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The feasibility of soluble Fms-Like Tyrosine kinase-1 (sFLT-1) and Placental Growth Factor (PlGF) ratio biomarker in predicting preeclampsia and adverse pregnancy outcomes among medium to high risk mothers in Kuala Lumpur, Malaysia

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Abstract

Background

Preeclampsia significantly contributes to maternal and perinatal morbidity and mortality. It is imperative to identify women at risk of developing preeclampsia in the effort to prevent adverse pregnancy outcomes through early intervention. Soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) level changes are noticeable several weeks before the onset of preeclampsia and its related complications. This study evaluated the feasibility of the sFlt-1/PlGF biomarker ratio in predicting preeclampsia and adverse pregnancy outcomes using a single cut-off point of >38.

Methods

This is a prospective cohort study conducted at a single tertiary centre, in an urban setting in Kuala Lumpur, Malaysia, between December 2019 and April 2021. A total of 140 medium to high risk mothers with singleton pregnancies were recruited at ≥20 weeks' gestation. sFlt-1/PlGF ratio was measured and the participant monitored according to a research algorithm until delivery. The primary outcome measure was incidence of preeclampsia and the secondary outcome measure was incidence of other adverse pregnancy outcomes.

Results

The overall incidence of preeclampsia was 20.7% (29/140). The mean sFlt-1/PlGF ratio was significantly higher in preeclampsia (73.58 ± 93.49) compared to no preeclampsia (13.41 ±

21.63) (p = 0.002). The risk of preeclampsia (adjusted OR 28.996; 95% CI 7.920–106.164; p<0.001) and low Apgar score (adjusted OR 17.387; 95% CI 3.069–98.517; p = 0.028) were significantly higher among women with sFlt-1/PlGF ratio >38 compared with sFLT-1/PlGF ratio ≤38. The area under the receiver-operator characteristic curve (AUC) for a combined approach (maternal clinical characteristics and biomarker) was 86.9% (p<0.001, 95% CI 78.7–95.0) compared with AUC biomarker alone, which was 74.8% (p<0.001, 95% CI 63.3–86.3) in predicting preeclampsia. The test sensitivity(SEN) was 58.6%, specificity (SPEC) 91%,positive predictive value (PPV) 63% and negative predictive value (NPV) 89.3% for prediction of preeclampsia. For predicting a low Apgar score at 5 minutes, the SEN was 84.6%, SPEC 87.4%, PPV 40.7%, and NPV 98.2%; low birth weight with SEN 52.6%,SPEC 86.0%, PPV 37.0%, NPV 92.0%; premature delivery with SEN 48.5%, SPEC 89.5%, PPV 59.3%, NPV 84.7% and NICU admission with SEN 50.0%, SPEC 85.8%, PPV 37.0% and NPV 91.2%.

Conclusions

It is feasible to use single cut-off point of >38 ratio of the biomarkers sFlt-1/PlGF in combination with other parameters (maternal clinical characteristics) in predicting preeclampsia and adverse pregnancy outcomes among medium to high risk mothers without restricting outcome measurement period to 1 and 4 weeks in a single urban tertiary centre in Kuala Lumpur, Malaysia.

Introduction

Preeclampsia complicates 2–10% of pregnancies worldwide and significantly contributes to maternal, fetal, and newborn morbidity and mortality [1–5]. Preeclampsia is characterized by the combined presentation of hypertension with proteinuria and/or maternal organ dysfunction, such as renal insufficiency, liver involvement, neurological or haematological complications, and uteroplacental dysfunction as evidenced by fetal growth restriction (FGR) [2, 6].

Early detection and prevention of preeclampsia will reduce both prevalence of the disease and health service costs [7]. Early identification of women at risk of developing preeclampsia for commencement of aspirin and calcium as prophylaxis, close monitoring, and timely delivery, are critical steps in the management, in order to prevent adverse pregnancy outcomes [8, 9]. A huge challenge in managing preeclampsia is in predicting its development and severity [10].

The pathophysiology of preeclampsia remains unclear but placental dysfunction is believed to be the primary insult [11, 12]. Incomplete maternal spiral artery remodeling in early pregnancy leads to placental hypoperfusion. Oxidative stress within the placenta leads to changes in biomarker levels, with increased serum soluble Fms-like tyrosine kinase-1 (sFlt-1) and reduced placental growth factor (PlGF) [13].

PlGF is a proangiogenic factor [14] that has been found to be a biomarker of preeclampsia [15, 16] It is produced by the placenta, mainly the syncytiotrophoblast, and the endothelium [16, 17]. PlGF plays a major role in the growth of placental blood vessels [18, 19]. It can be detected in maternal blood from 8 weeks' gestation up to the second trimester of pregnancy, after which PlGF decreases progressively until delivery [20]. In preeclampsia, PlGF remains persistently low [16, 21, 22]. Infusion of recombinant human PlGF using intraperitoneal

osmotic mini-pumps can eliminate the progression of hypertension in a rat model of pre-eclampsia [23].

Vascular endothelial growth factor (VEGF) is important for vascular homeostasis which stimulates both VEGF receptor-1 (VEGFR-1) and -2 (VEGFR-2), VEGF and endothelial nitric oxide synthase (eNOS), are essential for angiogenesis [24–28]. sFlt-1 antagonises VEGF and PlGF, preventing interaction of VEGF and PlGF, by preventing interaction with the endothelial receptors and causing endothelial dysfunction [29–31].

Changes in the levels of sFlt-1 and PlGF are noticeable several weeks before the onset of pre-eclampsia and its related complications [11]. sFlt-1/PlGF ratios of 38 or lower carry a negative predictive value (NPV) of 99.3% (95% CI 97.9–99.9) in the subsequent week, with 80.0% sensitivity (95% CI 51.9–95.7) and 78.3% specificity (95% CI 74.6–81.7) [32], whereas ratios above 38 carry a positive predictive value (PPV) of 36.7% (95% CI 28.4–45.7) within 4 weeks, with 66.2% sensitivity (95% CI 54.0–77.0) and 83.1% specificity (95% CI 79.4–86.3) [32]. In the Malaysian clinical practice guidelines (CPG), current preeclampsia screening relies on clinical characteristics and proteinuria only [33], which are of poor predictive value [34]. At present, women with suspected preeclampsia are unnecessarily admitted to hospital [35, 36], whilst asymptomatic severe cases are overlooked and managed as outpatient, resulting in late presentation with irreversible complications [35].

Serum sFlt-1/PlGF ratio testing (from 20 weeks' gestation onwards) has been suggested by the latest Malaysia CPG in order to predict preeclampsia [33], however it is not widely implemented especially in government health clinics setting, despite widespread adoption in several countries [35]. The National Institute for Health and Care Excellence (NICE), United Kingdom (UK), has recommended sFlt-1/PlGF ratio ≤38 to be used as a rule-out test for suspected pre-eclampsia between 20 and 34 weeks' gestation [37]. The German Society of Gynecology and Obstetrics (DGGG) recommended use of second trimester sFLT-1/PlGF ratio with uterine artery Doppler for risk assessment of preeclampsia development and prognostic assessment [38].

These biomarkers may help improve early diagnosis of preeclampsia and enable prediction of maternal and fetal outcomes [11]. We set out to assess the feasibility of sFlt-1/PlGF ratio in predicting preeclampsia and adverse pregnancy outcomes using the recently proposed single cut-off values of 38 and preeclampsia screening algorithm developed.

Materials and methods

A prospective cohort study was conducted at a single tertiary centre in an urban setting in Kuala Lumpur, Malaysia, from December 2019 until April 2021. The aim was to evaluate the feasibility of using sFlt-1/PlGF immunoassay ratio for early identification of preeclampsia and related adverse pregnancy outcomes. The study participants were monitored based on the algorithm shown in Fig 1 from enrolment into the study at ≥20 weeks' gestation until delivery. If the sFLT-1/PlGF ratio test was negative (≤38), the blood test will be repeated weekly for continued monitoring. If the result was positive (>38), the test will be repeated four-weekly until delivery or development of preeclampsia. Maximum 4 sample of sFlt-1/PlGF ratio test each participant for the purposed of controlling research cost. The outcome measure was preeclampsia and related adverse pregnancy outcomes as a comparison between positive and negative tests.

