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Prediction of preeclampsia using combination of biomarkers at 18–23 weeks of gestation: A nested case-control study

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ABSTRACT

Objective: To evaluate the combination of plasma activated endothelial microparticles (CD62e), serum Copeptin (CPP) and placental growth factor (PlGF) levels at 18–23 weeks of gestation for prediction of preeclampsia (PE) in primigravid women.

Methods: This was a nested case-control study from a prospective cohort of 1115 primigravid women attending antenatal care clinic. Plasma levels of CD62e and serum Copeptin, PlGF levels were measured by flow cytometry and ELISA, respectively. Data were presented as median (Interquartile range) and biomarker levels were compared between patients and controls using Mann-Whitney Test. Using binary logistic regression, predictive potential of a combination of biomarkers for PE prediction was determined.

Results: Women who developed PE 41 (3.97%) showed significantly increased levels of plasma CD62e [799.33 (546.86–1249.29) versus 384.08 (245.03–576.00), $p < 0.0001$], serum Copeptin [303.42 (226.01–484.18) versus 207.24 (169.73–276.46), $p < 0.0001$] and reduced level of PlGF [238.38 (161.36–312.62) versus 947.21 (466.7–1428.56), $p < 0.0001$] compared to controls at 18–23 weeks of gestation. None of the marker showed statistically significant alteration in levels in fetal growth restriction (FGR) group 68 (6.58%) compared to controls. Using binary logistic regression analysis, AUC, Sensitivity, specificity, PLR, NLR, PPV, and NPV of combination of CD62e, Copeptin and PlGF for prediction of PE at 18–23 weeks of gestation was 0.969, 92.3%, 90.3%, 9.73, 0.08, 79.17%, and 96.94%, respectively.

Conclusion: At 18–23 weeks, Combination of CD62e microparticles, copeptin, and PlGF levels can effectively identify women at risk of developing PE later in gestation.

1. Introduction

Preeclampsia represents the complex obstetric condition, clinically characterized by sudden onset of hypertension and proteinuria after 20 weeks of gestation [1]. It affects 2–8% pregnancies and prevalence are higher in developing countries. It is one of the leading causes of maternal and fetal mortality and morbidity with long term effects on maternal and fetal health [2]. Inadequate trophoblast invasion by maternal spiral artery and subsequent hypoperfusion of placenta and hypoxia hypothesized in the pathogenesis of PE [3,4]. Maternal inflammatory response and vascular endothelial injury further lead to the clinical manifestation of the disorder. Endothelial microparticles are proposed as a new marker for evaluation of endothelial damage [5]. Circulating microparticles (cMPs) are submicronic particles (0.1–1 μm) released from various cells upon activation or apoptosis by cell membrane blebbing [6]. Endothelial dysfunction had been attributed as a probable mechanism for the characteristic pathological changes associated with preeclampsia such as edema, proteinuria, end-organ dysfunctions, etc [7,8]. Endothelial cells upon damage releases microparticles in the circulation. These microparticles exhibit endothelial cell-specific surface markers such as CD31 and CD62e. Presence of endothelial cell-derived cMPs in plasma can indicate endothelial damage [9,10]. Increased levels of endothelial microparticle have been reported in patients with cardiovascular and cerebrovascular disease and levels have been positively correlated with altered blood pressure indicating an association of endothelial microparticles in the assessment of endothelial damage [11,12]. Studies reported elevated levels of CD62E+ and other endothelial cell-derived microparticles in women

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with PE along with association between pathological hallmarks of PE and cMPs levels [13,14]. Thus, suggesting the role of endothelial cMPs in clinical symptoms of PE and need of evaluation of predictive potential in PE prediction.

Various prospective cohort and case-control studies evaluated the decreased concentration of PlGF, alone or in combination with soluble Fms like tyrosine kinase 1 (sFlt-1), as early predictive marker in women with PE across gestational age [15–20]. Also known as anti-diuretic hormone, Arginine vasopressin (AVP) is one of the important hormones of the hypothalamic-pituitaryadrenal axis. The primary function of AVP includes regulation of blood pressure and osmotic homeostasis [21,22]. AVP leads to vasoconstriction via activation of V1a receptors on vascular walls whereas facilitating water reabsorption by V2 receptors on kidney tubules. Several pathways have been proposed to elucidate the association between vasopressin and development of PE [22–24]. However, with a biological half-life of 5–20 min [25] and instability in body fluids hampers the assessment of AVP as a reliable marker. Copeptin is 39 amino acid glycopeptide co-synthesized with AVP in 1:1 ratio and has no physiological known function. Copeptin has been proposed as a surrogate marker for AVP due to its stability in plasma/serum and ease of measurement in body fluids [26–28]. Studies have reported increased maternal levels of copeptin as a biomarker, both before and after clinical diagnosis of PE [29–32].

