Angiogenic and Placental factors as Predictors for Gestational Hypertensive Disorders: Which is appropriate?

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Short title

Angiogenic and Placental factors as Predictors for Gestational Hypertensive Disorders.

Abstract

Objectives: To assess the value of sequential estimation of biomarkers for prediction of gestation hypertension (GHTN) and preeclampsia (PE) and to stratify PE according to time of development and severity.

Patients & Methods: 80 normotensive (NT) newly pregnant women who continued pregnancy and were free of hypertensive manifestation (NT group), and the GHTN group included 80 newly pregnant NT women who developed high blood pressure (BP) measures without proteinuria after the 20th gestational week (GW). Early-onset PE (EO-PE) was diagnosed if a GHTN woman developed proteinuria before the 34th GW, but after the 34th GW, it is Late-onset PE (LO-PE). PE was diagnosed as mild if BP was <160/110 with +1 proteinuria on the dipstick, otherwise it is severe PE. Blood samples were obtained at the 12th, 24th, 32nd, and 36th GW for ELISA estimation of serum levels of placental growth factor (PLGF), soluble fms-like tyrosine kinase-1 (sFlt-1), pregnancy protein 13 (PP13), and pregnancy-associated plasma protein-A (PAPP-A) levels. The outcome is the ability of the estimated biomarkers' levels to distinguish women liable to develop GHTN or PE among NT pregnant women.

Results: 9 women developed EO-PE and 25 had LO-PE. 20 women had mild and 14 women had severe PE. Statistical analyses defined a high sFlt-1/PLGF ratio as specific, and low PAPP-A level as a screening predictor for GHTN at the 12th GW, and at the 24th GW low serum levels of PP13 were defined as the highly significant screening, and high sFlt-1/PLGF as a highly significant specific predictor for EO-PE. In the 32nd GW sample, low PP13 levels were defined as the most significant screening predictor, and high sFlt-1/PLGF ratio was the specific predictor for LO-PE.

Conclusion: Sequential estimation of multiple serum

biomarkers for screening of pregnant women to distinguish women vulnerable to developing HPD is required for early detection of these women, especially the high-risk women. The best policy is an estimation of serum PP13 at the 12th GW and an estimation of PP13, sFlt-1, and PLGF to calculate the sFlt-1/PLGF ratio at the 24th and 32nd GW to define women vulnerable to develop PE and to stratify them according to time and severity of PE.

Keywords: Gestational hypertension, Preeclampsia, Early prediction, Serum Biomarkers.

Introduction

Hypertensive pregnancy disorders (HPD) are associated with negative pregnancy outcomes and may be the leading cause of maternal and perinatal deaths worldwide. During pregnancy, uterine vasculature undergoes significant remodeling in the form of vascular growth both in length and circumference to accommodate for the increased blood volume to the fetoplacental unit [(1)].

Preeclampsia (PE) is a multifactorial pathology that makes clinical diagnosis a challenge [(2)]. PE has no cure and is associated with a compromised uterine vascular adaptation to pregnancy sufficient to induce fetal mal-development [(1)].

There is accumulating evidence demonstrating the importance of placental proteins and anti-angiogenic factors for the prediction of PE [(2)]. Galectin 13 (Placental Protein-13; PP13) is a pregnancy-specific galectin protein, which is localized on the syncytiotrophoblast surface and released as placental-associated extracellular vesicles to the maternal uterine vein [(3)]. PP13 is a regulatory protein that has bidirectional action because it has a role in extending the immune tolerance of the mother to the growing fetus [(4)] and because it is involved in pregnancy-induced uterine vascular system remodeling [(3)].

Pregnancy-associated plasma protein-A (PAPP-A) is a metalloprotease secreted by mesenchymal stromal cells and increases the availability of insulin-like growth factor by cleaving it from its binding protein [(5)]. PAPP-A has proven to be a reliable prenatal screening marker and reduced PAPP-A levels have a strong positive predictive value for small gestational age and intrauterine growth restriction [(6)].

The placental growth factor (PLGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) are altered in complicated pregnancies and are associated with PE mostly through induction of endothelial dysfunction which is an important component of PE [(7)].

Objectives

This study tried to assess the value of sequential estimation of an array of biomarkers for prediction of the possibility of getting gestation hypertension (GHTN) and progress of GHTN to PE and to stratify the risky women according to the possible time of developing PE and its probable severity.

Design

Prospective comparative selective clinical trial.

Setting

Departments of Obstetrics & Gynecology and Clinical Pathology, Faculty of Medicine, Benha University.

Ethical Consideration

The detailed study protocol was introduced for approval by the departmental committee before committing to case collection. Thereafter, the protocol was freely discussed with all the attendants of the Obstetrics outpatient clinic, and those accepted to participate in the study signed a fully informed written patients' consent. After the

completion of case collection and the end of the estimation of the assigned biomarkers, the final approval of the study protocol by the Local Ethical Committee, Benha University was obtained and registered by the code: RC:3-11-23.

Study participants

All newly pregnant women who attended the Obstetrics outpatient clinic for assurance of being pregnant were evaluated for determination of demographic data including age, weight, and height for calculation of body mass index (BMI) as the result of dividing weight in kg by height in meter square with BMI of <25 kg/m2 is the cutoff point for defining average weight women. History taking included the number of previous pregnancies and its outcome, as regards the development of HPD, diabetes mellitus (DM), or anemia, mode of previous delivery, neonatal data and the number of living offspring. Medical history was also inquired to ensure the absence of essential hypertension, manifest DM, kidney, cardiac or hepatic diseases, and blood diathesis and coagulopathies.