The minimum sample size was calculated based on the formula for prospective cohort studies by Fleiss (1981) [39], with a power of 80% and confidence interval (CI) of 95% [40]. A minimum of 120 samples were required.

Fig 1. Preeclampsia screening algorithm.

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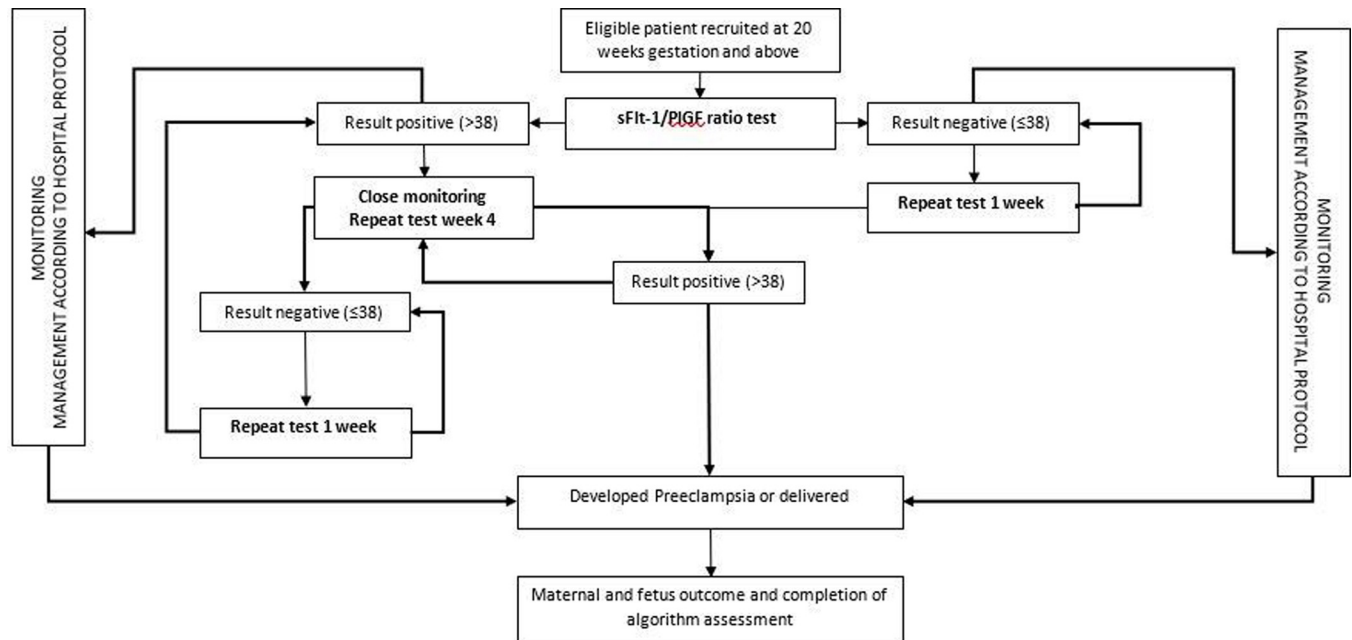
We included women aged ≥18 years, with systolic blood pressure (SBP) of 140–160 mmHg or diastolic blood pressure (DBP) of 90–100 mmHg or gestational hypertension or underlying chronic hypertension, and singleton pregnancies of ≥20 weeks gestation. We excluded women with confirmed diagnosis of preeclampsia or eclampsia, fetal congenital malformation, and those who refused.

The study received ethical approval from the Ministry of Health Malaysia (NMRR-19-1104-46049(IIR)) and UKM (FF-2019-371). The study conducted according to the Declaration of Helsinki. Written informed consents were obtained from all participants.

Whole blood was collected in a 5 ml plain vacutainer tube on the recruitment day. The blood was centrifuged, then subjected to a calibrated **cobas e** 411 immunoassay analyser tests (Roche Diagnostics). Quality control was performed for each test by using PRECICONTROL multimarker reagent. The blood analysis was performed by a trained medical laboratory technician under the supervision of a chemical pathologist. The laboratory result was entered in an integrated laboratory management system (ILMS) linked to the hospital information system.

Primary outcome measure

Our primary outcome measure was preeclampsia as defined by the International Society for the Study of Hypertension in Pregnancy (ISSHP) [2], i.e. gestational hypertension accompanied by at least one of the following new onset conditions at or after 20 weeks' gestation: proteinuria and/or maternal organ dysfunction (including acute kidney injury, altered liver function, neurological complications, haematological complications or uteroplacental dysfunction).

Secondary outcomes measure

Maternal and fetal adverse pregnancy outcome including maternal death, maternal intubation, emergency caesarean section, disseminated intravascular coagulation (DIVC), placental

abruption, acute heart failure, low birth weight, neonatal death, intrauterine death, neonatal intensive care unit (NICU) admission, premature delivery, and low Apgar score. The percentages of completed the preeclampsia screening algorithm developed is also measured as secondary outcome (as shown in Fig 1).

The operational definition of maternal death is death of a woman during pregnancy or up to 42 days post-partum. Maternal intubation is defined as the need for maternal intubation as a result of preeclampsia and related complications. Emergency caesarean section is defined as emergency caesarean section with preeclampsia as the main indication. Disseminated intravascular coagulation (DIVC), abruptio placenta, and acute heart failure are defined as complications resulting directly from preeclampsia. A low-birth-weight infant are defined as baby's birth weight of <2.5 kg. Intrauterine death is defined as death of a fetus in utero after 22 weeks of gestation. Neonatal death is defined as death of a baby within the first 28 days of life. Neonatal intensive care unit (NICU) admission is defined as requirement for NICU admission after birth. Premature delivery is defined as birth <37 weeks of gestation. A low Apgar score is defined as Apgar score <7 at 5 minutes after birth.

Independent variables

Our independent variables comprise sociodemographic characteristics (age, race, occupation, household income, education level), clinical features, Body mass index (BMI) classification, antenatal risk, gravida classification, parity classification, gestation at recruitment, anaemia, pre-gestational diabetes mellitus (DM), gestational DM, renal disease, autoimmune disease, previous history of hypertensive disorders in pregnancy, chronic hypertension, and sFlt-1/PlGF ratio.

Age was defined in years at recruitment based on date of birth, and categorized into two groups (>35 years, ≤ 35 years). Race was categorized into Malay and non-Malay according to major ethnicities in Malaysia. Occupation was classified according to International Standard Classification of Occupations and further divided into professional and non-professional groups. Household income was defined as overall income that was earned by household members, whether in cash or kind, and can be referred to as gross income. Household income was categorized into three groups Top 20 (> RM 10 960), Middle 40 (RM 4850–10959) and Below 40 (<RM4849). Education level was categorised into two groups (tertiary, secondary).

Body mass index (BMI) calculated as body weight in kg divided by height in meter squared (kg/m2), classified according to international World Health Organization (WHO) BMI classifications and further categorized into two groups (BMI ≥25, BMI ≤24.9). Antenatal risk was categorized into yellow (high risk) and green (medium risk), according to the Malaysian antenatal risk assessment colour coding system [41]. Gravida was categorized into three groups (Gravida 1, 2–5, ≥6). Parity was categorized into three groups (Para 0, 1–4, ≥5). Gestation at recruitment was defined as period of gestation (in weeks) at recruitment, either based on the date of the last menstrual period or on ultrasonography. The gestation at recruitment was categorized into two groups (≥27 weeks, 20–26 weeks). Diseases in pregnancy i.e. anaemia, pre-gestational and gestational DM, renal disease, autoimmune disease, history of hypertensive disorder in pregnancy, and chronic hypertension were dichotomously categorized (yes, no). sFlt-1/PlGF ratio was also dichotomously categorized (positive >38, negative ≤38).