The objective of present study is to investigate the predictive potential of plasma CD62e, serum copeptin and PlGF, alone and in combination, at 18–23 weeks of gestation for identification of women who are at the risk of development of PE later in pregnancy.

2. Materials and methods

2.1. Study population

The nested case-control study was derived from a prospective cohort comprising primigravid women attending routine antenatal care unit at Nowrosjee Wadia Maternity Hospital, Mumbai, India from March 2014 to June 2017. The details of participant selection are given in Fig. 1. The visit included recording of maternal clinical characteristics such as age, family history of pregnancy complications, mode of conception, obesity, hypertension, diabetes, and other disorders. The gestation age of 18–23 weeks was determined by measurement of fetal crown-rump length at 11–13 weeks of gestation. Women included in the study gave written informed consent for participation. Women with other pre-existing conditions such as gestational diabetes, chronic hypertension, chronic renal failure, hypo/hyperthyroidism or known immunological disorders in the prepregnant state were excluded from the study. For the nested case-control study, patient: control ratio of 1:2 is used for inclusion of controls [33]. Gestational age-matched primigravid women delivering at term, without any pregnancy and pre-pregnancy complications such as hypertension, diabetes, hyper/hypothyroidism, were included as controls.

5 mL each of venous blood was collected into tri-sodium citrated and plain vacutainers at 18–23 weeks of gestation. Platelet-poor plasma for MP analysis was extracted by centrifugation of Citrated blood samples at 1500*g* for 15 min at room temperature. Serum for copeptin and PlGF ELISA was extracted from Blood samples collected in plain vacutainers by centrifugation at 1500*g* for 15 min at 4 °C. Plasma and serum samples were stored at −80 °C until analysis. Women were followed up till delivery and grouped into PE, FGR and control groups depending on the outcome of the delivery. After identification of PE and FGR cases and gestational age at blood collection and storage time matched controls, plasma/serum samples were retrieved from storage and analyzed for CD62e MPs, PlGF and Copeptin. All analyses of plasma and serum samples were performed in a blind fashion.

2.2. Measurement of biomarker concentrations

Measurement of serum concentrations of PlGF and copeptin were done by using commercial ELISA kits (RayBiotech, Georgia, USA; BT Bioassay Technology Laboratory, Shanghai, China). Plasma CD62e levels were analyzed by standardized method on Becton Dickinson (BD) fluorescence activated cell sorting (FACS) Aria by participating in the "Vascular Biology Scientific and Standardization committee workshop: Standardization of flow cytometry (FCM) – based platelet MPs (PMP) enumeration" [34]. The detailed methodology for CD62e MPs analysis was described in the previous study by our group [35].

2.3. Definition of clinical outcome

PE and FGR cases were diagnosed based on the standard American College of Obstetricians and Gynaecologists criteria [1]. PE was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg on two occasions at least 4 h apart, developing after 20 weeks of gestation in previously normotensive women with proteinuria of 300 mg or more in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens, if 24-hour urine collection was not available. FGR was defined as a fetus with an estimated fetal weight < 10th centile on ultrasound, confirmed at delivery (birth weight < 10th centile for age and gender) and not associated with aneuploidy and structural anomalies. Reference curve for the calculation of fetal birth weight percentile has been referred for identification of FGR cases [36].

2.4. Statistical analysis

CD62e MPs, PlGF and CPP levels were given as median (interquartile range). Comparison of clinical characteristics and CD62e MPs, PlGF, CPP levels between PE, FGR, and control groups were done using Mann-Whitney test. P value of < 0.05 was considered as statistically significant. The final analysis included all the data generated for patient and control groups. The data was adjusted for the body mass index using linear regression analysis for individual marker in each patient group. The Receiver–operating characteristics (ROC) analysis was done to determine and compare area under curve (AUC), sensitivity, specificity, Positive likelihood ratio (PLR), Negative likelihood ratio (NLR), Positive predictive value (PPV), and Negative predictive value (NPV) of individual and combination of markers in different study groups. AUC cut off value of 0.6 was used to select clinically significant marker. The predictive utility of the combination of biomarkers was evaluated by using binary logistic regression. The data analysis was done using statistical software SPSS version 20 (SPSS Inc, Chicago, IL, USA).

2.5. Ethics approval

The study was approved by Institutional Ethics Committee Review Boards of National Institute of Immunohaematology (ICMR) (NIIH/IEC/27-2013) and Nowrosjee Wadia Maternity Hospital (IEC-NWMH/AP/2014/001-V2) for research on human Subjects in accordance with the Declaration of Helsinki.

