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Pregnancy-Associated Placental Protein A2/Placental Growth Factor Ratio Might Discriminate Normotensive Pregnant Women Who Are Liable to Develop Early-Onset Preeclampsia

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Abstract

Objectives: Evaluation of the ability of serum Pregnancy-associated Placental Protein A2 (PAPP-A2), Placental growth factor (PLGF) and soluble fms-like tyrosine kinase 1 (sFlt-1) levels estimated at the 12th gestational weeks (GW) to predict the possibility of development of preeclampsia (PE) in the normotensive pregnant women.

Patients: 66 PE and 66 No-PE women gave blood samples at the 12th GW for ELISA estimation of study biomarkers. During pregnancy, 21 women had early-onset and 19 women had severe PE. Study outcome is the ability of serum cytokines' levels estimated at the 12th GW for prediction of oncoming PE development.

Results: Blood pressure (**BP**) measures were significantly **higher** in PE women than their baseline measures and corresponding measures of women of No-PE group. Serum levels of **PAPP-A2** and **sFlt-1** were significantly **higher**, while levels of **PLGF** were significantly **lower** in PE than in No-PE women. ROC curve analysis defined **PAPP-A2/PLGF** ratio **>0.51** as discriminator point for **early-onset PE with moderate accuracy** (AUC=0.662; 95% CI: 0.264-0.982) and sensitivity rate of 90.48% and negative predictive value of 94.12%.

Conclusion: Determination of **PAPP-A2/PLGF** ratio in serum at the **12 GW** might be used as **early predictor for PE and ratio at >0.51** could predict early-onset PE with **moderate accuracy**.

Keywords: Preeclampsia, Early-onset, Pregnancy-associated placental protein A2, Placental growth factor, Soluble fms-Like Tyrosine Kinase-1, Early predictors

1. Introduction

Preeclampsia (PE) is a pregnancy-specific syndrome characterized by hypertension, proteinuria and edema, which resolves on placental delivery [(1)]. PE complicates up to 5% of pregnancies, and is associated with deleterious effects to the gravid women, the fetus and the neonate [(2)].

Adequate placentation, placental tissue remodeling and vascularization is essential for the success of gestation and optimal fetal growth [(3)]. Abnormal maternal serum levels of certain cytokines could contribute to the impaired placentation due to a degree of angiogenic imbalance and endothelial dysfunction [(4)] leading to inadequate trophoblastic invasion of the maternal spiral arteries in pregnancies complicated by PE [(5)].

Vascular endothelial growth factor (VEGF) is a multifunctional cytokine, which regulates angiogenesis [(6)]. Placental growth factor (PLGF) is a member of VEGF family; by alternative splicing of RNA, two homodimeric glycoproteins isoforms are generated and can bind with high affinity to sFlt-1 [(7)]. Soluble Flt-1 (sFlt-1) is a secreted splice variant of Flt-1 that antagonizes VEGF and PLGF by binding, and preventing their interaction with endothelial receptors on the cell surface [(8)]. Increased levels of

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antiangiogenic factors such as sFlt-1 while decreased concentrations of pro-angiogenic factor; PLGF in the circulation occurs weeks before the onset of PE [4] and so higher sFlt-1/PLGF ratio is associated with increased risk of PE [9].

Pregnancy-associated plasma protein-A2 (PAPP-A2) is a placental-enriched gene, which is important for normal human placentation and defects in the gene can cause complications in pregnancy [10]. PAPP-A2 is an insulin growth factor binding protein (IGFBP) protease, which is abundantly expressed in the human placenta, and proteolyses both IGFBP-4 and 5 [11]. The IGFBP, which are the substrate of PAPP-A2, is expressed in some placental components including endothelium of maternal blood vessels, in the syncytiotrophoblast layer of placental villi in the first trimester and may contribute to the release of IGF-II to promote feto-placental growth [12]. PAPP-A2 is also detectable in the circulation of pregnant women during the first trimester raising the possibility that PAPP-A2 may be a useful biomarker of placental dysfunction [13, 14].

2. Methods

Aim of the study

The current study targets to evaluate the ability of serum PAPP-A2, PLGF and sFlt-1 levels estimated at the 12th gestational weeks (GW) to predict the possibility of development of PE in the newly pregnant normotensive women and/or to discriminate PE according to time of development.