Statistical analysis

Data were analysed using the IBM Statistical Package for the Social Sciences (SPSS) version 22. Incidence of preeclampsia was calculated from the number of preeclampsia cases divided by the total recruited sample. The characteristics of the variables are described using frequency

(n) and percentage (%). Simple logistic regression and Pearson Chi-square was used for bivariate analysis and further analysed using multiple logistic regression to control for potential confounders. All p-values resulted from two-sided statistical tests. Values of $p<0.05$ were considered statistically significant.

The diagnostic validity for sFlt-1/PlGF ratio was calculated and presented as sensitivity, specificity, PPV, NPV, positive likelihood ratio, negative likelihood ratio and post-test probability. The receiver operator characteristic (ROC) curves were calculated and the area under the curves (AUCs) were reported.

Result

Study population

A total of 140 women were recruited between December 2019 and April 2021 as shown in Fig 2. The numbers of potentially eligible were 200. After being examined for eligibility, only 180 women were confirmed eligible. However, 40 women refused to involve in the study due to other life commitments. Only 57% (n = 80) completed the research algorithm. A total of 140 women were included in the full analysis population according to first blood sample taken.

Incidence of preeclampsia among medium to high risk mothers was 20.7% (29/140). The mean sFlt-1/PlGF ratio was significantly higher in women who developed preeclampsia (73.58 ± 93.49) compared with those who did not (13.41 ± 21.63), p = 0.002. The percentages of women who developed preeclampsia according to sFlt-1/PlGF ratio versus week of assessment are shown in **Table 1**. A total of 5 (62.5%) women developed preeclampsia within a week of positive sFlt-1/PlGF ratio (>38). At the first hospital booking and in the first week after initial blood screening, 29.4% and 23.5% of cases were screened positive and developed preeclampsia respectively. The percentage of patient developed preeclampsia among positive sFlt-1/PlGF ratio test subsequently reduced from week to week.

Predictors of preeclampsia

Table 2 shows factors associated with preeclampsia as defined by the ISSHP [2]. Simple logistic regression analysis indicated that occupation and positive sFlt-1/PlGF ratio (>38) at first blood sample are significantly associated with preeclampsia. On performing multivariate analyses using multiple logistic regression, adjusted analysis showed that women working as professionals with positive sFlt-1/PlGF ratio had a higher risk of developing preeclampsia compared to non-professionals and negative sFlt-1/PlGF ratio respectively.

The association between positive sFlt-1/PlGF ratio and adverse pregnancy outcome

Table 3 shows the association between positive sFlt-1/PlGF ratio test and adverse pregnancy outcome. After controlling with potential confounders including maternal characteristics, antenatal risk, underlying medical illness, gestational age and preeclampsia, adjusted analysis showed that the risk of low Apgar score (<7) at 5 minutes, low birth weight, premature delivery and baby admitted to NICU are significantly higher among positive sFlt-1/PlGF ratio test (>38) as compared to negative sFlt-1/PlGF ratio test (≤38).

The validity of sFlt-1/PlGF ratio in predicting preeclampsia and low Apgar score at 5 minutes

Table 4 shows the validity assessment of positive sFlt-1/PlGF ratio in predicting preeclampsia and adverse pregnancy outcome. The sensitivity of the test was low (58.6%) but the specificity
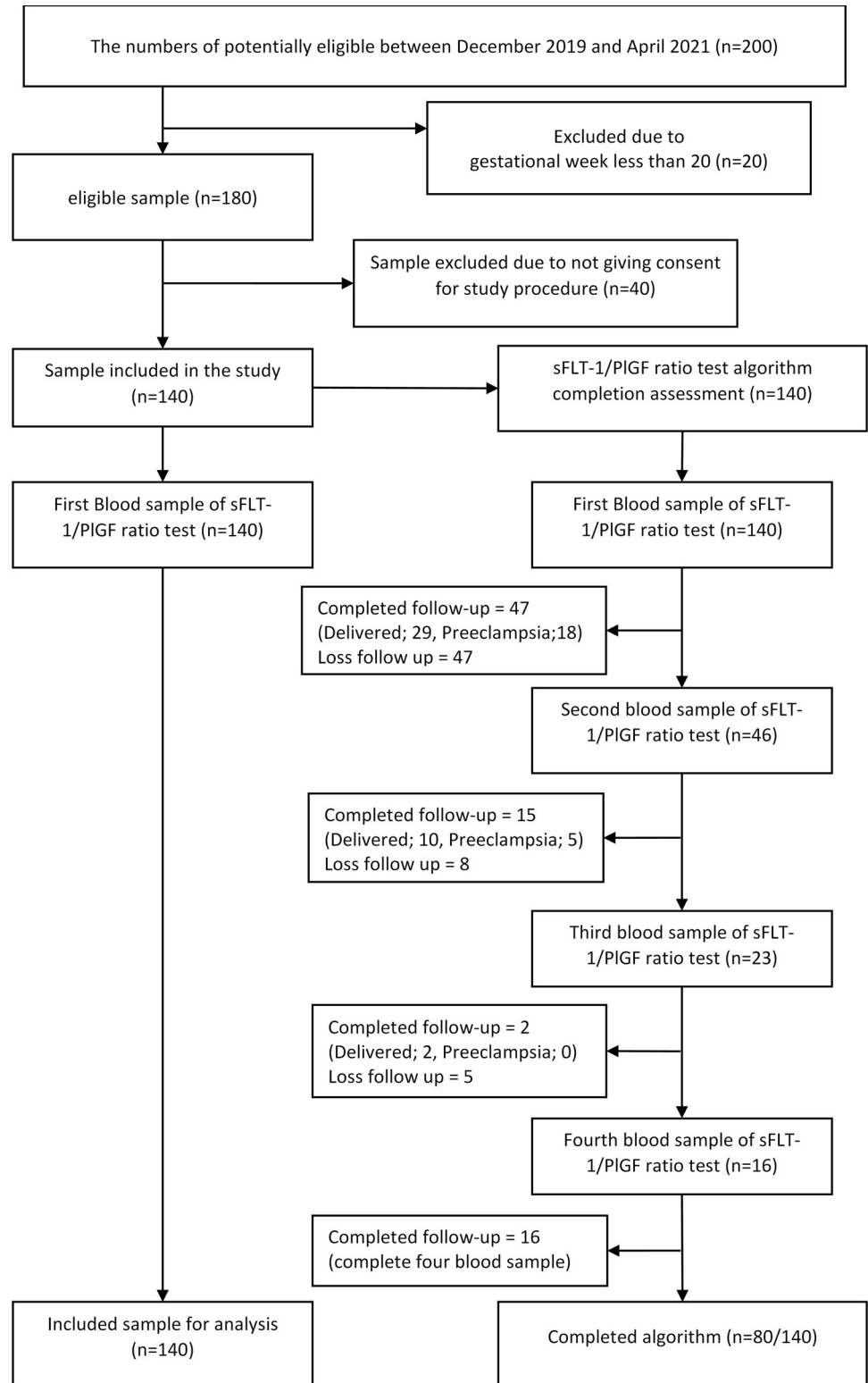
Fig 2. Study participants flow chart.

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Table 1. The number of cases screened as preeclampsia by week of assessment.

Assessment week	sFlt-1/PlGF ratio test		Total
	Positive (>38)	Negative (≤38)	
	n(%)	n(%)	
0	5(62.5%)	3(37.5%)	8(27.6%)
1	4(57.1%)	3(42.9%)	7(24.1%)
2	3(100.0%)	0(0.0%)	3(10.3%)
3	2(50.0%)	2(50.0%)	4(13.8%)
4	2(100.0%)	0(0.0%)	2(6.9%)
5	0(0.0%)	2(100.0%)	2(6.9%)
6	0(0.0%)	1(100.0%)	1(3.4%)
7	1(100.0%)	0(0.0%)	1(3.4%)
8	0(0.0%)	0(0.0%)	0(0.0%)
9	0(0.0%)	1(100.0%)	1(3.4%)
Total	17	12	29(100.0%)

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was high (91.0%) in predicting preeclampsia. **Fig 3** shows the comparison of ROC curve combined approach (maternal characteristic and sFlt-1/PlGF ratio) and sFlt-1/PlGF ratio alone in predicting both preeclampsia and significant adverse pregnancy outcome (low Apgar score). The area under the ROC curve (AUC) for combined approach was 86.9% ($p<0.001$, 95% CI 78.7–95.0) compared with AUC for sFlt-1/PlGF ratio alone 74.8% ($p<0.001$, 95% CI 63.3–86.3) to predict preeclampsia, the test having high sensitivity (84.6%) and specificity (87.4%) to predict low Apgar score at 5 minutes. The AUC for combined approach was 87.8% ($p<0.001$, 95% CI 75.2–100.0) compared with AUC sFlt-1/PlGF ratio alone 86.0% ($p<0.001$, 95% CI 74.2–97.9) to predict low Apgar score at 5 minutes.