3. Results

3.1. Descriptive statistics

The study population included 1115 primigravid women and 82 cases lost due to non-availability of pregnancy outcome data. Out of remaining study population, 41 (3.97%) developed PE and 68 (6.58%) developed FGR later in pregnancy. 105 women who have normal pregnancy outcome with matched gestational age of blood collection

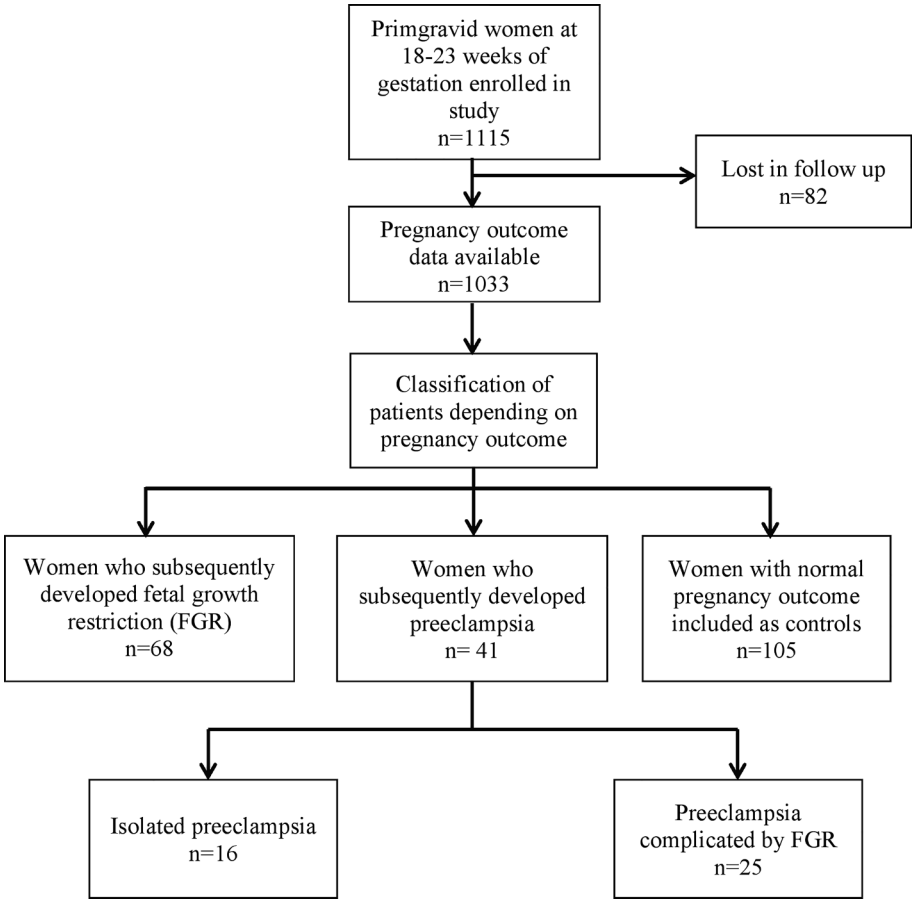

Fig. 1. Flow diagram of study participant selection.

Table 1
Clinical Characteristics and biomarker levels in patient and control groups.

Clinical Characteristics	PE (n = 41)	FGR (n = 68)	Isolated PE (n = 16)	PE + FGR (n = 25)	Controls (n = 105)
Maternal age (years)	25 (23–28)	25 (23–27)	26 (23–28.25)	24 (23–28)	24 (23–28)
BMI (Kg/m^2)	24.43 (21.16–27.07)†	20.25 (17.97–23.55)	24.53 (20.84–28.10)†	24.43 (22.02–26.63)†	21.93 (19.47–23.32)
Average time of diagnosis from sample collection (days)	108.50 (102.20–115.50)	102.90 (92.40–109.03)	115.50 (109.03–120.40)	102.90 (101.50–108.50)	–
GA at delivery (weeks)	37.20 (36.10–38)*	38.20 (36.70–39.18)*	38 (37.43–38.73)†	36.60 (36–37.20)*	39.40 (38.53–40.10)
Caesarean Delivery (%)	90.24% (37/41)*	57.57% (38/66)*	87.5% (14/16) *	92% (23/25)*	24.76% (26/105)
Birth weight (g)	2230 (2000–2750)*	2238 (2028–2400)*	2905 (2490–3162)	2125 (1815–2230)*	2895 (2757–3192)
SBP (mm Hg)	150 (150–160)*	110 (110–120)	150 (140–150)*	150 (150–160)*	110 (110–120)
DBP (mm Hg)	100 (100–110)*	70 (70–80)	100 (100–110)*	100 (100–110)*	70 (70–80)
MAP (mm Hg)	120 (116.70–120)*	83.30 (83.30–90)	120 (116.7–123.3)*	120 (116.70–120)*	83.30 (83.30–90)
Proteinuria	2+	ND	2+	2+	ND
Biomarker levels at 18–23 weeks of gestation					
CD62e (MPs/μL)	799.33 (546.86–1249.29)*	535.66 (306.52–861.25)	837.7 (286.95–1357.29)*	799.33 (672.16–1240.06)*	384.08 (245.03–576.00)
PlGF (pg/mL)	238.38 (161.36–312.62)*	892.98 (346.41–1674.8)	252.55 (192.87–305.83)*	214.4 (85.62–312.62)*	947.21 (466.7–1428.56)
Copeptin (ng/L)	300.75 (225.44–424.4)†	223.05 (184.08–260.71)	300.34 (225.73–478.17)†	300.75 (225.58–335.65)†	207.24 (169.73–276.46)