Study setting

Obstetrics & Gynecology Department in conjunction with Clinical Pathology Department, Benha University Hospital and Medical Biochemistry Department, Cairo University.

Study design

Prospective comparative case-control study.

Characteristics of participants

The protocol for this study was approved by Benha ethical committee. All women who attended the outpatient clinic of obstetrics at Benha University Hospital for assuring of pregnancy were evaluated clinically for demographic data including age, gravidity, parity, weight and height for calculation of body mass index (BMI) as kg/m^2 with full history taking about previous pregnancies that were complicated by gestational hypertensive disorders or diabetes.

Exclusion criteria

1) Multiple gestational sacs on ultrasonography. 2) Uterine abnormalities. 3) Essential hypertension. 4) Manifested diabetes mellitus. 5) Vascular diseases. 6) Previous deep venous thrombosis, and/or coagulopathy. 7) History of gestational hypertensive disorders. 8) Gestational diabetes mellitus. 9) Small-for-gestational age. 10) Recurrent fetal loss.

Inclusion criteria

Normotensive women with **singleton** gestational sac who attended the clinic at the **6th GW** and free of exclusion criteria were enrolled in the study.

Diagnosis of PE

PE was defined as development of **hypertension** and **proteinuria** in normotensive pregnant women and was stratified according to guidelines of the American College of Obstetricians and Gynecologists, as **mild PE (MPE)** if systolic (**SBP**) and diastolic (**DBP**) blood pressure (BP) 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 sample [15]. PE was categorized as **early-onset PE** (**EPE**) if diagnosed **prior to 34th GW** and **late onset PE** (LPE) if diagnosed **after the 34th GW** [16, 17].

Study protocol and grouping.

All enrolled women were asked to attend the antenatal care clinic for assurance of normal progress of pregnancy, estimation of BP and to give blood samples for estimation of the studied biomarkers and urine samples after their approval for evaluation of presence and severity of proteinuria using the dipstick test. Follow-up visits were assigned to be at the 12th, 24th, 30th and 36th GW. Women who developed increased BP measures with proteinuria were collected as PE group and were categorized as mild or severe and early-onset or late-onset. A number of women who continued their pregnancy free of hypertensive manifestations similar to number of women who developed PE with cross-matched age and BMI were collected as Non-PE group.

Blood sampling

Blood samples (5 ml) were withdrawn under complete aseptic conditions at the 12th GW, allowed to clot and then centrifuged at 3000 rpm for 10 minutes to separate serum that was collected in sterile Eppendorf tube and stores at -20^{o}C till be assayed. Blood samples were collected and numbered by an assistant who was blinded about diagnosis.

Laboratory investigations

Serum cytokines levels were measured using enzyme linked immunosorbent assay (ELISA) kits

according to the manufacturer's instructions and were read using a 96 well microplate ELISA reader (Dynatech. MR 7000).

1. Human placental growth factor (PLGF) was measured with an ELISA kit (catalogue no. ab100629, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique [18].
2. Human soluble fms-like tyrosine kinase-1 (sFlt-1) was measured with an ELISA kit (catalogue no. ab289705, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique [19].
3. Human placental-associated pregnancy protein-A2 (PAPP-A2) was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab235647, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique [20].

Study outcome

The ability of serum cytokines levels estimated at the 12th GW for prediction of oncoming PE and to predict early-onset PE development.

Statistical analysis

The statistical analysis was conducted using the software, Statistical Package for Social Science (SPSS Inc. Released 2009- PASW Statistics for windows version 21.0. Chicago: SPSS Inc.). Data were presented as mean, standard deviation, numbers, and percentages. Inter-group differences were evaluated using paired t-test and intra-group differences were evaluated using One-way ANOVA test. Non-parametric data were evaluated using Chi-square and Mann-Whitney tests. Correlations between biomarkers levels estimated in sample samples obtained at the 12th GW and the development of PE and early-onset were evaluated. The Receiver Operating Characteristic (ROC) Curve was conducted to evaluate the optimum cutoff point to differentiate between early- and late-onset PE and was defined as the cutoff point which maximized the area under curve (AUC) value. Test validity characters of the proposed cutoff point were determined.