Discussion

We found that the mean sFlt-1/PlGF ratio was significantly higher among women who developed preeclampsia compared to women without preeclampsia, when using sFlt-1/PlGF ratio cut-off value >38 (sensitivity 58.6%; specificity 91.1%; PPV 63%; NPV 89.3% and AUC 74.8%, $p<0.001$; 95% CI 63.3–86.3). With combined approach (by including maternal characteristics and clinical features) the AUC improved towards 86.9% ($p<0.001$, 95% CI 78.7–95.0) compared with AUC sFlt-1/PlGF ratio alone in predicting preeclampsia. Other findings suggest that sFlt-1/PlGF ratio with or without clinical characteristics can help second or third trimester prediction of both early onset and late onset preeclampsia [11]. The discrepancy of these findings may be due to the relatively small sample size in our study, whereas other studies reported that the combined approach had superior predictive value compared to biomarkers in the first trimester alone [42–44]. Therefore, our study supports the predictive value of sFlt-1/PlGF ratio >38 for preeclampsia among Malaysian women with significantly higher PPV (63%) than the reported PPV (32–36%) by PROGNOSIS and PROGNOSIS Asia Study [32, 45].

The PROGNOSIS study, a large multicenter, prospective observational study conducted in 14 countries, validated a single cut-off point of sFlt-1/PlGF ratio >38 in predicting preeclampsia [32]. However, less than 10% of the study population were Asian. Another research followed to validate the cut-off point (sFlt-1/PlGF ratio >38) among Asian women (China, Hong Kong, Japan, Singapore, South Korea, and Thailand) between December 2014 and December 2016 but Malaysia was not involved [46]. As sFlt-1 and PlGF levels may be influenced by ethnicity [47], there is a need to validate the test in Malaysia. Both researchers assessed the value of the sFlt-1/PlGF ratio for ruling out preeclampsia within a week and ruling in preeclampsia

Table 2. Factors associated with preeclampsia.

Variable	Preeclampsia n (%)	Non preeclampsia n (%)	Crude OR	95% CI	p	Adj. OR	95% CI	p
Age groups					0.139			
More than 35 years old	12(41.4%)	63(56.8%)	0.538	(0.235;1.232)				
≤ 35 years old	17(58.6%)	48(43.2%)	1.000					
Race								
Malay	26(89.7%)	88(79.3%)	2.265	(0.630; 0.149)	0.177			
Others	3(10.3%)	23(20.7%)	1.000					
Occupational classification					<0.001			<0.001
Professional	15(51.7%)	18(16.2%)	5.536	(2.282; 13.428)		6.694	(2.247;19.937)	
Non-professional	14(48.3%)	93(83.8%)	1.000			1.000		
Household incomed class (median income)					0.266			
Top 20 (> Ringgit Malaysia 10 960)	4(13.8%)	7(6.3%)	3.214	(0.761; 13.572)	0.112			
Middle 40 (Ringgit Malaysia 4850–10959)	17(58.6%)	59(53.2%)	1.621	(0.642; 4.090)	0.306			
Below 40 (< Ringgit Malaysia 4849)	8(27.6%)	45(40.5%)	1.000					
Education level					0.541			
Tertiary	23(79.3%)	82(73.9%)	0.738	(0.273; 1.992)				
Secondary	6(20.7%)	29(26.1%)	1.000					
Body mass index (BMI) class, kg/m^2								
BMI ≥25	22(75.9%)	87(78.4%)	0.867	(0.331; 2.271)	0.773			
BMI ≤24.9	7(24.1%)	24(21.6%)	1.000					
Antenatal risk coded					0.445			
Yellow (high risk)	4(13.3%)	22(19.8%)	0.647	(0.204; 2.052)				
Green (medium risk)	26(86.7%)	89(80.2%)	1.000					
Gravida classification					0.940			
Gravida 1	6(20.7%)	25(22.5%)	1.200	(0.117; 12.267)	0.785			
Gravida 2–5	22(75.9%)	81(73.0%)	1.358	(0.151; 12.233)	0.878			
Gravida 6 and more	1(3.4%)	5(4.5%)	1.000					
Parity classification					0.983			
Multipara	22(75.9%)	84(75.7%)	1.010	(0.389;2.624)				
Nulliparaous	7(24.1%)	27(24.3%)	1.000					
POG at recruitment					0.482			
20–26 weeks	3(10.3%)	17(15.3%)	0.638	(0.174;2.346)				
≥27 weeks	26(89.7%)	94(84.7%)	1.000					
Anaemia					0.335			
Yes	7(24.1%)	18(16.2%)	1.644	(0.611; 4.420)				
No	22(75.9%)	93(83.8%)	1.000					
Diabetes mellitus					0.612			
Yes	2(6.9%)	5(4.5%)	1.570	(0.289; 8.539)				
No	27(93.1%)	106(95.5%)	1.000					
Gestational DM					0.494			
Yes	9(31.0%)	42(37.8%)	0.739	(0.308; 1.774)				
No	20(69.0%)	69(62.2%)	1.000					
Kidney disease					0.607			
Yes	1(3.4%)	2(1.8%)	1.946	(0.170; 22.245)				

(*Continued*)

Table 2. (Continued)

Variable	Preeclampsia n (%)	Non preeclampsia n (%)	Crude OR	95% CI	p	Adj. OR	95% CI	p
No	28(96.6%)	109(98.2%)	1.000					
Autoimmune disease					0.607			
Yes	1(3.4%)	2(1.8%)	1.946	(0.170; 22.245)				
No	28(96.6%)	109(98.2%)	1.000					
History of HDP					0.827			
Yes	2(6.9%)	9(8.1%)	0.840	(0.171; 4.116)				
No	27(93.1%)	102(91.9%)	1.000					
Chronic hypertension					0.054			
Yes	7(24.1%)	48(43.2%)	0.418	(0.165; 1.058)				
No	22(75.9%)	63(56.8%)	1.000					
sFlt-1/PlGF ratio					<0.001			<0.001
Positive >38	17(58.6%)	10(9.0%)	14.308	(5.349; 38.277)		15.063	(5.206;43.557)	
Negative≤38	12(41.4%)	101(91.0%)	1.000			1.000		

Final model using Backward Stepwise (Likelihood Ratio)

Hosmer and Lemeshow Test 0.343

Nagelkerke R Square 42.1%

Classification table 82.9%

within four weeks, with an established clinical value for the short-term prediction of pre-eclampsia in women with suspected preeclampsia, potentially helping to prevent unnecessary hospitalization and intervention. Our study evaluated the feasibility of the sFlt-1/PlGF ratio >38 to predict preeclampsia and adverse pregnancy outcome throughout pregnancy without limiting the period of outcome assessment.

A previous study on the same urban population in Kuala Lumpur revealed that women with gestational hypertension had significantly lower PlGF and higher sFlt-1 levels compared with normotensive women [48]. However, the study did not assess these biomarkers level among women with preeclampsia. Another study among 84 high risk Malaysian pregnant women who had at least one risk factor for preeclampsia observed a significantly higher median sFlt-1 and sFlt-1/PlGF ratio from 25 to 28 weeks of gestation and sFlt-1/PlGF ratio from 29 to 36 weeks in high risk women who developed preeclampsia [49]. The sFLT-1/PlGF ratio in the third trimester showed the best AUC of 87.3% (p<0.001, 95% CI 77.3–97.3) compared with second trimester AUC, which was 68.8% (p = 0.038, 95% CI 52.0–85.5). The optimized cut-off value for sFLT-1/PlGF ratio was 5.50 (sensitivity 92%; specificity 68%; PPV 32%; NPV 98%) with AUC 87.3% (95% CI, 77.3–97.3) [49]. However, the predictive value of these markers could not be clearly established due to a small samples size.