Exclusion criteria

The presence of a history of adverse pregnancy outcomes, especially PE, the presence of essential hypertension, manifest DM, kidney diseases, hemoglobinopathies, autoimmune disorders, or maintenance of immunosuppressive therapy are the exclusion criteria. Women who had multiple gestational sacs on US diagnosis of pregnancy, and women who attended after the end of the 1st trimester, refused to participate in the study or to sign the written consent or lost during follow-up were also excluded from the study.

Inclusion criteria

Newly pregnant women who attended the clinic early in pregnancy had singleton gestational sacs and were free of exclusion criteria were enrolled in the study.

Diagnosis and categorization of GHTN

Gestational hypertension was defined as the development of high systolic and diastolic blood pressure (SBP & DBP) measures reaching up to ≥140 and ≥90 mmHg, respectively, on two separate measurements at least 4–6 hours apart without detection of protein in the urine after the 20th GW in a previously normotensive (NT) woman at the time of pregnancy diagnosis [8]. PE was defined as the development of GHTN in association with proteinuria quantified as 1+ on the dipstick [9]. Concerning PE severity, PE was considered mild (MPE) if SBP and DBP were <160 and <110 mmHg, respectively, with proteinuria of 1+ and absence of systemic manifestations, while Severe PE (SPE) was diagnosed if SBP and DBP measures were ≥160 and ≥110 mmHg, respectively, with proteinuria ≥2+ on a voided random urine [10]. As regards the timing of PE development, PE was categorized as early-onset (EO-PE) and late-onset (LO-PE) if PE, irrespective of severity, was diagnosed before or after the 34th GW, respectively [11, 12].

Sample size calculation

The null hypothesis of the current study is the detection of significant differences in levels of serum biomarkers between women who completed their pregnancy and were free of gestational hypertensive manifestations (NT women), and women who developed GHTN or PE, irrespective of its timing and/or severity. Atakul (13) detected a significant difference between control (n=24), mild (n=32) and severe PE (n=32) women in serum levels of PAPP-A, and Nikuei et al. (14) reported a significant difference in sFlt-1/PLGF between PE and control women in a study included 23 women developed mild, 15 women developed severe PE, and 20 normal term pregnant. Using the G*Power

(Version 3.1.9.2) (15), the sample size was calculated to be 80 GHTN and 80 NT women. Considering the effect size of 0.20 by the F test model, the calculated sample size of 80 women per group was found to provide a study power of 80% using α-error 5 and ensure the certainty of the null hypothesis.

Study rationale

This selective-based case-control study aimed to collect 80 women who had developed GHTN during their pregnancy course, irrespective of being associated or not with proteinuria. The only condition for selection is that these women must be normotensive at time of diagnosis of pregnancy at the 6th gestational week (GW). On the other side, 80 women who were NT at the 6th GW and continued NT till the 36th GW were also collected.

Both SBP and DBP were measured and registered at the 12th GW (Baseline measure), at the 24th GW to distinguish GHTN women, at the 32nd GW with evaluation of the level of proteinuria to detect women who developed EO-PE among the women had GHTN and to assure that control women were still NT, and at the 36th GW to detect women who developed LO-PE among GHTN women and to assure normality of BP of NT women.

Blood samples were obtained aseptically at the 12th GW for estimation of baseline levels of the studied biomarkers and to evaluate its predictability for the development of GHTN. Other blood samples were withdrawn at the 24th and the 32nd GW to estimate the biomarkers' levels and to determine their distinguishing ability for women vulnerable to develop EO-PE and LO-PE, respectively among GHTN women. The last blood samples were collected at the 36th GW to estimate the biomarkers' levels and ensure the differences between NT and GHTN women and between EO-PE and LO-PE.

Blindness

One author, El Sayed LK, was responsible for case collection and clinical stratification of women according to blood pressure measurements and was blinded about the estimated biomarkers' levels. Another author, Rachwan MT, was responsible for blood sample collection and conduction of investigations and was blinded about which sample was obtained from case or control women and about the timing of sampling concerning gestation weeks. The 3rd author, Mohamed SA, was responsible for the interpretation of clinical and lab data to evaluate the study outcome.

Blood sample processing and storage

Blood samples were collected in plain tubes and were allowed to clot and centrifuged at 3000 rpm for 10 minutes to separate serum. The obtained serum was collected in a sterile numbered Eppendorf tube and stored at -20oC till it was assayed using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions and was read using a 96-well microplate ELISA reader (Dynatech. MR 7000).

Laboratory investigations

1. Human placental growth factor (PLGF) was measured with an Abcam ELISA kit (Cat. No. ab260056 Abcam Inc., Cambridge, USA) [16].
2. Serum levels of human soluble fms-like tyrosine kinase-1 (sFlt-1) were estimated using an Abcam ELISA kit (Cat. No. ab289705, Abcam Inc., Cambridge, USA) [17].
3. Serum human pregnancy protein 13 was assayed using an Abcam ELISA kit (Cat. No. ab100553, Abcam Inc., Cambridge, USA) [18].

4. Serum pregnancy-associated plasma protein-A levels were measured using an Abcam ELISA kit (Cat. No. ab235647, Abcam Inc., Cambridge, USA) [19].

Study outcomes

The outcome of the current study is the ability of the estimated biomarkers' levels for:

1. Distinguishing women liable to develop GHTN among NT pregnant women.
2. Identification of women vulnerable to progress to PE among GHTN women.
3. Differentiation between PE women according to timing and severity of PE.

Statistical analysis

One-way ANOVA test and Chi-square test (X2 test) were used to assess the significance of the intragroup comparisons. Evaluation of predictability was conducted using the Receiver characteristic curve. The significance of the area under the ROC curve (AUC) was assessed concerning the area under the reference curve. The significance of the predictors was assured using the Regression Analysis. Statistical analyses were conducted using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA). The significance of the results was determined using a P-value at a cutoff point of 0.05.