3. Results

During the study duration, 583 newly pregnant attended the outpatient clinic of Obstetrics at the 6th GW for clinical assurance of pregnancy, 58 women were excluded for not fulfilling the inclusion criteria and 525 women gave blood sample for baseline levels of the studied biomarkers. Throughout follow-up visits 66 women developed PE; 21 women had hypertensive manifestations with proteinuria before the 34th GW, early-onset PE and 45 women developed manifestations of PE after the 34th GW, late-onset PE. As regards PE severity, 47 women had mild manifestations, while 19 women had severe manifestations **(Fig. 1 & 2).** The remaining 459 women completed their pregnancy free of hypertensive manifestations and 66 women with cross-matched age and BMI at time of enrolment were collected as No-PE group. The enrolment data of women of both groups showed non-significant differences as shown in **(Table 1).**

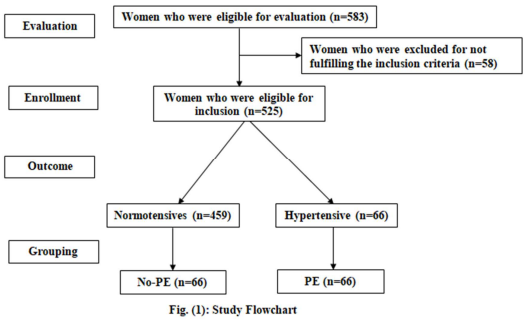

Fig. (1): Study Flowchart

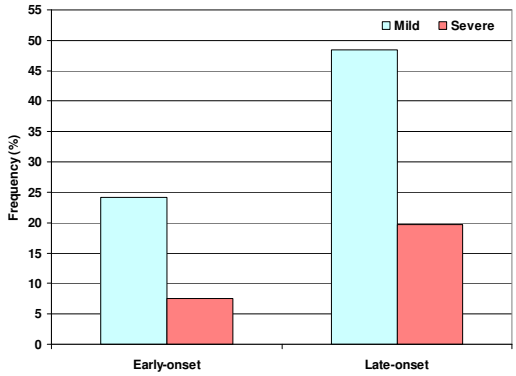

Fig. (2): Frequencies of PE women according to type and severity of PE

Table (1)
Patients' enrollment data

Variable / Group			No-PE (n=66)	PE (n=66)	*P*-value
Age (years)			28±4.1	27±4.7	0.198
Weight (kg)			80.7±8.1	81.4±8.4	0.592
Height (cm)			1658±4.6	166.7±4.9	0.323
	Strata	Overweight	36 (54.5%)	39 (59.1%)	0.598
		Obese I	30 (45.5%)	27 (40.9%)	
Body mass index (kg/m²)	Mean		29.3±2.4	29.3±2.7	0.975
Gravidity			3.4±1	3.6±0.9	0.258
Parity			2.1±0.8	2.3±1	0.181

Data were shown as mean, standard deviation, numbers, and percentages; p-value indicates the significance of difference between both groups; P<0.05 indicates significant difference; P>0.05 indicates non-significant difference, PE: preeclampsia, n: number, Kg: kilogram, cm: centimeter, kg/m2: kilogram per meter

During pregnancy course all women showed increased blood pressure (BP) measures than their baseline BP. However, BP measures were significantly higher in PE women in comparison to their baseline BP measures and to the corresponding measures of women of No-PE group **(Table 2).**

Moreover, BP measures were significantly higher in women had early-onset than those had late-onset PE and in women had severe PE compared to women had mild PE **(Table 3).**

Table (2)
Systolic and diastolic blood pressure measures determined during pregnancy in women of both groups

Variable Group		No-PE (n=66)	PE (n=66)	*P*-value
SBP (mmHg)	6th GW	119.1±4	119.7±3.2	0.329
	12th GW	121.7±3.7	124.7±3.1	0.794
	24th GW	123.9±4.2	133±5.5	0.00004
	32nd GW	127.2±5.1	142.4±19.9	0.0003
	36th GW	129.8±6.5	152.2±12.5	0.00005
DBP (mmHg)	6th GW	83.3±2.6	83.7±2.3	0.327
	12th GW	84.5±2.4	85.1±3	0.226
	24th GW	85.3±2.7	86.5±2.4	<0.0001
	32nd GW	86±2.7	94.4±11.4	<0.0001
	36th GW	85.3±2.2	103±9.9	<0.0001