The current standard of antenatal care in maternal and child health clinics in Malaysia stratifies women at risk using clinical risk factors and a colour coding system. This has been proven to be insufficient [50]. Our finding shows that the antenatal color coding is not a significant predictor for preeclampsia. There is limited evidence on the effectiveness of routine blood pressure and urine protein screening to identify women with preeclampsia [10]. The percentage of women without proteinuria who developed preeclampsia or who have proteinuria without hypertension preceding preeclampsia is unclear due to different approaches in proteinuria measurement [10]. Presence of proteinuria is associated with a worse pregnancy outcome as compared to preeclampsia without proteinuria. On the contrary, higher levels of

Table 3. Regression analysis between positive sFlt-1/PlGF ratio test and adverse pregnancy outcome.

sFlt-1/PlGF ratio	PREGNANCY OUTCOMES		Crude OR[a]	95% CI OR	p	Adjusted OR[b]	(95% CI)	p
	present	None						
	n (%)	n (%)						
Maternal death								
Positive	0(0%)	27(19.3%)	-	-	-			
Negative	0(0%)	113(80.7%)						
Intubation					0.561			
Positive	1(33.3%)	26(19.0%)	2.135	(0.186;4.445)				
Negative	2(66.7%)	111(81.0%)	1.000					
Caesarean section					0.386			
Positive	20(21.3%)	7(15.2%)	1.506	(0.586;3.871)				
Negative	74(78.7%)	39(84.8%)	1.000					
Neonatal death					0.512			
Positive	0(0.0%)	27(19.4%)	0.000	(0.000;—)				
Negative	1(100.0%)	112(80.6%)	1.000					
Low birth weight					<0.001			<0.001
Positive	10(52.6%)	17(14.0%)	6.797	(2.412;19.160)		6.841	(2.282;20.511)	
Negative	9(47.4%)	104(86.0%)	1.000					
Premature delivery					<0.001			<0.001
Positive	16(48.5%)	11(10.5%)	8.043	(3.188;20.289)		8.821	(3.620;21.494)	
Negative	17(51.5%)	94(89.5%)	1.000					
Apgar score at 5 minutes <7					0.001			0.028
Positive	11(84.6%)	16(12.6%)	38.156	(7.741;188.084)		17.387	(3.069;98.517)	
Negative	2(15.4%)	111(87.4%)	1.000			1.000		
DIVC					-			
Positive	0(0%)	27(19.3%)	-	-	-			
Negative	0(0%)	113(80.7%)	-					
Placenta abruptio					-			
Positive	1(100.0%)	26(18.7%)	-	-				
Negative	0(0.0%)	113(81.3%)	-					
Acute heart failure					-			
Positive	0(0%)	27(19.3%)	-	-				
Negative	0(0%)	113(80.7%)	-					
NICU admission					<0.001			<0.001
Positive	10(50.0%)	17(14.2%)	7.081	(2.591; 19.352)		8.305	(2.815;24.501)	
Negative	10(50.0%)	103(85.8%)	1.000					
Intrauterine death								
Positive	0(0%)	27(19.3%)	-	-				
Negative	0(0%)	113(80.7%)	-					

[a] Crude odd ratio using simple logistic regression.

[b] Adjusted odd ratio (multiple logistic regression using Backward likelihood method, Hosmer and Lemenshow test p-value >0.05)

proteinuria among women diagnosed with preeclampsia does not indicate a higher risk of severe adverse outcomes [51]. The presence or absence of proteinuria is more important than the amount of proteinuria.

The progress of preeclampsia is often unpredictable and can lead to rapid deterioration in maternal and fetal conditions. Therefore, close surveillance is mandatory during antenatal visits [36]. A decision must be made at the time of diagnosis whether to manage the patient as an

Table 4. The validity of sFLT-1/PlGF ratio test in predicting preeclampsia and low Apgar score at 5 minutes.

Outcome	Sensitivity	Specificity	PPV	NPV	LR + (95% CI)	LR- (95% CI)	cOR (95% CI)	aOR (95% CI)
Preeclampsia	0.586	0.910	0.630	0.893	6.507 (3.344;12.660)	0.455(0.293;0.704)	14.308 (5.349;38.277)	22.000 (6.448;75.069
Apgar score at 5 minutes <7	0.846	0.874	0.407	0.982	6.716 (4.020;11.223)	0.176(0.049;0.631)	38.156 (7.741;188.084)	17.387 (3.069;98.517)
Low birth weight	0.526	0.860	0.370	0.920	3.746 (2.029;6.918)	0.551(0.341;0.890)	6.797 (2.412;19.160)	6.841 (2.282;20.511)
Premature delivery	0.485	0.895	0.593	0.847	4.628 (2.391; 8.959)	0.575(0.411;0.806)	8.043 (3.188;20.289)	8.821 (3.620;21.494)
NICU admission	0.500	0.858	0.370	0.912	3.529 (1.896;6.570)	0.583(0.374;0.909)	7.081 (2.591; 19.352)	8.305 (2.815;24.501)

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outpatient or inpatient [36]. In the absence of proteinuria, the mother may be managed as an outpatient [36]. However, it is now recognized that preeclampsia may be present even without proteinuria. A highly predictive, pre-emptive, and low-cost diagnostic test is needed to guide management [52]. Early preeclampsia detection through universal or targeted screening methods may help reduce health related adverse events, particularly for newborns [53]. We found positive sFlt-1/PlGF ratio (>38) to have 15 times higher odds of developing preeclampsia and 6 to 17 times higher odds of having a newborn with poor outcomes. The majority of positive
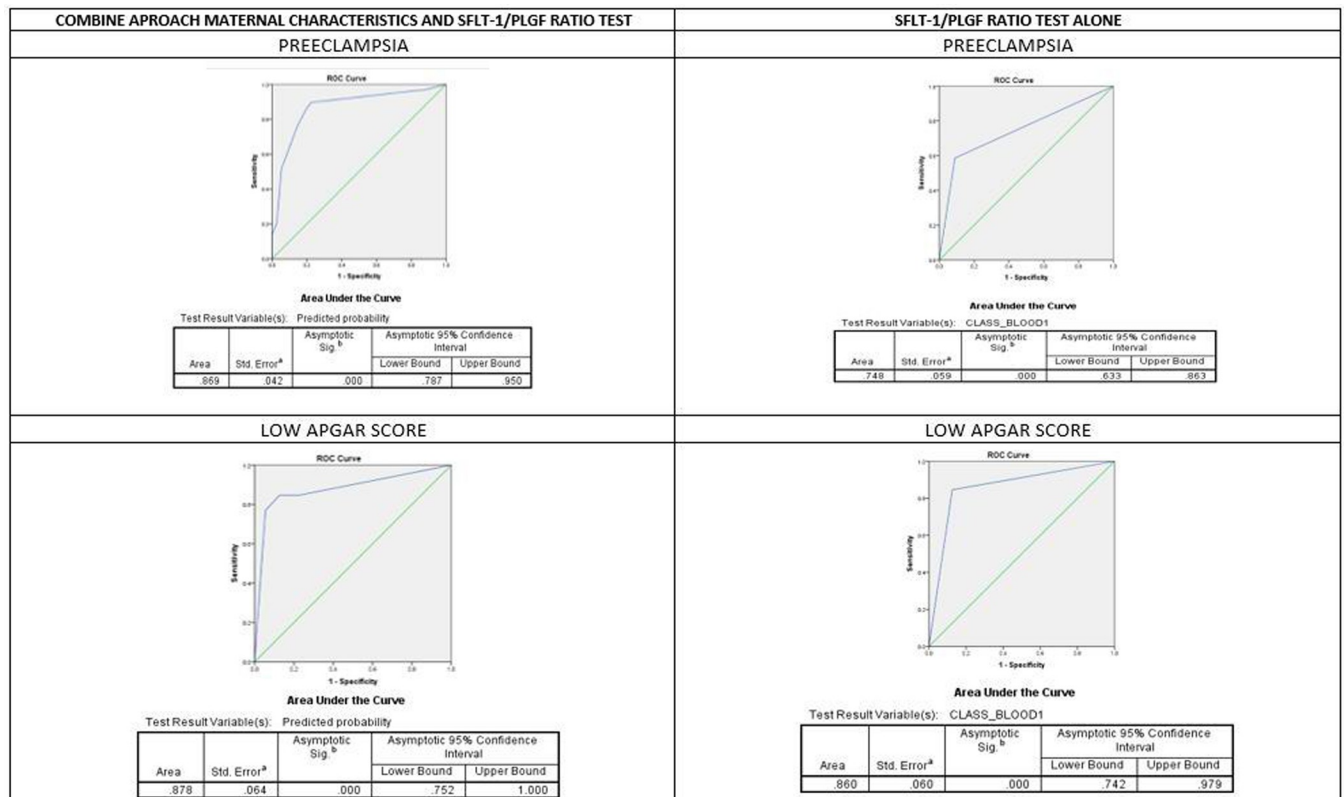