Results

Throughout the study duration and considering the selective basis of the study, 80 pregnant women were selected from those who continued their pregnancy free of GHTN (NT group). Also, 80 women who developed GHTN and were diagnosed at the 24th GW were collected as the GHTN group. The diagnostic flowchart is shown in Figure 1, and the enrolment data of women of both groups are shown in Table 1.

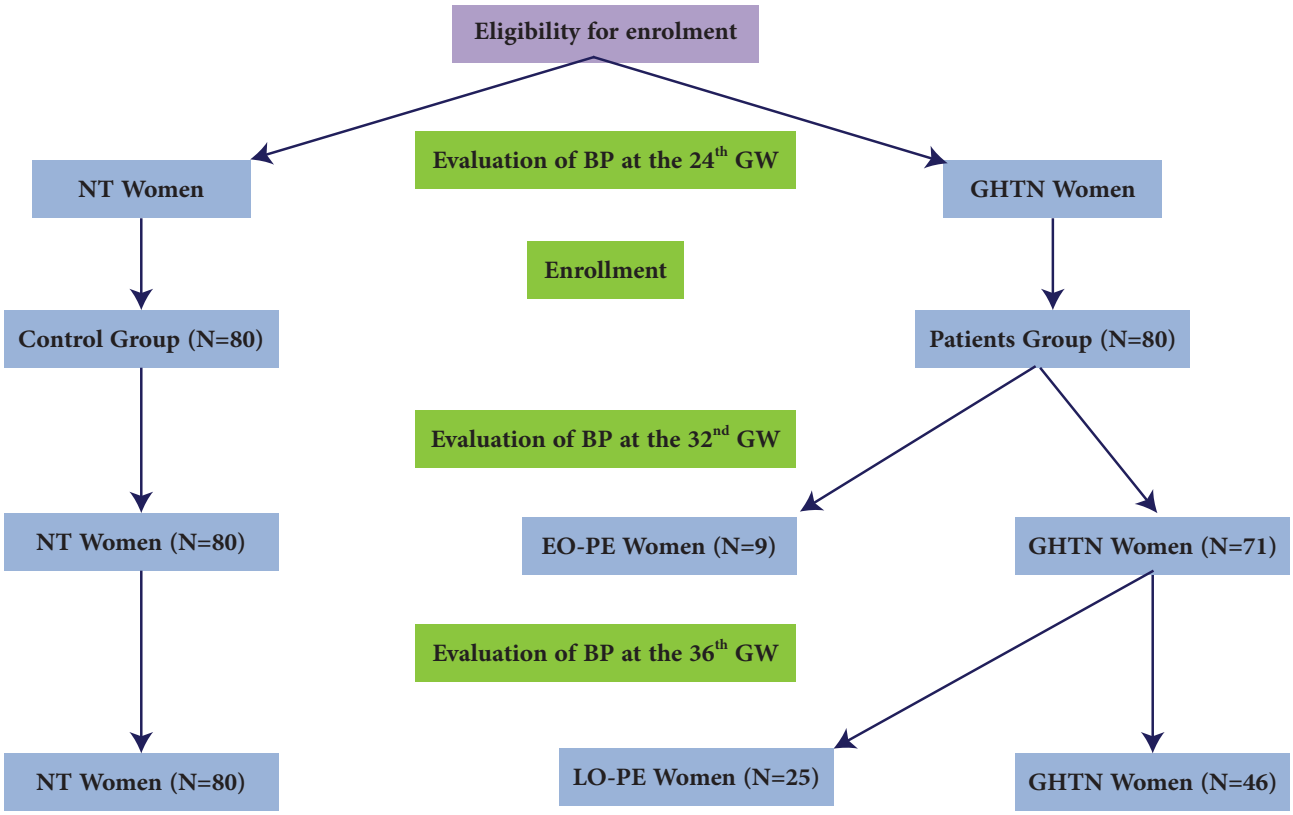


Figure 1: Study Flow Chart

Table 1: Women's enrolment data

Data	Group	NT (n=80)	GHTN (n=80)	P-value
Age (years)	Average (SD)	27.8 (3.7)	28.5 (3.6)	0.226
BMI (kg/m²)	Overweight	43 (53.75%)	35 (43.75%)	0.206
	Obesity	37 (46.25%)	45 (56.25%)	
	Average (SD)	29.3 (2.9)	29.6 (2.6)	0.488
Gravidity	Primigravida	47 (58.75%)	54 (67.5%)	0.251
	Multigravida	33 (41.25%)	45 (32.5%)	
Parity among multigravida	Para-1	14 (42.4%)	18 (69.2%)	0.077
	Para-2	13 (39.4%)	7 (26.9%)	
	Para-3	6 (18.2%)	1 (3.9%)	
Number of living offspring	No	2 (10.5%)	1 (12.5%)	0.648
	One	8 (42.2%)	5 (62.5%)	
	Two	7 (36.8%)	1 (12.5%)	
	Three	2 (10.5%)	1 (12.5%)	