Data were shown as mean, standard deviation, numbers, and percentages; p-value indicates the significance of difference between both groups; P<0.05 indicates significant difference; P>0.05 indicates non-significant difference, PE: preeclampsia, n: number, SBP: systolic blood pressure, mmHg: millimeter mercury, DBP: diastolic blood pressure

Table (3)
Systolic and diastolic blood pressure measures determined during pregnancy in PE women who were categorized according to timing and severity of PE

Variables		Timing of PE development			Severity of PE		
Variables	Timing	Early	Late	*P*-value	Mild	Severe	*P*-value
SBP (mmHg)	6th GW	120.4±3.4	119.4±3.1	0.231	119.6±3.3	120±3	0.666
	12th GW	126.2±2.7	123.9±3	0.005	124.4±3	125.3±3.3	0.311
	24th GW	138.5±5.5	130.5±3.3	<0.0001	132.6±4.7	134.2±7.3	0.303
	32nd GW	158.7±8.9	134.5±4.3	<0.0001	142.6±8.7	148.2±16.6	0.078
	36th GW	141.2±9.9	157.4±10.2	<0.0001	147±8.7	165.2±11.2	<0.0001
DBP (mmHg)	6th GW	83.5±2.4	83.8±2.3	0.956	83.3±2.3	83±2.9	0.675
	12th GW	85.1±2.8	85.1±3.1	0.962	84.9±2.5	85.5±2.1	0.347
	24th GW	86.2±2	86.6±2.6	0.603	86.5±2.2	87.3±1.9	0.169
	32nd GW	109.9±6.4	87.1±2	<0.0001	94±9.29	97.1±13.1	0.280
	36th GW	92.3±8.4	108±5.6	<0.0001	99.9±8.7	110±9.1	0.0007

Data were shown as mean, standard deviation; p-value indicates the significance of difference between both groups; P<0.05 indicates significant difference; P>0.05 indicates non-significant difference, PE: preeclampsia, SBP: systolic blood pressure, mmHg: millimeter mercury, DBP: diastolic blood pressure

Serum levels of PAPP-A2 and sFlt-1 were significantly higher and serum levels of PLGF were significantly lower in samples of PE women in comparison to No-PE women. Subsequently, the PAPP-A2/PLGF and sFlt-1/PLGF ratios were significantly higher in PE women than in women of the No-PE group **(Table 4).**

Table (4):
Serum levels of studied biomarkers in the 12th GW samples of studied women categorized according to development of PE

Biomarkers	No-PE group (n=66)	PE group (n=66)	*P*-value
PAPP-A2 (mg/ml)	65±28.5	105.4±44.7	<0.0001
Flt-1 (pg/ml)	20.72.9±694.5	2610.8±899.4	0.0005
PLGF (pg/ml)	285.6±87.5	185.8±59	<0.0001
PAPP-A2/PLGF	0.285±0.21	0.73±0.57	<0.0001
sFlt-1/PLGF	8.9±6.11	17.6±12.6	<0.0001

Data are shown as mean, standard deviation; p-value indicates the significance of difference between both groups; P<0.05 indicates significant difference; P>0.05 indicates non-significant difference, PE: preeclampsia, n: number, PAPP-A2: Pregnancy-associated plasma protein-A2, mg/ml: milligram per milliliter, sFlt-1: soluble fms-Like Tyrosine Kinase-1, pg/ml: picogram per milliliter, PLGF: Placental growth factor

Development of PE positively and significantly correlated with serum levels of PAPP-A2, PAPP-A2/PLGF ratio, sFlt-1/PLGF ratio, SBP, serum levels of sFlt-1 in the 12th GW samples, in decreasing order of significance, while showed negative significant correlation with serum PLGF levels estimated in the 12th GW sample and the correlation was positive but non-significant with DBP estimated at the 12th WG. ROC curve analysis assured these relations and its significance **(Table 5, Fig. 3).**

Table (5)
Statistical analyses of blood pressure measures and serum levels of studied biomarkers at the 12th GW as predictors for development of PE

