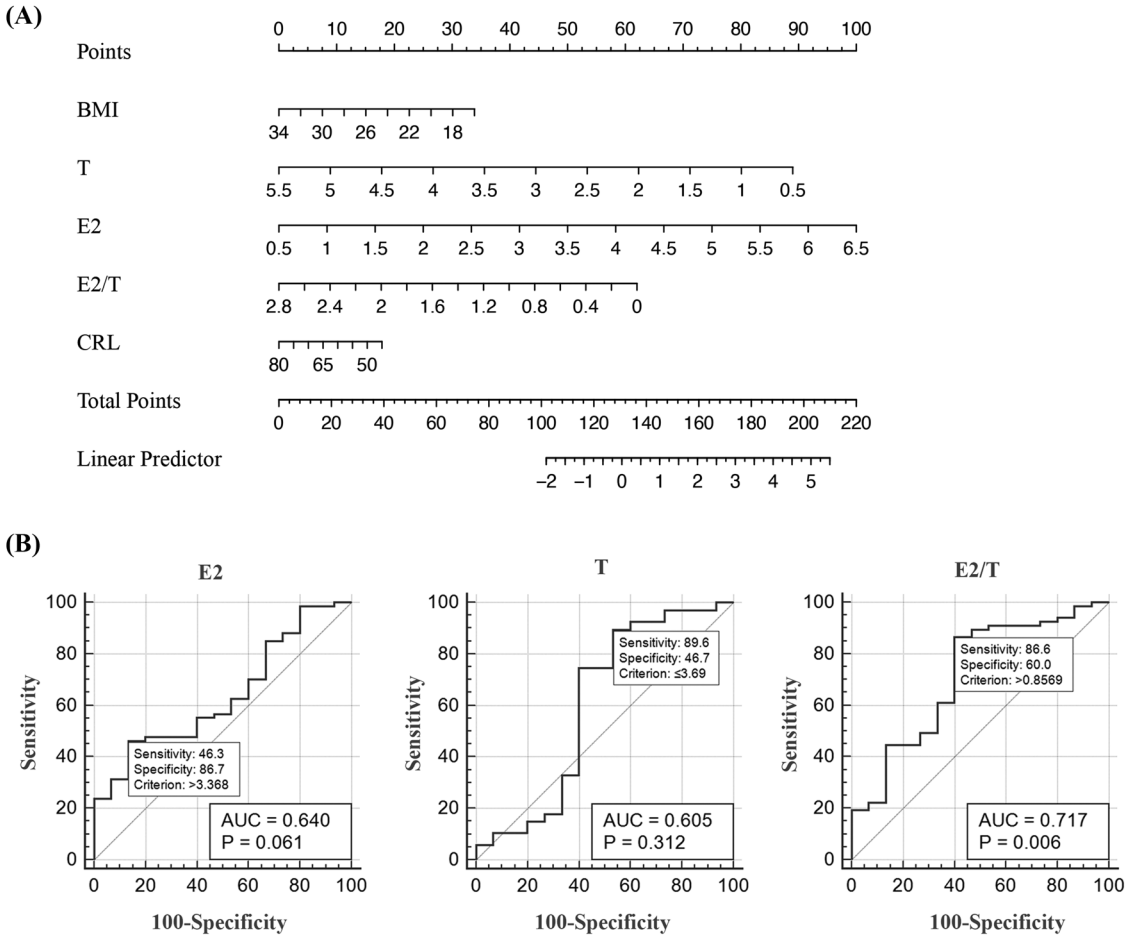

FIGURE 3 Predictive model for sPE. (A). Nomogram for predicting preeclampsia; (B). ROC curve of T, E2, or E2/T predictive sPE.

miR-22-mediated pathways accounted for the imbalanced production of T/E2 in the PE placenta.[9] In addition, the expression of aromatase P450, which converts T to estrogens, was decreased in the sPE placenta.[33] It could be speculated that the E2/T ratio is more indicative of the pathogenesis of PE than the concentration of T or E2 alone. Epidemiological studies demonstrated that fetal sex was associated with different patterns of ultrasonic markers of placentation,[34] placental pathology,[35] the risk of perinatal death,[36] and PE.[28,37,38] Nevertheless, there were also reports that women with early preterm PE (< 34 weeks) had higher odds with a female fetus.[39] It was reported that the levels of T and E2 may be correlated with fetal sex. In contrast, we found that there was no differences in CRL, NT, T, E2, PAPPA, and β-HCG between sPE and control groups with the same fetal sex (data not shown). Therefore, the imbalanced T and E2 levels could have a certain intrinsic relevance to the sPE status in the patients.

It is well-known that E2 and T are associated with vascular remodeling.[40,41] Accordingly, abnormal E2 or T may affect the placental function and lead to PE in the early stage of placental development, but the mechanism remains to be further studied. Our study revealed that sPE may be related to the concentration of T and E2, but especially the balance of E2/T. Considering that the sample size of this study was relatively small after being filtered through strict inclusion and exclusion criteria, and was a single-center trial, the research had certain regional limitations. Predictive models could be further verified by multicenter and large sample data. In summary, we identified serum E2/T ratio as a novel indicator for predicting PE at the early stage of pregnancy, which we believe could hold potential in contributing to clinical decision-making.

AUTHOR CONTRIBUTIONS

All authors contributed to either conceptualization. Software: Yongmei Shen and Jiasong Cao. Resources: Liying Yao. Ultrasonography: Xiaomin Zhao and Lei Zhang. Serum detection: Shanshan Li and Wen Li. Formal analysis: Yongmei Shen and Jianxi Wang. Writing-original draft preparation: Yongmei Shen. Writing-review and editing: Jianxi Wang. Supervision: Jiasong Cao and Zhuo Wei. Project administration: Ying Chang and Liying Yao. Funding acquisition: Yongmei Shen and Zhuo Wei. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

This study was supported by National Natural Science Foundation of China (82001579), Natural Science Foundation of Tianjin (21JCQNJC00040), Tianjin Key Medical Discipline (Specialty)

Construction Project (TJWJ2021QN053), Open Fund of Tianjin Central Hospital of Gynecology Obstetrics/Tianjin Key Laboratory of human development and reproductive regulation (2022XH10).

CONFLICT OF INTEREST

Yongmei Shen, Jiasong Cao, Liying Yao, Shanshan Li, Xiaomin Zhao, Wen Li, Zhuo Wei, Lei Zhang, Jianxi Wang, Ying Chang declare that they have no conflict of interest.

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How to cite this article: Shen Y, Cao J, Yao L, et al. Serum estradiol to testosterone ratio as a novel predictor of severe preeclampsia in the first trimester. *J Clin Hypertens*. 2023;25:53–60. https://doi.org/10.1111/jch.14601