P indicates the significance of intergroup differences at a cutoff point of 0.05

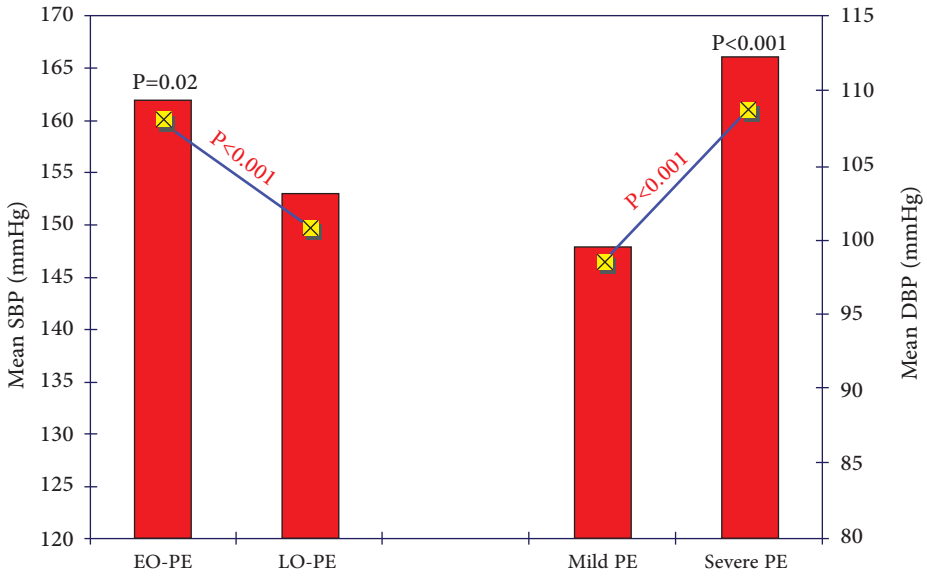
Blood pressure measures estimated at the time of enrolment and the 12th GW of women of both groups showed insignificant differences. At the 24th GW, BP measures were significantly (P<0.001) higher in GHTN than in NT women. Throughout the duration from the 24th to the 32nd GW, 9 GHTN women showed significantly higher BP measures than the remaining 71 GHTN women, developed proteinuria, and were grouped as EO-PE. Moreover, the BP measures of the remaining 71 GHTN women were significantly (P<0.001) higher in comparison to the BP measures of NT women. During follow-up between the 32nd and 36th GW, 25 GHTN women showed flared-up BP measures that were significantly (P=0.0005 & <0.001 for SBP & DBP, respectively) higher in comparison to the corresponding measures of GHTN women (n=46) as shown in table 2. Further, these 25 women developed proteinuria and regarding the development of PE after the 34th GW, these 25 women were grouped as LO-PE (Fig. 1, Table 2).

Collectively, there were 34 PE women; 9 had early and 25 had late PE, and women who had EO-PE showed significantly higher SBP (P=0.02) and DBP (P=0.0061) than women who had LO-PE. As regards the severity of PE; 20 women had mild and 14 women had severe PE, irrespective of the timing of PE development. Women who had severe PE showed significantly (P<0.001) higher BP measures than women who had mild PE as shown in Table 2, figure 2.

Table 2: Time-course blood pressure measures of the studied women categorized according to the detected blood pressure disorders

Variate	Group Parameter	SBP (mmHg)	P-value	DBP (mmHg)	P-value
Enrolment	NT group (N=80)	116.3 (6.3)	0.267	75.6 (4.5)	0.306
	GHTN group (n=80)	115.1 (7.3)		74.9 (4.1)	
12th GW	NT group (N=80)	118.4 (7.3)	0.110	79.9 (5.5)	0.086
	GHTN group (n=80)	119.9 (4)		81.5 (6.2)	
24th GW	NT group (N=80)	120 (8.4)	<0.001	81.5 (6)	<0.001
	GHTN group (n=80)	140.2 (7.9)		94.4 (4.9)	
32nd GW	NT group (N=80)	120.9 (8.2)	<0.001	82 (5.3)	<0.001
	GHTN group (n=71)	144 (4.9)		95 (2.2)	
36th GW	NT group (N=80)	122.1 (6.8)	<0.001	82.7 (5.2)	<0.001
	GHTN group (n=46)	146 (5.5)		94.5 (2.8)	
32nd GW	GHTN group (N=71)	144.8 (5.2)	<0.001	95 (1.8)	<0.001
	EO-PE group (n=9)	162 (11.8)		108 (8.5)	
36th GW	GHTN group (N=46)	147 (3.6)	0.0005	95.5 (1.4)	<0.001
	LO-PE group (n=25)	153 (8.5)		100.8 (5.4)	
Timing of PE	EO-PE (n=9)	162 (11.8)	0.020	108 (8.5)	0.0061
	LO-PE (n=25)	153 (8.5)		100.8 (5.4)	
Severity of PE	Mild PE (n=20)	147.9 (2.4)	<0.001	98.5 (4.2)	<0.001
	Severe PE (n=14)	166.1 (6.7)		108.8 (5.5)	

The P-value indicates the significance of the difference in BP measures at a cutoff point of 0.05



Fif. (2) Mean SBP & DBP measures in PE women categorized according timing and severity

Estimated serum levels of the studied biomarkers in samples obtained at enrolment showed insignificant differences between the enrolled women. Serum levels of PP-13 and PAPP-A estimated in women's samples were significantly (P<0.001) lower in GHTN women than NT women throughout the pregnancy course. Moreover, serum PP-13 and PAPP-A levels were significantly lower in samples of EO-PE and severe PE women than levels estimated in samples of LO-PE and mild PE women, respectively (Table 3).