Data given as median (interquartile range) or otherwise indicated; *P value < 0.0001; †P value < 0.01.
BMI: Body mass index; DBP: Diastolic blood pressure; FGR: Fetal growth restriction; GA: Gestational age; ISOPE: Isolated PE cases; MAP: Mean arterial pressure; ND: Not detected; PE: Preeclampsia; PE + FGR: PE cases complicated by FGR; SBP: Systolic blood pressure.

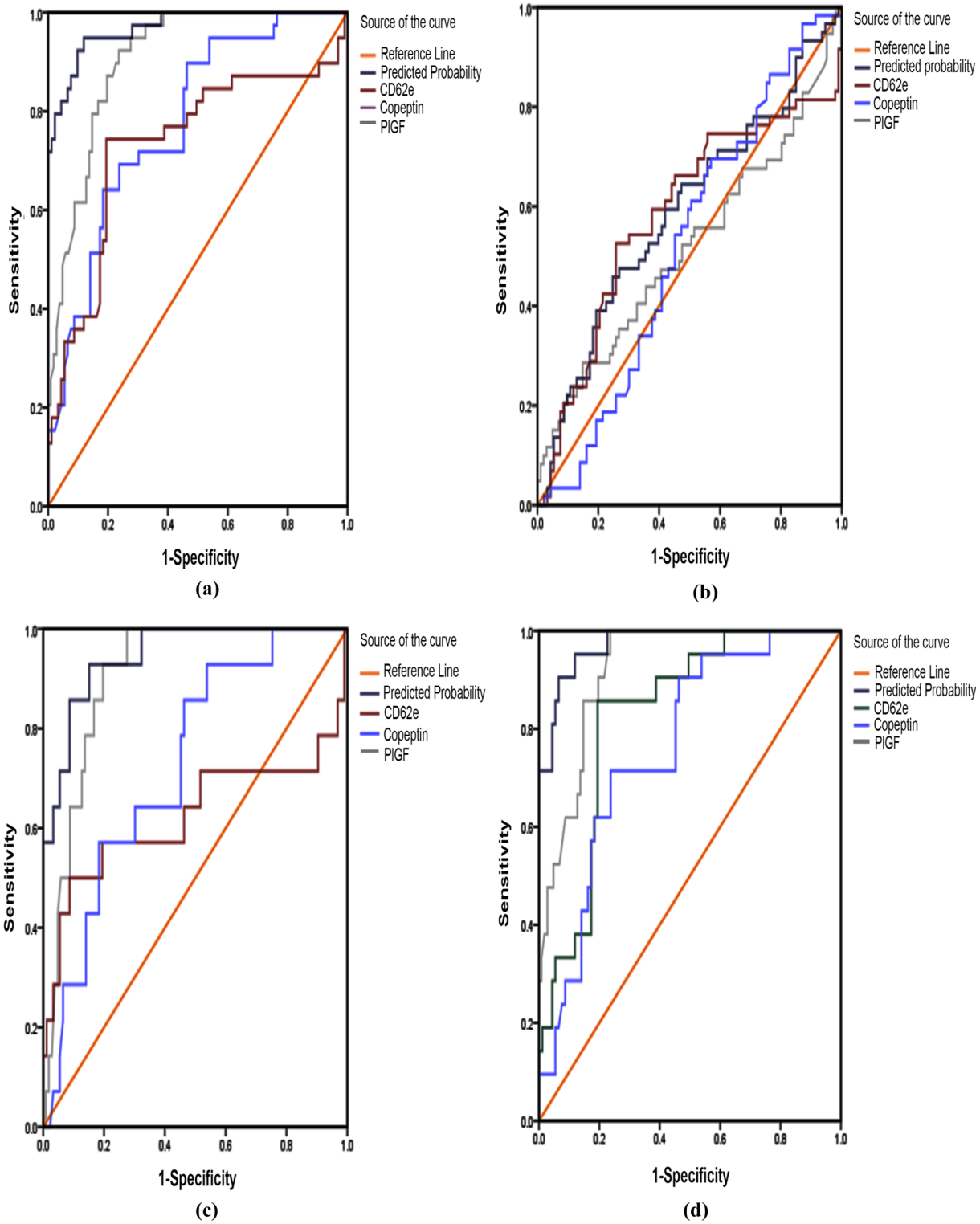

Fig. 2. Receiver operating characteristic curve showing the screening characteristics of endothelial MPs (CD62e), PlGF and CPP, alone and in combination for prediction of (a) PE, (b) FGR, (c) Isolated PE and (d) PE complicated by FGR cases at 18–23 weeks of gestation.

and storage time of sample were included as controls. Depending on the presence or absence of FGR in cases of PE, PE cases were further classified as isolated PE (16/41) and PE complicated by FGR (25/41) (Fig. 1). Clinical characteristics and biomarker levels for PE, FGR, and control groups are given in Table 1. Overall PE cases along with all subgroups and FGR showed significantly lower gestational age of delivery and higher incidence of Caesarean delivery compared to controls. Except isolated PE group, other patient groups showed significantly lower birthweight ($p < 0.0001$) compared to healthy controls. PE, isolated PE and PE cases complicated by FGR showed significantly higher body mass index (BMI) ($p < 0.01$), Systolic blood pressure (< 0.0001), diastolic blood pressure (< 0.0001), mean arterial pressure (MAP) (< 0.0001) compared to healthy controls. (Table 1)

Table 2
Receiver–operating characteristics curve analysis showing area under curve (AUC) and diagnostic performance characteristics of biomarkers for the prediction of PE and FGR.