Fig 3. ROC curve comparison between combine maternal and biomarker approach and biomarker alone.

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test mothers (62.5%) manifest preeclampsia within a week. With good NPV (89.3%) it is effective in identifying patients who will benefit from admission, close monitoring, and antenatal corticosteroids commencement in anticipation of preterm delivery [54, 55]. Thus, the test may improve maternal and child health care by early identification, early intervention and prevent subsequent bad outcomes.

Placental dysfunction causes a range of perinatal pathologies, including preeclampsia. Angiogenesis-related factors, including sFlt-1 and PlGF, play an important role in placental dysfunction. Altered levels are detectable several weeks prior to the onset of pregnancy complications [11, 56]. We found that, among mothers who developed preeclampsia within a week of assessment, 62% (5/8) had sFLT-1/PlGF ratio >38 (positive). A total of 59% (17/29) women with such positive results developed preeclampsia within a week, and majority (94%) developed preeclampsia within four weeks. However, 37.9% women who developed preeclampsia were among women with sFlt-1/PlGF ratio ≤38 (negative) with a small percentage of 25% (3/12) progressing to preeclampsia in less than a week of assessment. Besides the ability to predict preeclampsia, the positive test can predict Apgar score < 7 at 5 minutes postnatally with a sensitivity of 84.6%, specificity of 87.4%, PPV of 40.7% and NPV of 98.2%. The AUC was 86.0% (p<0.001, 95% CI 74.2–97.9) using sFlt-1/PlGF ratio alone. The AUC and predictive values showed improvement based on multiple logistic regression predictive values.

An interesting finding in this study was that women working as professionals were at higher risk of developing preeclampsia with adjusted odds ratio (aOR) 8.79 (95% CI 2.80–27.58). This finding is supported by a previous study that the risk of preeclampsia increased 3.1-fold (95% CI 1.2–7.8) among women employed in high-stress jobs (high psychological demand, low decision latitude) [57]. The aOR was 2.0 (95% CI 1.0–4.3) for low-stress jobs compared to non-working women [57]. In another study, being exposed to physically demanding and stressful occupational conditions at the onset of pregnancy increased the risk of preeclampsia [58]. Women working more than five successive days without a day off had aOR 3.0 (95% CI 1.0–9.5) [58]. A significant association exists between acute and chronic occupational/domestic stress with major depression/current depressive symptoms among married professionals and managerial employees [59]. We therefore propose adding occupation as a criteria in the list of current guidelines for risk assessment of preeclampsia in order to improve preeclampsia prediction and thus prevention.

The sFlt-1/PlGF ratio is useful for clinical decision-making with regard to hospitalization [60]. In addition to improving clinical care, assessment of the sFlt-1/PlGF ratio helps avoid unnecessary stress and anxiety for the patient [54]. Malaysia practices a mixed public-private healthcare system [61]. Nonetheless, public hospitals and clinics are the primary source of care in Malaysia. The public sector provides for the bulk (65%) of the population, but is served by only 45% of all registered doctors, and even fewer specialists (25–30%) [62]. The heavily subsidized public sector is borne by budget provisions, with patients paying minimal fees for access to both outpatient and hospitalized care [61, 62]. Thus, a reduction in hospitalization may contribute substantially to cost-saving, and reduce the financial and workload burden in the Malaysian healthcare system [63–65].

The strength of our study lies in the prospective cohort study design, hence the temporal effect between the sFlt:PlGF and onset of preeclampsia and adverse pregnancy outcome can be established according to Hills criteria. The limitation of this study is that it was restricted to a single urban tertiary centre, hence producing low external validity. A number of study participants (43%) were lost to follow-up especially after the first test due to several reasons, including government enforcement of the movement control order in response to the COVID-19 pandemic, work and family commitments, and logistic issues. Therefore, the findings should be interpreted cautiously if it were to be applied to a different population setting.

Conclusions

We found a higher PPV value for sFlt-1/PlGF compared to previous observations. Interestingly, we also found that the sFlt-1/PlGF ratio is a significant marker for predicting adverse pregnancy outcomes among newborns of medium to high risk mothers. In summary, this study highlighted for the first time, the utility of the sFlt-1/PlGF ratio as a feasible biomarker to predict preeclampsia in the Malaysian population, in combination with other parameters (maternal clinical characteristics), provided the outcome measurement period is not restricted to 4 weeks.

Supporting information

S1 Data.
(XLSX)

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Writing – review & editing: Rosnah Sutan, Zaleha Abdullah Mahdy, Rahana Abd Rahman, Dian Nasriana Nasuruddin.

References

1. Ananth C, Lavery J, Friedman A, Wapner R, Wright J. Serious maternal complications in relation to severe pre-eclampsia: a retrospective cohort study of the impact of hospital volume. BJOG: An International Journal of Obstetrics & Gynaecology. 2017; 124(8):1246–53. https://doi.org/10.1111/1471-0528.14384 PMID: 27770512

2. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. Hypertension. 2018; 72(1):24–43. https://doi.org/10.1161/HYPERTENSIONAHA.117.10803 PMID: 29899139

3. Hutcheon JA, Lisonkova S, Joseph K. Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy. Best practice & research Clinical obstetrics & gynaecology. 2011; 25(4):391–403. https://doi.org/10.1016/j.bpobgyn.2011.01.006 PMID: 21333604

4. Stubert J, Ullmann S, Bolz M, Külz T, Dieterich M, Richter D-U, et al. Prediction of preeclampsia and induced delivery at <34 weeks gestation by sFLT-1 and PlGF in patients with abnormal midtrimester uterine Doppler velocimetry: a prospective cohort analysis. BMC Pregnancy and Childbirth. 2014; 14 (1):292.

5. Umesawa M, Kobashi G. Epidemiology of hypertensive disorders in pregnancy: prevalence, risk factors, predictors and prognosis. Hypertension Research. 2017; 40(3):213–20. https://doi.org/10.1038/hr.2016.126 PMID: 27682655

6. Tranquilli A, Dekker G, Magee L, Roberts J, Sibai B, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP. Pregnancy hypertension. 2014; 4(2):97. https://doi.org/10.1016/j.preghy.2014.02.001 PMID: 26104417

7. Park F, Deeming S, Bennett N, Hyett J. Cost effectiveness analysis of a model of first trimester prediction and prevention for preterm preeclampsia against usual care. Ultrasound in Obstetrics & Gynecology. 2020.

8. Von Dadelszen P, Magee LA. Preventing deaths due to the hypertensive disorders of pregnancy. Best Practice & Research Clinical Obstetrics & Gynaecology. 2016; 36:83–102.

9. Perez-Cuevas R, Fraser W, Reyes H, Reinharz D, Daftari A, Heinz CS, et al. Critical pathways for the management of preeclampsia and severe preeclampsia in institutionalised health care settings. BMC Pregnancy and Childbirth. 2003; 3(1):6. https://doi.org/10.1186/1471-2393-3-6 PMID: 14525621

10. Henderson JT, Thompson JH, Burda BU, Cantor A, Beil T, Whitlock EP. Screening for preeclampsia: a systematic evidence review for the US Preventive Services Task Force. 2017.