Table 3: Time-course serum levels of PP-13 and PAPP-A estimated in samples of the studied women categorized according to the detected blood pressure disorders

Variate	Group Biomarker	PP-13 (ng/ml)	P-value	PAPP-A (mIU/ml)	P-value
Enrolment	NT (N=80)	135 (13.8)	0.158	1194 (217.3)	0.204
	GHTN (N=80)	132 (13)		1145 (265.9)	
12th GW	NT (N=80)	157.5 (15.8)	<0.001	1857 (339.6)	<0.001
	GHTN (N=80)	137.3 (9.8)		1042 (252.4)	
24th GW	NT (N=80)	216.4 (31.4)	<0.001	2014 (341.9)	<0.001
	GHTN (N=80)	161.2 (13.9)		955.5 (266.4)	
32nd GW	NT (N=80)	240 (35.2)	<0.001	2056 (464.4)	<0.001
	GHTN (N=71)	196 (20.7)		822.6 (241)	
36th GW	NT (N=80)	298 (27.2)	<0.001	2200 (490.6)	<0.001
	GHTN (N=46)	236 (21.6)		788 (248.3)	
Timing of PE	EO-PE (N=9)	97.8 (4.1)	0.0127	529 (112.4)	0.0084
	LO-PE (N=25)	112.9 (16.8)		670.2 (134.5)	
Severity of PE	Mild PE (N=20)	115.8 (17.4)	0.0014	744.5 (86)	<0.001
	Severe PE (N=14)	99 (5.1)		510 (108.1)	

The P-value indicates the significance of differences in BP measures at a cutoff point of 0.05

Throughout the pregnancy course, serum levels of sFlt-1 progressively increased and serum PLGF progressively decreased with progressive increase of sFlt-1/PLGF ratio in samples of GHTN than NT women. The difference between serum levels of sFlt-1 and PLGF, and the in-between ratio between PE women differentiated according to time of development of PE were insignificant, while the differences according to the severity of PE were significant (Table 4).

Table 4: Time-course serum levels of sFlt-1 and PLGF with the sFlt-1/PLGF ratio estimated in samples of the studied women categorized according to the detected blood pressure disorders

Variate	Biomarker Group	sFlt-1	P	PLGF	P	sFlt-1/ PLGF	P
Enrolment	NT (N=80)	145.1 (19.4)	0.188	33 (7.7)	0.461	4.63 (1.2)	0.614
	GHTN (N=80)	149 (17.9)		34.1 (10.9)		4.73 (1.4)	
12th GW	NT (N=80)	555 (127.5)	0.0003	156 (52.6)	0.015	4.2 (2.3)	0.027
	GHTN (N=80)	645.4 (175.6)		138.1 (38.9)		5 (2.15)	
24th GW	NT (N=80)	658.1 (187.9)	<0.001	280.8 (71.8)	<0.001	2.58 (1.25)	<0.001
	GHTN (N=80)	1022.2 (391.1)		179.7 (46)		6.2 (3.6)	
32nd GW	NT (N=80)	1000.2 (219.3)	<0.001	505 (128.5)	<0.001	2.2 (1.14)	<0.001
	GHTN (N=71)	2417.8 (558.8)		98 (20.7)		25.25 (6)	
36th GW	NT (N=80)	1426 (443.2)	<0.001	600.4 (129.2)	<0.001	2.5 (0.85)	<0.001
	GHTN (n=46)	2001.4 (561.3)		83 (20.6)		24.8 (7.4)	
Timing of PE	EO (n=9)	3055.7 (415.8)	0.299	73.8 (8.8)	0.062	42 (7.7)	0.061
	LO (n=25)	2815.7 (630.4)		82 (11.6)		35.2 (9.4)	
Severity of PE	Mild (n=20)	2694 (588)	0.045	83.4 (11.6)	0.016	33.1 (8.6)	0.0031
	Severe (n=14)	3090 (476)		74 (9)		42.3 (7.6)	

The P-value indicates the significance of differences in BP measures at a cutoff point of 0.05

Statistical analysis of the estimated biomarkers' levels in the 12th GW samples using ROC analysis defined high serum sFlt-1 and high sFlt-1/PLGF ratio as specific predictors for oncoming GHTN, but despite the equal AUC, its significance concerning the area under the reference line was higher for sFlt-1/PLGF ratio. Also, ROC curve analysis stratified other biomarkers as screening parameters for oncoming GHTN according to the AUC as follows: low serum levels of PAPP-A, PP13, and PLGF (Table 5, Fig.3). Regression analysis defined low PAPP-A as the predictor with persistently significant standardized coefficient, followed by low serum levels of PP13 (Table 5). To verify the predictability of low serum levels of PP13 and PAPP-A, the paired-sample area difference under the ROC curves showed a significant (P<0.001) difference between AUCs of PP13 and PAPP-A (AUC difference= 0.103; Std. Error difference=0.196; 95% CI: 0.049-0.157) in favor of PAPP-A.

Table 5: Statistical analyses for the serum levels of the studied biomarkers estimated in the 12th GW samples as predictors for oncoming GHTN

	Receiver Operating Characteristic Curve				Regression analysis	
	AUC	Std.	P	95% CI	β	P
PP13	0.131	0.028	<0.001	0.075-0.186	-0.247	<0.001
PAPP-A	0.028	0.010	<0.001	0.007-0.048	-0.674	<0.001
sFlt-1	0.659	0.043	0.001	0.574-0.744	0.083	0.068
PLGF	0.401	0.048	0.031	0.308-0.495	-0.128	0.003
sFlt-1/PLGF	0.659	0.045	<0.001	0.571-0.747	0.088	0.057

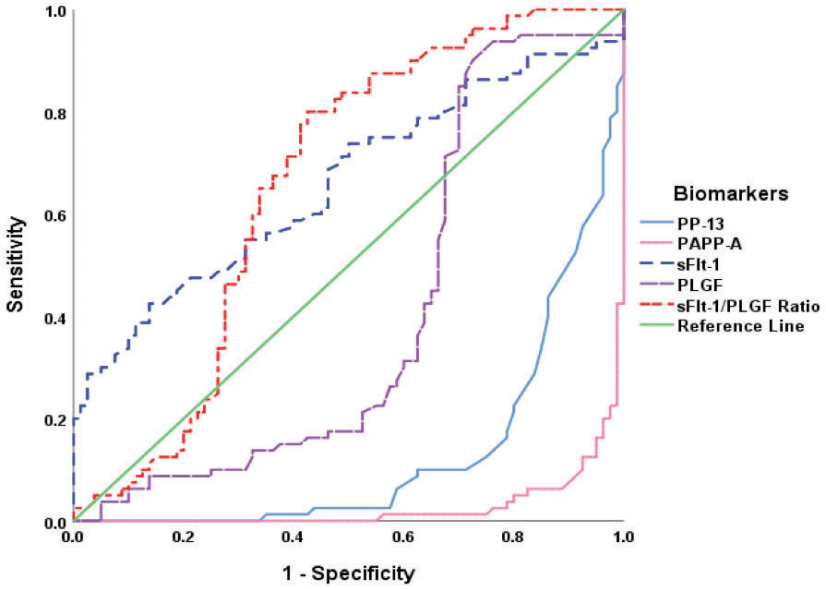


Fig. 3: ROC curve analysis of biomarkers' levels estimated in the 12th GW samples for prediction of oncoming GHTN