Test Result Variable(s)	CD62e	PlGF	CPP	CD62e + PlGF + CPP	CD62e	PlGF	CPP	CD62e + PlGF + CPP
	PE (41)				FGR (68)			
AUC	0.734	0.921	0.778	0.969 (0.942–0.996)	0.583	0.534	0.52	0.589 (0.493–0.684)
(95% CI)	(0.629–0.839)	(0.877–0.965)	(0.695–0.861)		(0.484–0.682)	(0.435–0.634)	(0.428–0.612)	
Std. Error[a]	0.054	0.022	0.042	0.014	0.051	0.051	0.047	0.049
Asymptotic Sig[b]	0.000	0.000	0.000	0.000	0.085	0.476	0.673	0.066
Sensitivity	74.4	89.7	71.8	92.3 (80.08–98.46)	61.0	52.5	54.2	59.3 (46.23–70.63)
(95% CI)	(59.70–87.64)	(76.87–97.28)	(54.46–83.87)		(47.70–71.97)	(40.45–65.17)	(41.88–66.55)	
Specificity	80.6	81.7	70	90.3 (83.18–95.34)	58.1	54.8	54.8	58.1 (48.07–67.66)
(95% CI)	(72.13–87.96)	(73.19–88.74)	(60.78–78.98)		(48.07–67.66)	(45.22–64.95)	(45.22–64.95)	
PLR	3.97	4.99	2.40	9.73 (5.36–17.66)	1.44	1.18	1.22	1.40 (1.04–1.90)
(95% CI)	(2.58–6.11)	(3.28–7.58)	(1.68–3.42)		(1.07–1.94)	(0.87–1.61)	(0.90–1.65)	
NLR	0.30	0.12	0.42	0.08 (0.03–0.24)	0.68	0.85	0.83	0.71 (0.51–0.98)
(95% CI)	(0.17–0.52)	(0.05–0.30)	(0.25–0.68)		(0.49–0.96)	(0.63–1.16)	(0.60–1.13)	
PPV	60.78	66.07	48.33	79.17 (67.88–87.33)	48.24	43.37	44.05	47.62 (40.23–55.11)
(95% CI)	(50.18–70.46)	(56.15–74.76)	(39.61–57.16)		(40.92–55.62)	(36–51.05)	(36.74–51.62)	
NPV	89.47	95.56	86.05	96.94 (91.40–98.95)	69.32	64.44	65.17	68.54 (61.10–75.14)
(95% CI)	(83.11–93.63)	(89.40–98.21)	(79.04–90.98)		(61.78–75.95)	(57.19–71.09)	(57.81–71.87)	

a: Under the nonparametric consideration; b: Null hypothesis: true area = 0.5.
AUC: Area under curve; CD62e: Endothelial microparticles; CPP: Copeptin; FGR: Fetal growth restriction; NPV: Negative predictive value; NLR: Negative Likelihood ratio; PE: Preeclampsia; PlGF: Placental growth factor; PLR: Positive Likelihood ratio; PPV: Positive predictive value.