11. Stepan H, Hund M, Andraczek T. Combining biomarkers to predict pregnancy complications and redefine preeclampsia: the angiogenic-placental syndrome. Hypertension. 2020; 75(4):918–26. https://doi.org/10.1161/HYPERTENSIONAHA.119.13763 PMID: 32063058

12. Marshall SA, Cox AG, Parry LJ, Wallace EM. Targeting the vascular dysfunction: Potential treatments for preeclampsia. Microcirculation. 2019; 26(4):e12522.

13. Armaly Z, Jadaon JE, Jabbour A, Abassi ZA. Preeclampsia: Novel Mechanisms and Potential Therapeutic Approaches. Frontiers in physiology. 2018;9. https://doi.org/10.3389/fphys.2018.00009 PMID: 29467662

14. Ahmed A. New insights into the etiology of preeclampsia: identification of key elusive factors for the vascular complications. Thrombosis research. 2011; 127 Suppl 3:S72–5. https://doi.org/10.1016/S0049-3848(11)70020-2 PMID: 21262447

15. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nature medicine. 2006; 12(6):642–9. https://doi.org/10.1038/nm1429 PMID: 16751767

16. Staff AC, Benton SJ, von Dadelszen P, Roberts JM, Taylor RN, Powers RW, et al. Redefining preeclampsia using placenta-derived biomarkers. Hypertension. 2013; 61(5):932–42. https://doi.org/10.1161/HYPERTENSIONAHA.111.00250 PMID: 23460278

17. Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG. Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. Proceedings of the National Academy of Sciences of the United States of America. 1991; 88(20):9267–71. https://doi.org/10.1073/pnas.88.20.9267 PMID: 1924389

18. Iwasaki H, Kawamoto A, Tjwa M, Horii M, Hayashi S, Oyamada A, et al. PlGF repairs myocardial ischemia through mechanisms of angiogenesis, cardioprotection and recruitment of myo-angiogenic competent marrow progenitors. PLoS One. 2011; 6(9):e24872. https://doi.org/10.1371/journal.pone.0024872 PMID: 21969865

19. De Falco S. The discovery of placenta growth factor and its biological activity. Experimental & molecular medicine. 2012; 44(1):1–9. https://doi.org/10.3858/emm.2012.44.1.025 PMID: 22228176

20. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. American journal of obstetrics and gynecology. 2003; 188(1):177–82. https://doi.org/10.1067/mob.2003.111 PMID: 12548214

21. George EM, Granger JP. Recent insights into the pathophysiology of preeclampsia. Expert review of obstetrics & gynecology. 2010; 5(5):557–66. https://doi.org/10.1586/eog.10.45 PMID: 21170149

22. Kar M. Role of biomarkers in early detection of preeclampsia. Journal of clinical and diagnostic research: JCDR. 2014; 8(4):BE01. https://doi.org/10.7860/JCDR/2014/7969.4261 PMID: 24959436

23. Spradley FT, Tan AY, Joo WS, Daniels G, Kussie P, Karumanchi SA, et al. Placental growth factor administration abolishes placental ischemia-induced hypertension. Hypertension. 2016; 67(4):740–7. https://doi.org/10.1161/HYPERTENSIONAHA.115.06783 PMID: 26831193

24. Ferrara N. Vascular Endothelial Growth Factor: Basic Science and Clinical Progress. Endocrine Reviews. 2004; 25(4):581–611. https://doi.org/10.1210/er.2003-0027 PMID: 15294883

25. Ahmad S, Hewett PW, Wang P, Al-Ani B, Cudmore M, Fujisawa T, et al. Direct evidence for endothelial vascular endothelial growth factor receptor-1 function in nitric oxide–mediated angiogenesis. Circulation research. 2006; 99(7):715–22. https://doi.org/10.1161/01.RES.0000243989.46006.b9 PMID: 16946136

26. Sison K, Eremina V, Baelde H, Min W, Hirashima M, Fantus IG, et al. Glomerular structure and function require paracrine, not autocrine, VEGF–VEGFR-2 signaling. Journal of the American Society of Nephrology. 2010:ASN. 2010030295.

27. Bertuccio C, Veron D, Aggarwal PK, Holzman L, Tufro A. Vascular endothelial growth factor receptor 2 direct interaction with nephrin links VEGF-A signals to actin in kidney podocytes. Journal of Biological Chemistry. 2011; 286(46):39933–44. https://doi.org/10.1074/jbc.M111.241620 PMID: 21937443

28. Veron D, Villegas G, Aggarwal PK, Bertuccio C, Jimenez J, Velazquez H, et al. Acute podocyte vascular endothelial growth factor (VEGF-A) knockdown disrupts alphaVbeta3 integrin signaling in the glomerulus. PloS one. 2012; 7(7):e40589. https://doi.org/10.1371/journal.pone.0040589 PMID: 22808199

29. Onda K, Tong S, Beard S, Binder N, Muto M, Senadheera SN, et al. Proton pump inhibitors decrease soluble fms-like tyrosine kinase-1 and soluble endoglin secretion, decrease hypertension, and rescue endothelial dysfunction. Hypertension. 2017; 69(3):457–68. https://doi.org/10.1161/HYPERTENSIONAHA.116.08408 PMID: 28115513

30. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. The New England journal of medicine. 2004; 350(7):672–83. https://doi.org/10.1056/NEJMoa031884 PMID: 14764923

31. Maynard SE, Min J-Y, Merchan J, Lim K-H, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. The Journal of Clinical Investigation. 2003; 111(5):649–58. https://doi.org/10.1172/JCI17189 PMID: 12618519

32. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, et al. Predictive value of the sFlt-1: PlGF ratio in women with suspected preeclampsia. New England Journal of Medicine. 2016; 374(1):13–22.

33. Kementerian Kesihatan Malaysia. 5th Edition Clinical Practice Guideline Management of Hypertension 2018 [cited 2019 23 December 2019]. Available from: http://www.acadmed.org.my/index.cfm?menuid=67.

34. Wright D, Tan MY, O'Gorman N, Poon LC, Syngelaki A, Wright A, et al. Predictive performance of the competing risk model in screening for preeclampsia. American Journal of Obstetrics and Gynecology. 2019; 220(2):199.e1–.e13. https://doi.org/10.1016/j.ajog.2018.11.1087 PMID: 30447210

35. Hurrell A, Beardmore-Gray A, Duhig K, Webster L, Chappell L, Shennan A. Placental growth factor in suspected preterm pre-eclampsia: a review of the evidence and practicalities of implementation. BJOG: An International Journal of Obstetrics & Gynaecology. 2020; 127(13):1590–7.

36. Malaysia NTCCEIMD. Training Manual Hypertensive Disorders in Pregnancy Revised 2018. Ministry of Health Malaysia: Family Health Development Division Ministry of Health, Malaysia; 2018. p. 13.

37. Saffer C, Olson G, Boggess KA, Beyerlein R, Eubank C, Sibai BM, et al. Determination of placental growth factor (PlGF) levels in healthy pregnant women without signs or symptoms of preeclampsia. Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health. 2013; 3(2):124–32. https://doi.org/10.1016/j.preghy.2013.01.004 PMID: 26105949

38. Stepan H, Kuse-Föhl S, Klockenbusch W, Rath W, Schauf B, Walther T, et al. Diagnosis and Treatment of Hypertensive Pregnancy Disorders. Guideline of DGGG (S1-Level, AWMF Registry No. 015/018, December 2013). Geburtshilfe Frauenheilkd. 2015; 75(9):900–14. https://doi.org/10.1055/s-0035-1557924 PMID: 28435172

39. Sullivan K, Soe M. Sample Size for a Cross-Sectional, Cohort, or Clinical Trial Studies Sample Size for Cross-Sectional & Cohort Studies & Clinical Trials. Artical; 2007.