ROC curve analysis differentiated the estimated biomarkers' levels in the 24th GW samples for prediction of EO-PE as screening biomarkers with highly significant AUC; PP13, PLGF, and PAPP-A in decreasing order of significance and sFlt-1/PLGF ratio and serum sFlt-1 levels as specific predictors for EO-PE (Table 6, Fig. 4a). Regression analysis assured that low serum levels of PP13 is highly significant screening biomarker for EO-PE and excluded both of PLGF and PAPP-A, while defined high sFlt-1/PLGF as highly significant specific predictor for EO-PE and excluded high serum sFlt-1 (Table 6).

Evaluation of the ability of estimated biomarkers' levels in the 32nd GW sample for predicting the development of LO-PE using the ROC curve defined low PP13 as the most significant screening predictor, while low serum levels of PAPP-A and PLGF could predict LO-PE but with low significant AUC. On the other side, high serum sFlt-1 and high sFlt-1/PLGF ratio could define patients liable to develop LO-PE with nearly equal AUC (Table 6, Fig. 4b). Verification of these biomarkers using Regression Analysis defined low PP13 as the only significant screening predictor and high sFlt-1/PLGF ratio as the specific predictor (Table 6)

Table 6: Statistical analyses for the serum biomarkers' levels estimated in the 24th & the 32nd GW samples as predictors for oncoming PE

		Receiver Operating Characteristic Curve				Regression analysis	
Sample time	Biomarkers	AUC	Std.	P	95% CI	β	P
The 24th GW (for prediction of EO-PE)	PP13	0.000	0.000	<0.001	0.000	-0.230	<0.001
	PAPP-A	0.040	0.022	<0.001	0.000-0.084	-0.093	0.419
	sFlt-1	0.997	0.004	<0.001	0.989-1.000	-0.054	0.641
	PLGF	0.002	0.003	<0.001	0.000-0.007	0.231	0.052
	sFlt-1/PLGF	1.000	0.000	<0.001	1.000-1.000	0.758	<0.001
The 32nd GW (for prediction of LO-PE)	PP-13	0.000	0.000	<0.001	0.000	-0.857	<0.001
	PAPP-A	0.291	0.060	0.004	0.172-0.409	-0.135	0.846
	sFlt-1	0.759	0.069	<0.001	0.623-0.895	0.055	0.255
	PLGF	0.349	0.068	0.037	0.217-0.482	0.078	0.727
	sFlt-1/PLGF	0.772	0.073	<0.001	0.630-0.914	0.126	0.020

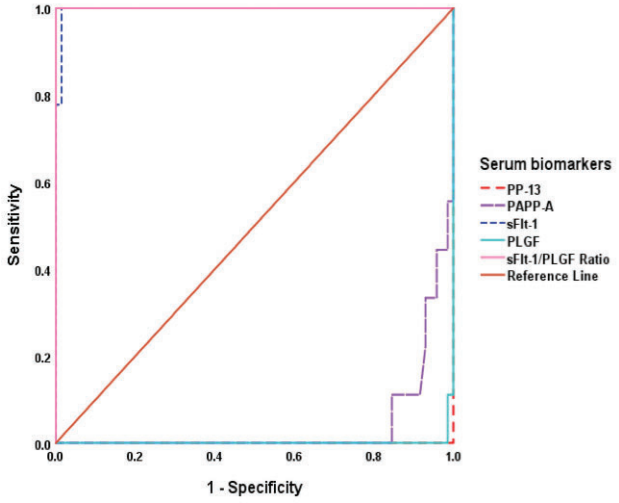


Fig. 4a: ROC curve analysis of biomarkers' levels estimated in the 24th GW samples for prediction of oncoming EO-PE

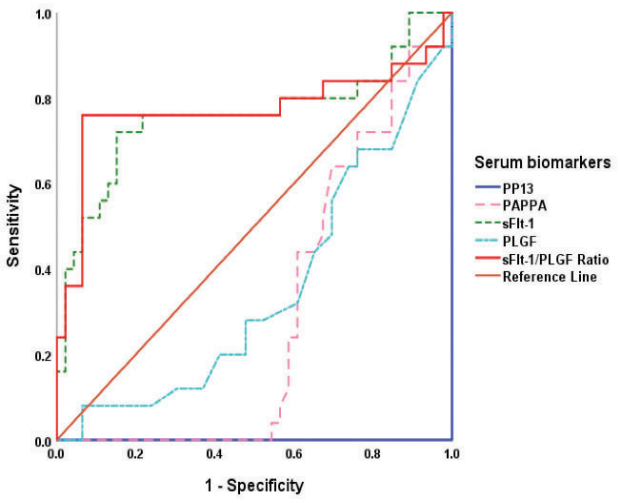


Fig. 4b: ROC curve analysis of biomarkers' levels estimated in the 24th GW samples for prediction of oncoming LO-PE

Using the ROC curve analysis for serum biomarkers' levels estimated in the 24th GW samples, low serum levels of PP13 and PAPP-A were found to be significant screening predictors for severe EO-PE (Fig. 5a), but Regression Analysis excluded PAPP-A and assured the utility of PP13 as a screening biomarker for severe EO-PE. According to the estimated serum biomarkers' levels in the 32nd GW samples, ROC curve analysis defined low serum PP13 and PPAP-A as the significant screening and high sFlt-1/PLGF ratio as the significant specific predictor for severe LO-PE (Fig. 5b). However, Regression Analysis defined low PP13 serum levels in the 32nd GW sample as the significant screening predictor for severe LO-PE (Table 7).