3.2. Serum and plasma concentrations of biomarkers

The serum and plasma levels of biomarkers were correlated with body mass index (Supplementary table 1). None of the markers in PE, isolated PE, PE cases complicated by FGR and isolated FGR cases showed significant correlation with BMI. Thus, BMI was not included further in the generation of the predictive model. Elevated levels of plasma CD62e MPs and serum copeptin; reduced serum PlGF levels were observed in PE cases compared to FGR and control (Table 1). At 18–23 weeks of gestation, overall PE group showed elevated plasma CD62e ($p < 0.0001$), serum Copeptin ($p < 0.0001$) and reduced level of PlGF ($p < 0.0001$) compared to controls. Similar to Overall PE, isolated PE cases showed reduced serum PlGF levels ($p < 0.0001$) and elevated plasma CD62e ($p < 0.0001$), serum copeptin levels (p-0.0028) compared to controls. In PE cases complicated by FGR, plasma CD62e ($p < 0.0001$) and serum copeptin levels (p-0.001) were significantly elevated; serum PlGF concentration ($p < 0.0001$) was reduced compared to controls. None of the markers showed significant alteration in FGR group.

The ROC analysis of different patient groups for CD62e MPs, PlGF and CPP, alone and in combination, are given in Fig. 2. In PE group, the values for AUC, Sensitivity, specificity, PLR, NLR, PPV, and NPV for CD62e were 0.734, 74.4%, 80.6%, 3.97, 0.30, 60.78%, and 89.47%, respectively; the values for PlGF were 0.921, 89.7%, 81.7%, 4.99, 0.12, 66.07%, 95.56%, respectively; the values for copeptin were 0.778, 71.8%, 70%, 2.40, 0.42, 48.33%, and 86.05%, respectively at 18–23 weeks. Using the binary logistic regression, the combination of CD62e, PlGF and copeptin showed improved predictive power with 0.969 AUC, 92.3% sensitivity, 90.3% specificity, 9.73 PLR, 0.08 NLR, 79.17% PPV, and 96.94% NPV. The details of ROC analysis for FGR and PE subgroups are given in Tables 2 and 3.

4. Discussion

4.1. Main findings

The finding of this study demonstrated that primigravid women who are at risk of development of PE later in gestation can be effectively identified at 18–23 weeks of gestation using combination of Plasma CD62e, Serum PlGF, and copeptin levels. The combination can predict 92.3%, 92.9% and 95.2% cases of PE, isolated PE and PE with FGR respectively. The low predictive values for FGR cases (0.589 AUC, 59.3% sensitivity, 58.1% specificity, 1.40 PLR, 0.71 NPR, 47.62% PPV and 68.54% NPV) suggested specific nature of biomarkers for prediction of PE, thus can differentiate PE cases from FGR, which represent more severe form of pregnancy complication, early in gestational age.

Reduced concentration of plasma/serum PlGF, alone and in combination with anti-angiogenic factors, have been proposed as a predictive marker in the second trimester for PE prediction [17–19]. A prospective cohort study evaluating the concentration of PlGF in pregnant women at 22–26 weeks of gestation, reported combination of abnormal uterine artery Doppler velocimetry (UADV) and maternal plasma PlGF of < 280 pg/mL identifies women at risk of development of early-onset PE, severe PE and PE [17]. In a case-control study, authors reported significantly reduced plasma concentration of PlGF in preeclamptic women in the second trimester and sFlt-1/PlGF ratio improved prediction with 78% specificity and diagnostic sensitivity of 80.4% [18]. Similarly, a longitudinal cohort study evaluating plasma concentration of PlGF and anti-angiogenic factors in singleton pregnant women reported the predictive utility of slope of PlGF/sEng ratio for prediction of PE at 20–25 weeks of gestation with a high positive likelihood ratio, low negative likelihood ratio [19]. In congruence with the literature, our data showed serum PlGF concentration can predict PE (PLR: 4.99, NLR: 0.12), isolated PE (PLR: 4.69, NLR: 0.08) and PE cases complicated by FGR (PLR: 5.25, NLR: 0) at 18–23 weeks as it gave a high positive likelihood ratio and low negative likelihood ratio for each group.