40. Abalos E, Cuesta C, Carroli G, Zahida Q, Widmer M, Vogel J, et al. Pre-eclampsia, eclampsia and adverse maternal and perinatal outcomes: a secondary analysis of the World Health Organization Multi-country Survey on Maternal and Newborn Health. BJOG: An International Journal of Obstetrics & Gynaecology. 2014; 121:14–24.

41. Yeoh PL, Hornetz K, Ahmad Shauki NI, Dahlui M. Assessing the Extent of Adherence to the Recommended Antenatal Care Content in Malaysia: Room for Improvement. PloS one. 2015; 10(8):e0135301. https://doi.org/10.1371/journal.pone.0135301 PMID: 26270471

42. O'Gorman N, Wright D, Syngelaki A, Akolekar R, Wright A, Poon LC, et al. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11–13 weeks gestation. American journal of obstetrics and gynecology. 2016; 214(1):103. e1–. e12. https://doi.org/10.1016/j.ajog.2015.08.034 PMID: 26297382

43. Tan M, Wright D, Syngelaki A, Akolekar R, Cicero S, Janga D, et al. Comparison of diagnostic accuracy of early screening for pre-eclampsia by NICE guidelines and a method combining maternal factors and biomarkers: results of SPREE. Ultrasound in Obstetrics & Gynecology. 2018; 51(6):743–50. https://doi.org/10.1002/uog.19039 PMID: 29536574

44. O'Gorman N, Wright D, Poon L, Rolnik DL, Syngelaki A, de ALVARADO M, et al. Multicenter screening for pre-eclampsia by maternal factors and biomarkers at 11–13 weeks' gestation: comparison with NICE guidelines and ACOG recommendations. Ultrasound in Obstetrics & Gynecology. 2017; 49 (6):756–60. https://doi.org/10.1002/uog.17455 PMID: 28295782

45. Ohkuchi A, Saito S, Yamamoto T, Minakami H, Masuyama H, Kumasawa K, et al. Short-term prediction of preeclampsia using the sFlt-1/PlGF ratio: a subanalysis of pregnant Japanese women from the PROGNOSIS Asia study. Hypertension Research. 2021; 44(7):813–21. https://doi.org/10.1038/s41440-021-00629-x PMID: 33727707

46. Bian X, Biswas A, Huang X, Lee KJ, Li TK, Masuyama H, et al. Short-Term Prediction of Adverse Outcomes Using the sFlt-1 (Soluble fms-Like Tyrosine Kinase 1)/PlGF (Placental Growth Factor) Ratio in Asian Women With Suspected Preeclampsia. Hypertension. 2019; 74(1):164–72. https://doi.org/10.1161/HYPERTENSIONAHA.119.12760 PMID: 31188674

47. Khalil A, Maiz N, Garcia-Mandujano R, Penco J, Nicolaides K. Longitudinal changes in maternal serum placental growth factor and soluble fms-like tyrosine kinase-1 in women at increased risk of pre-eclampsia. Ultrasound in Obstetrics & Gynecology. 2016; 47(3):324–31. https://doi.org/10.1002/uog.15750 PMID: 26387758

48. Nadarajah VD, Min RG, Judson JP, Jegasothy R, Ling EH. Maternal plasma soluble fms-like tyrosine kinase-1 and placental growth factor levels as biochemical markers of gestational hypertension for Malaysian mothers. Journal of Obstetrics and Gynaecology Research. 2009; 35(5):855–63. https://doi.org/10.1111/j.1447-0756.2009.01037.x PMID: 20149032

49. Hanita O, Alia N, Zaleha A, Azlin MN. Serum soluble FMS-like tyrosine kinase 1 and placental growth factor concentration as predictors of preeclampsia in high risk pregnant women. The Malaysian journal of pathology. 2014; 36(1):19. PMID: 24763231

50. North RA, McCowan LM, Dekker GA, Poston L, Chan EH, Stewart AW, et al. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. Bmj. 2011; 342:d1875. https://doi.org/10.1136/bmj.d1875 PMID: 21474517

51. Homer CS, Brown MA, Mangos G, Davis GK. Non-proteinuric pre-eclampsia: a novel risk indicator in women with gestational hypertension. Journal of hypertension. 2008; 26(2):295–302. https://doi.org/10.1097/HJH.0b013e3282f1a953 PMID: 18192844

52. Lockwood CJ. Incremental progress in predicting preeclampsia: understanding the biological mechanisms behind it will lead to better treatment. Contemporary OB/GYN. 2016 2016/04//:6.

53. Henderson JT, Thompson JH, Burda BU, Cantor A, Beil T, Whitlock EP. U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews. Screening for Preeclampsia: A Systematic Evidence Review for the US Preventive Services Task Force. Rockville (MD): Agency for Healthcare Research and Quality (US); 2017.

54. Caillon H, Tardif C, Dumontet E, Winer N, Masson D. Evaluation of sFlt-1/PlGF ratio for predicting and improving clinical management of pre-eclampsia: Experience in a specialized perinatal care center. Annals of laboratory medicine. 2018; 38(2):95. https://doi.org/10.3343/alm.2018.38.2.95 PMID: 29214752

55. Verlohren S, Galindo A, Schlembach D, Zeisler H, Herraiz I, Moertl MG, et al. An automated method for the determination of the sFlt-1/PlGF ratio in the assessment of preeclampsia. American journal of obstetrics and gynecology. 2010; 202(2):161. e1–. e11. https://doi.org/10.1016/j.ajog.2009.09.016 PMID: 19850276

56. Stepan H, Herraiz I, Schlembach D, Verlohren S, Brennecke S, Chantraine F, et al. Implementation of the sFlt-1/PlGF ratio for prediction and diagnosis of pre-eclampsia in singleton pregnancy: implications for clinical practice. Ultrasound in Obstetrics & Gynecology. 2015; 45(3):241–6. https://doi.org/10.1002/uog.14799 PMID: 25736847

57. Klonoff-Cohen HS, Cross JL, Pieper CF. Job stress and preeclampsia. Epidemiology. 1996; 7(3):245–9. https://doi.org/10.1097/00001648-199605000-00005 PMID: 8728436

58. Haelterman E, Marcoux S, Croteau A, Dramaix M. Population-based study on occupational risk factors for preeclampsia and gestational hypertension. Scand J Work Environ Health. 2007; 33(4):304–17. https://doi.org/10.5271/sjweh.1147 PMID: 17717623

59. Phelan J, Schwartz JE, Bromet EJ, Dew MA, Parkinson DK, Schulberg HC, et al. Work stress, family stress and depression in professional and managerial employees. Psychological Medicine. 2009; 21 (4):999–1012.

60. Klein E, Schlembach D, Ramoni A, Langer E, Bahlmann F, Grill S, et al. Influence of the sFlt-1/PlGF ratio on clinical decision-making in women with suspected preeclampsia. PloS one. 2016; 11(5): e0156013. https://doi.org/10.1371/journal.pone.0156013 PMID: 27243815

61. Loganathan T, Rui D, Ng C-W, Pocock NS. Breaking down the barriers: Understanding migrant workers' access to healthcare in Malaysia. PloS one. 2019; 14(7):e0218669. https://doi.org/10.1371/journal.pone.0218669 PMID: 31269052

62. Quek D, editor The Malaysian healthcare system: a review. Intensive workshop on health systems in transition; 2009.

63. Hodel M, Blank PR, Marty P, Lapaire O. sFlt-1/PlGF Ratio as a Predictive Marker in Women with Suspected Preeclampsia: An Economic Evaluation from a Swiss Perspective. Disease Markers. 2019; 2019:4096847. https://doi.org/10.1155/2019/4096847 PMID: 31485276

64. Schlembach D, Hund M, Schroer A, Wolf C. Economic assessment of the use of the sFlt-1/PlGF ratio test to predict preeclampsia in Germany. BMC health services research. 2018; 18(1):603. https://doi.org/10.1186/s12913-018-3406-1 PMID: 30081904

65. Ohkuchi A, Masuyama H, Yamamoto T, Kikuchi T, Taguchi N, Wolf C, et al. Economic evaluation of the sFlt-1/PlGF ratio for the short-term prediction of preeclampsia in a Japanese cohort of the PROGNOSIS Asia study. Hypertension Research. 2021:1–8.