Table 7: Statistical analyses for the serum levels of the studied biomarkers estimated in the 24th & the 32nd GW samples as predictors for severity of oncoming PE

		Receiver Operating Characteristic Curve				Regression analysis	
Sample time	**Biomarkers**	AUC	Std.	P	95% CI	β	P
The 24th GW (for prediction of severe PE among women who had EO-PE)	**PP13**	0.000	0.000	<0.001	0.000	-0.230	<0.001
	PAPP-A	0.040	0.022	<0.001	0.000-0.084	-0.093	0.419
	sFlt-1	0.997	0.004	<0.001	0.989-1.000	-0.054	0.641
	PLGF	0.002	0.003	<0.001	0.000-0.007	0.231	0.052
	sFlt-1/PLGF	1.000	0.000	<0.001	1.000-1.000	0.758	<0.001
The 32nd GW (for prediction of severe PE among women who had LO-PE)	**PP13**	0.000	0.000	<0.001	0.000	-0.857	<0.001
	PAPP-A	0.291	0.060	0.004	0.172-0.409	-0.135	0.846
	sFlt-1	0.759	0.069	<0.001	0.623-0.895	0.055	0.255
	PLGF	0.349	0.068	0.037	0.217-0.482	0.078	0.727
	sFlt-1/PLGF	0.772	0.073	<0.001	0.630-0.914	0.126	0.020

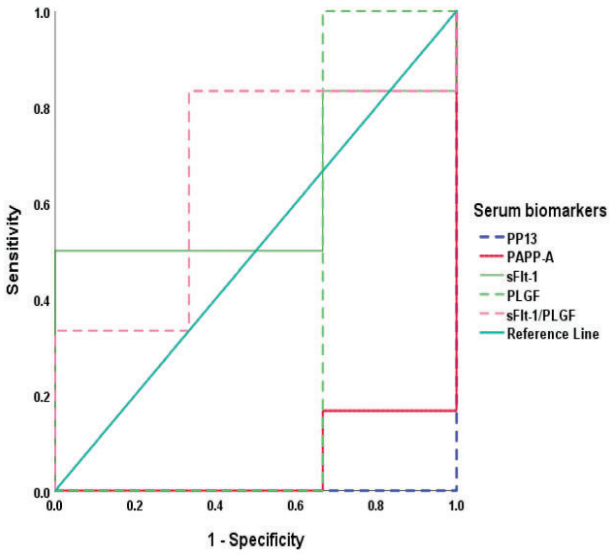


Fig. 5a: ROC curve analysis of biomarkers' levels estimated in the 32nd GW samples for prediction of oncoming severe EO-PE

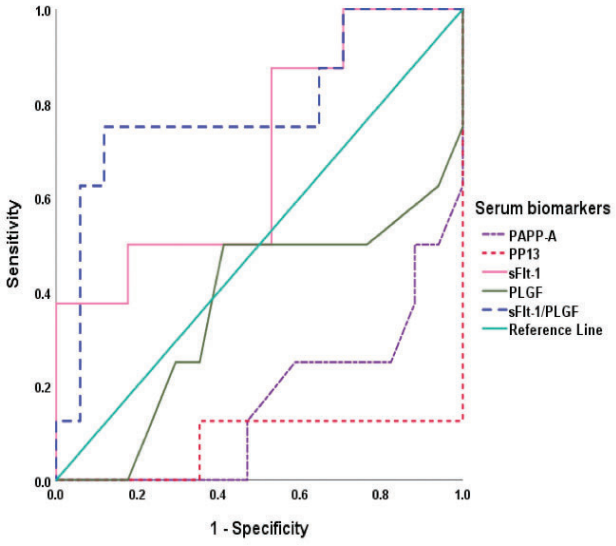


Fig. 5b: ROC curve analysis of biomarkers' levels estimated in the 32nd GW samples for prediction of oncoming severe LO-PE

Discussion

The results of the current study shed light on two important points; first, no single serum biomarker could predict the oncoming GHTN, the possibility of progress of GHTN to PE or stratify patients according to timing and severity of PE. Second, a single sample for estimation of serum biomarkers' levels could falsely exclude the possibility of committing GHTN or define women as vulnerable to developing PE. In support of these findings, serum biomarkers' levels estimated throughout the pregnancy course showed significant differences between NT and GHTN women, thus just estimation of any biomarker in a single sample can give fake results, if not interpreted with levels of other biomarkers and clinical findings.

Moreover, ROC curve analysis for serum biomarkers' levels estimated in the 12th GW samples showed its ability to be used as early predictors for GHTN with varied AUC, but Regression Analysis defined low serum levels of PAPP-A and PP13 in the 12th GW samples as the biomarkers that might distinguish women vulnerable to develop GTHN at or after the 24th GW and any of them could be used as early screening test for hypertensive pregnancy disorders (HPD).