Arginine Vasopressin (AVP) dependent hypertension and low circulating renin-angiotensin system activity has been reported in non-pregnant population comprising blacks, elderly and patients with chronic renal or heart failure. Compared to non-eclamptic pregnant women, low circulating renin-angiotensin system activity has been exhibited by women with PE. This suggested the role of elevated AVP in the development of PE [37–39]. The C terminal cleavage product of pre-pro-AVP, copeptin, has been proposed as a surrogate marker for AVP. Elevated maternal plasma levels of copeptin have been implicated in PE pathogenesis [29–31]. In a case-control study authors reported elevated maternal plasma copeptin concentration throughout the pregnancy. The ROC analysis showed high predictive values of copeptin with second trimester AUC of 0.90, 81% sensitivity and 84% specificity.

Table 3
Receiver–operating characteristics curve analysis showing area under curve (AUC) and diagnostic performance characteristics of biomarkers for the prediction of isolated PE and PE cases complicated by FGR.

Test Result Variable(s)	CD62e	PlGF	CPP	CD62e + PlGF + CPP	CD62e	PlGF	CPP	CD62e + PlGF + CPP
	ISOPE (16)			PE + FGR (25)				
AUC (95% CI)	0.624 (0.411–0.837)	0.921 (0.869–0.974)	0.728 (0.601–0.856)	0.948 (0.896–0.999)	0.828 (0.744–0.913)	0.937 (0.894–0.979)	0.764 (0.661–0.866)	0.974 (0.947–1.000)
Std. Error[a]	0.109	0.027	0.065	0.026	0.043	0.022	0.052	0.014
Asymptotic Sig[b]	0.086	0.000	0.006	0.000	0.000	0.000	0.000	0.000
Sensitivity (95% CI)	64.3 (35.43–84.80)	92.9 (69.77–99.84)	64.3 (35.43–84.90)	92.9 (69.77–99.84)	85.7 (68.78–97.45)	100 (86.28–100)	71.4 (50.61–87.93)	95.2 (79.65–99.90)
Specificity (95% CI)	53.8 (44.28–64.04)	80.2 (71.07–87.17)	69.9 (59.78–78.13)	84.9 (76.44–91.03)	80.6 (72.13–87.96)	80.6 (72.13–87.96)	76.3 (66.89–83.96)	88.2 (80.89–93.95)
PLR (95% CI)	1.37 (0.89–2.11)	4.69 (3.13–7.01)	2.05 (1.27–3.30)	6.15 (3.85–9.83)	4.62 (3.04–7.03)	5.25 (3.54–7.79)	3.02 (1.99–4.60)	8.40 (4.90–14.39)
NLR (95% CI)	0.69 (0.36–1.33)	0.08 (0.01–0.52)	0.54 (0.28–1.03)	0.07 (0.01–0.49)	0.15 (0.05–0.43)	0.000	0.37 (0.19–0.70)	0.05 (0.01–0.31)
PPV (95% CI)	17.24 (11.90–24.31)	41.67 (32.31–51.66)	23.81 (16.24–33.49)	48.39 (36.98–59.96)	52.38 (41.95–62.51)	55.56 (45.73–64.96)	41.86 (32.10–52.30)	66.67 (53.86–77.41)
NPV (95% CI)	90.48 (83.13–94.82)	98.82 (92.63–99.82)	92.41 (86.45–95.87)	98.89 (93.02–99.83)	96.59 (90.71–98.80)	100 (86.28–100)	91.95 (85.80–95.58)	98.94 (93.16–99.84)

a: Under the nonparametric consideration; b: Null hypothesis: true area = 0.5.
AUC: Area under curve; CD62e: Endothelial microparticles; CPP: Copeptin; FGR: Fetal growth restriction; ISOPE: Isolated PE cases; NPV: Negative predictive value; NLR: Negative Likelihood ratio; PE: Preeclampsia; PE + FGR: PE cases complicated by FGR; PlGF: Placental growth factor; PLR: Positive Likelihood ratio; PPV: Positive predictive value.

The study recommended Copeptin as an independent predictor for PE throughout pregnancy [29]. Similar studies evaluating copeptin, independent and in combination with biophysical marker such as uterine artery Doppler, as predictive marker reported promising results across gestational age [30,31]. Our results showed high PLR (2.40), low NLR (0.42) and high NPV (86.05%) for copeptin at 18–23 weeks of gestation. Similar to Yeung et al. [30], our study reported specific nature of copeptin in the prediction of PE as elevated copeptin levels did not show significant difference in FGR group.