Statistical analyses Variable	Correlation		ROC curve		
	Rho.	*P*-value	AUC (±SE)	*P*-value	95% CI
SBP (mmHg)	0.394	<0.0001	0.726 (0.044)	<0.0001	0.641-0.812
DBP (mmHg)	0.146	0.094	0.584 (0.05)	0.094	0.486-0.682
PAPP-A2 (mg/ml)	0.472	<0.0001	0.772 (0.04)	<0.0001	0.695-0.850
sFlt-1 (pg/ml)	0.313	<0.0001	0.681 (0.046)	<0.0001	0.590-0.771
PLGF (pg/ml)	-0.509	<0.0001	0.206 (0.038)	<0.0001	0.132-0.281
PAPP-A2/PLGF	0.493	<0.0001	0.785 (0.038)	<0.0001	0.709-0.860
sFlt-1/PLGF	0.440	<0.0001	0.754 (0.041)	<0.0001	0.673-0.853

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; PAPP-A2: Pregnancy-associated plasma protein A2; sFlt-1: PLGF: Placental growth factor; Rho: Spearman's correlation coefficient; ROC: Receiver Operating Characteristic; AUC: Area under curve; SE: Standard error; CI: Confidence interval; P-value indicates the significant of variable; P<0.05 indicates significant value; P>0.05 indicates non-significant value, SBP: systolic blood pressure, mmHg: millimeter mercury, DBP: diastolic blood pressure, PAPP-A2: Pregnancy-associated plasma protein-A2, mg/ml: milligram per milliliter, sFlt-1: soluble fms-Like Tyrosine Kinase-1, pg/ml: picogram per milliliter, PLGF: Placental growth factor

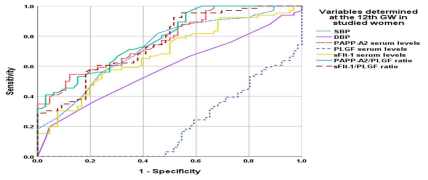

Figure (3): ROC curve analysis for the predictability of BP measures and serum levels of studied biomarkers estimated at the 12th GW for the oncoming development of PE

ROC curve analysis defined **PAPP-A2/PLGF ratio** at **>0.51** could predict **early-onset PE** with moderate accuracy and AUC=0.662; 95CI: 0.264-0.982 (Fig. 4). Using PAPP-A2/PLGF ratio >0.51 as a cutoff point, it could predict early-onset PE with **sensitivity** rate of **90.48%** (95%CI: 69.92-98.83%), **specificity** rate of **71.11%** (95CI: 69-83.63%), negative predictive value of 94.12% (95CI: 80.86-98.38%) and accuracy rate of 77.27% (95CI: 65.3-86.69%).

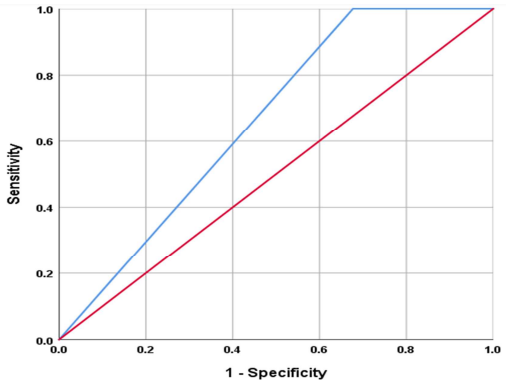

Figure (4): ROC curve analysis for the predictability of PAPP-A2/PLGF ratio at cutoff point 0.51 as predictor for the oncoming early-onset PE

4. Discussion

Estimated serum levels of PLGF were significantly lower, while serum levels of PAPP-A2 and sFlt-1 were significantly higher in women who developed PE during pregnancy (PE group) in comparison to women who completed their pregnancy free of hypertensive manifestations (No-PE group). These data indicated a role for significantly lower angiogenic factors as PLGF, early in pregnancy for initiation of development of abnormal-placentation related pregnancy disorders. In line with this assumption, **Pihl et al.** [21] and **Law & Wei** [22] found high mean arterial pressure and low PLGF in maternal serum at the 1st trimester are markers for the possibility of development of PE.

Recently, **Vikraman & Elayedatt** [2] screened newly pregnant women in first trimester for PE and found estimation of serum levels of PLGF can predict all PE by sensitivity rate of 54%, early-onset PE by sensitivity of 96%, late-onset PE by 77% and term PE by 38% with 10% false positive rate. Also, **Huang et al.** [23] found PE women had significantly lower PLGF, in the first and second trimesters, which could predict early- and late-onset PE with sensitivity rates of 76% and 67%, respectively and 20% false positive rate.