In line with this finding, multiple previous studies assured the applicability of estimated serum PAPP-A levels early in pregnancy as a predictor for oncoming pregnancy adverse events, where Luewan et al. (19) documented that pregnant women with low 1st-trimester PAPP-A levels must be managed as PE high-risk women, and Ramezani et al. (20) found the risk for developing gestational diabetes mellitus (GDM) was 3.98-fold higher in women had low PAPP-A during the 11-14 GW than women had normal serum PAPP-A levels, and concluded that low PAPP-A level is a risk factor that might be used for early prediction of GDM. Also, Genc et al. (21) documented that the 1st-trimester screening PAPP-A test could be used for early prediction of pregnancy complications, and Gupta et al. (22) detected a significant difference between serum levels of PAPP-A and PLGF in samples obtained during the 1st-trimester from normotensive and PE women. Thereafter, Melamed et al. (23) found the adjusted relative risk using low PAPP-A serum level estimated during the 1st trimester as a predictor for oncoming placenta-mediated complications including PE was 2.48 versus 2.28 for low PLGF.

Despite the ability of estimated serum PAPP-A levels at the 12th GW to early predict GHTN, its levels estimated at the 24th and 32nd GW failed to predict PE or to stratify it according to timing. Similarly, Jie et al., (2023) (24) detected an increased risk for PE in women who had normal PAPP-A levels during the 2nd trimester as judged by abnormal uterine artery Doppler findings.

Contrary to PAPP-A, low serum levels of PP13 in the 12th GW sample could be used for screening pregnant women for the possibility of development of GHTN and the 24th and the 32nd GW samples could be used as screening tests with high sensitivity for distinguishing women liable to develop EO and LO-PE, respectively among GHTN women and to define women who will develop severe PE, irrespective of time of having PE among women vulnerable to develop PE.

In line with the usefulness of low serum PP13 as a biomarker for the predictive screening for HPD, Wu et al. (25) in a meta-analysis documented that decreased expression levels of PP13 expression is one of the earliest signs of PE development with high predictive performance. Also, Piskun et al. (26) using immunohistochemistry detected lower expression of PP13 in syncytiotrophoblast of chorionic villi of placentae of PE women than in placenta of normal pregnancies and concluded that the lowest production of PP13 is accompanied by placental dysfunction and PE. Recently, Palalioglu & Erbiyik (27) documented the relation between endothelial

dysfunction parameters; PP13, P-selectin, and E-selectin and the development of PE.

Experimentally using an animal model, PP13 was found to have a dilatory action on uterine and placental vasculature in late pregnancy through a pathway consisting of small conductance Ca2+-activated K+ channels- Nitric Oxide- big conductance Ca2+-activated K+ channels. So the reduced PP13 levels lead to a diminution of this dilatory action with the subsequent release of vasoactive substances and induction of hypertensive disorders (1).

The calculated sFlt-1/PLGF ratio, according to levels estimated in the 24th and the 32nd GW samples, could be used as a specific predictor for the development of EO and LO-PE, irrespective of the severity of PE. These findings go in hand with the previous retrospective studies that documented the ability of high sFlt-1/PLGF ratio to identify women who were liable to develop PE among GHTN women and to predict PE development within the oncoming 4 weeks (28, 29). Moreover, Rowson et al (30) detected significantly lower serum PLGF and higher sFlt-1 with a high ratio in pregnant women who gave samples during the 2nd trimester and developed PE in comparison to women who did not develop PE and gave samples at the same time.

In support of the high diagnostic yield of high sFlt-1/PLGF ratio and its specificity for PE women among GHTN women, Miller et al (31) documented the superior clinical performance of sFlt-1/PlGF over hypertension and proteinuria alone to predict PE. Also, Kluivers et al. (32) detected significantly increased serum levels of sFlt-1 with high levels of sFlt-1/PLGF ratio in PE than non-PE women, and attributed these increased levels to upregulation of the expression levels of sFlt-1 that occurs synergistically with the progress of pregnancy and increases of blood pressure measures. In another study, Kurlak et al. (33) detected low levels of PLGF, and high sFlt-1 levels with a high in-between ratio in the maternal serum of PE women and found estimated levels of selenium, zinc and manganese were all lower, while copper levels were high in PE women and suggested a possible role for deregulated levels of these antioxidant micronutrients for the development of the antiangiogenic state in PE women.

The reported diagnostic value of each biomarker at a certain time during pregnancy indicated the need for more than one biomarker to verify the pregnant women according to the possibility of PE development, and to necessitate the sequential estimation of these biomarkers using multiple samples obtained during pregnancy. The only obstacle to applying a similar protocol as routine screening protocol is the cost of the ELISA estimation of these biomarkers. However, from the cost-benefit point of view, the cost of screening for early prediction and management of pregnancy-associated complications to provide safe pregnancy outcomes concerning the mother and the fetus is surely lower than the management of complications or confronting the risk of maternal and fetal morbidities and mortalities. In line with this suggestion, Garay et al. (34) also suggested the possibility of cost-saving with the implementation of biomarkers-based screening for PE through the reduction of unnecessary hospitalizations.

Conclusion

Sequential estimation of multiple serum biomarkers for screening of pregnant women to distinguish women vulnerable to developing HPD is required for early detection of these women, especially high-risk women. The best policy is an estimation of serum PP13 at the 12th GW and an estimation of PP13, sFlt-1, and PLGF to calculate the sFlt-1/PLGF ratio at the 24th and 32nd GW to define women vulnerable to developing PE and to stratify them according to time and severity of PE.

Limitation

The inclusion of women free of risk of hypertensive disorders is a limitation of the outcomes of this study, thus a similar study protocol was recommended to be tried for women with a high risk for HPD.

Recommendation

The inclusion of similar protocols for the prediction of other pregnancy-associated complications, especially small for gestational age, intrauterine growth restriction, GDM, gestational anemia, and postpartum PE is mandatory to accomplish a complementary screening protocol. Also, screening campaigns funded by great companies and hospitals are required to establish screening protocols using large sample-sized populations.

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