Preeclampsia is considered as a disorder of maternal endothelium. Hypoxia-mediated activation of endothelium contributes to the maternal inflammatory response. The endothelium cell activation should be detectable as circulating endothelial MPs. The endothelial MPs then contribute to the endothelial dysfunction and aggravate inflammatory response [40]. Role of cMPs of various cell origins as biomarkers for early prediction of PE still needs to be elucidated as only few studies investigated its predictive utility in the clinical setting [13,14,41,42]. In a prospective cohort study assessing cMPs in pregnant women at 24 weeks of gestation, authors did not find significant association of altered levels of endothelial and platelet-derived MPs and risk of preeclampsia [41]. Lok et al. [42] reported altered levels of various cell-derived cMPs in pregnant women at 28–36 weeks of gestation in women who subsequently developed PE [42]. In our study, we found significantly elevated endothelial MPs (CD62e). CD62e MPs appeared to be independent predictor for PE as overall PE (PLR: 3.97, NLR: 0.30, NPV: 89.47) group along with the sub classes of PE i.e. isolated PE (PLR: 1.37, NLR: 0.69, NPV: 90.47) and PE complicated by FGR (PLR: 4.62, NLR: 0.15, NPV: 96.59) showed good positive and negative likelihood ratio with high negative predictive values.

4.2. Strength and limitations

This is a first nested case-control study derived from a prospective cohort of primigravid women which showed promising results for prediction of PE using endothelial MPs (CD62e) in combination with serum PlGF and Copeptin. Being a heterogeneous, multi-system disorder, PE cannot be predicted by alteration in single biomarker in plasma/serum. The current study thus utilized markers representing different pathways involved in pathogenesis and clinical symptoms such as angiogenesis (PlGF), endothelial dysfunction (CD62e MPs) and hypertension (copeptin) in PE development. Though PlGF appears to be a marker significantly driving the performance of the combination, copeptin and CD62e significantly improved sensitivity, specificity and positive likelihood ratio for the PE subgroups. Confirmation of pregnancy outcome by hospital documents at the time of delivery represents the major strength of this study as it avoided misdiagnosis of patient groups included in the study. Previously, a case-control study not only reported elevated MPs in women with recurrent pregnancy loss (RPL) but also improvement of pregnancy outcome after administration of low molecular weight heparin. The study concluded administration of anticoagulant as a therapeutic intervention in reduction or normalization of MPs levels in RPL cases [43]. As elevated levels of endothelial MPs are positively correlated with the development of clinical signs of PE, therapy-based reduction of cMPs levels can be targeted for assessment of improvement in pregnancy outcome of PE cases.

Major limitation of this study is less number of PE patients and absence of syncytiotrophoblast derived MPs from the combination of biomarkers included in study; hence prospective cohort study comprising large number of patients is required for confirming the prognostic efficiency of other cell-derived MPs, alone or in combination, with other biochemical markers for PE prediction. With a large number of samples, comparative assessment of biomarker levels will be required to differentiate plasma/serum biomarker profile in early and late onset PE. Though the high negative predictive value of the combination of biomarkers facilitate identification of women not at risk of PE, it may result in missing 4% of patients at risk of developing PE. Thus, the

prospective application of these predictive tests in future studies will be required to define the risks and benefits.

4.3. Interpretation

The current study proposes the use of combination of endothelial (CD62e) MPs, serum PlGF and Copeptin as a predictive marker for prediction of PE but not FGR at 18–23 weeks of gestation. The combination showed slightly improved predictive values for PE cases complicated by FGR (0.974 AUC, 95.2% specificity, 88.2% specificity, 8.40 PLR, 0.05 NLR, 66.67 PPV and 98.94 NPV) than isolated PE cases (0.948 AUC, 92.9% specificity, 84.9% specificity, 6.15 PLR, 0.07 NLR, 48.39 PPV and 98.89 NPV) suggested improved prediction in severe form of pregnancy complication. Even though meta-analysis studies recommended prophylactic use of aspirin prior to 16 weeks of gestation for better pregnancy outcome [44,45], prediction of PE at 18–23 weeks will help in early identification and hence management of women at risk. High negative predictive value (96.94%) and low negative likelihood ratio (0.08) for PE cases suggested that combination of Plasma CD62e, Serum PlGF and copeptin can identify women who are not at risk for PE and hence can prevent unnecessary hospitalization of these patients, especially in resource-limited medical setup.

5. Conclusion

Even though tested in a nested case-control study, combination of cMPs of endothelial origin along with PlGF and copeptin showed immense promise in predicting PE early in gestation age. The combination can help in differentiating women at risk of developing PE than FGR cases at 18–23 weeks. Nonetheless, further work is required to validate the initial predictive potential of the combination of various cell-derived MPs with conventional serum markers for prediction of preeclampsia.

Declaration of interest

All authors contributed to the design of the study, data analysis and the writing of the manuscript. Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.04.006.

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