In line with obtained data concerning the detection of high serum levels of anti-angiogenic factors in PE women than in No-PE women, **Crosley et al.** [24] found the concentration of the PAPP-A2 was significantly higher in pregnancies that developed PE than those that did not. **Pihl et al.** [21] documented that high maternal serum sFlt-1 early in the 1st trimester is a significant marker of PE. Also, **Nagalla et al.** [25] and **Wang et al.** [26] detected high levels of PAPP-A2 and sFlt-1 with low PLGF in serum samples obtained during the 1st trimester of women who developed PE and were significantly associated with clinically defined PE. Moreover, **Neuman et al.** [27] found significantly higher levels of PAPP-A2 in serum and placental perfusate of PE women than non-PE women and concluded that PAPP-A2 showed significant potential to predict PE and might prove beneficial on top of the angiogenic markers.

In support of the diagnostic efficacy of the studied biomarkers, **Huhn et al.** [28] who evaluated women had high-risk of PE, found high PAPP-A2 and sFlt serum levels obtained four weeks before development of PE and low PLGF could predict PE with sensitivity rates of 87% 84% and 81%, and specificity rates of 77%, 91% and 83%, respectively.

ROC curve analysis defined PAPP-A2/PLGF at >0.51 could predict early-onset PE with moderate accuracy and test validity characters showed sensitivity and specificity rate of 90.48% and 71.11%, respectively and negative predictive value of 94.12%. These findings supported the previously reported that upregulation of PAPP-A2 appears to begin early in pregnancy, well before the symptoms develop [24].

Keikkala et al. [29] found high PAPP-A2/PLGF predicted early-onset PE by AUC = 0.701 and 95%CI of 0.562-0.840. Also, **Wang et al.** [30] evaluated a series of serum biomarkers for PE prediction and found the AUCs were 0.64 (PAPP-A2/PLGF), 0.63 (blood urea nitrogen), 0.63 (Creatinine), and 0.60 (PAPP-A2) and the positive predictive values of these serum markers range from 33.1 to 58.5%, while the negative predictive values were in range of 80.9- 89.5%.

Multiple studies tried to investigate the pathogenesis of increased PAPP-A2 levels in women who developed PE, **Chen et al.** [31] detected elevated expression levels of PAPP-A2 mRNA in the cytoplasm of primary trophoblasts and this attenuated trophoblast invasion and migration by restraining epithelial-mesenchymal translations via downregulation of the Hedgehog signaling pathway, thus may contribute to poor placentation and inadequate angiogenesis thereby leading to the development of preeclampsia. **Lamale-Smith et al.** [32] detected significantly higher serum levels of PAPP-A2 in pregnant women residing at high compared to those residing at low altitudes and these levels were positively correlated with the uterine artery pulsatility index in cases with early-onset PE, but not in normotensive women and attributed the

elevated levels PAPP-A2 at high altitude to its up-regulation by hypoxia.

5. Conclusion

Determination of **PAPP-A2/PLGF** ratio in serum at the **12th GW** might be used as early predictor for PE and ratio at **>0.51** could **predict early-onset PE** with moderate accuracy.

6. Declarations

Ethics approval and consent to participate

The protocol for this study was approved by Benha ethical committee (00285). All enrolled women were asked to attend the antenatal care clinic for assurance of normal progress of pregnancy, estimation of BP and to give blood samples for estimation of the studied biomarkers and urine samples after their approval.

Consent for publication

All authors approve the manuscript for publication.

Availability of data and materials

Data and materials related to this work are available upon request.

Competing interests

The authors declare that they have no competing interests.

Funding

Nil.

Authors contribution

The manuscript has been read and approved by all authors, authors declare that they have no conflict of interest. The requirement for authorship has been met and that each author has substantial contribution in this manuscript. Heba E. Abdel Raziq and Amira E. Khalil were responsible for data collection, estimation of blood pressure, follow up of patients. Hamasat A Alnoury were responsible for blood and urine sample collection and preparation for further investigations. Hamasat A Alnoury, George N.B. Morcos and Moataz Maher Kamel were responsible for all laboratory investigations and statistical analysis of the data.

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