Low Serum Pregnancy Protein 13 Early in Pregnancy might predict the oncoming Gestational Hypertensive Disorders, especially Early-onset Preeclampsia

Amr Sharafeldeen[a*] and Raafat R. Mohammed[b]

[a]Department of Obstetrics & Gynecology, Faculty of Medicine, Benha University, Egypt.
[b]Assistant Consultant of Medical Biochemistry, Hospital Lab, Clinical Pathology Department, Faculty of Medicine, Benha University, Egypt.

Abstract

Background: Gestational hypertensive disorders (GHD) are associated with maternal and neonatal complications. However, the prediction of GHD stills a dilemma especially in high-risk women.

Objectives: Evaluation of the role of estimated serum levels of pregnancy protein 13 (PP13) for the prediction of GHD in newly pregnant women.

Patients and methods: the attendants of the clinic with a one-missed period (T0) underwent determination of baseline blood pressure (BP) measures and gave blood samples for estimation of levels of PP13, placental growth factor (PLGF) and soluble fms-like tyrosine kinase-1 (sFlt-1). The same evaluations were repeated on the 6th, 24th, 32nd, and 36th gestational week (GW). Twenty non-pregnant women gave samples as control group for estimated biomarkers .

Results: PP13 was undetectable in sera of non-pregnant women but was detected in all T0 samples of pregnant women, but were significantly lower in PE women and continued to increase during pregnancy with significantly lower levels in PE women. The sFlt-1/PLGF ratio was significantly higher in PE women than in other women. Low serum PP13 levels and high sFlt-1/PLGF in T0 samples are significant predictors for high BP measures at the 24th GW and low serum PP13 at the cutoff point of 122 ng/ml can predict early-onset PE.

Conclusion: Low serum PP13 levels in T0 sample could predict women liable to develop GHD especially early-onset PE and low PP13 levels and high sFlt-1/PLGF ratio in the 6th GW samples will assure this suspicion.

Keywords: Gestational hypertensive disorders; Pregnancy protein 13; Placental growth factor; Early predictors; Early-onset Preeclampsia.

DOI: 10.21608/svuijm.2023.184698.1484
***Correspondence**: **amr.sharafeldin20018@gmail.com**
Received: 2 Novenber,2022.
Revised: 5 December,2022.
Accepted: 5 Jaunury,2023.
Cite this article as: Amr Sharafeldeen and Raafat R. Mohammed (2023). Low Serum Pregnancy Protein 13 Early in Pregnancy might predict the oncoming Gestational Hypertensive Disorders, especially Early-onset Preeclampsia. *SVU-International Journal of Medical Sciences*. Vol.6, Issue 1, pp: 465- 475 .

Introduction

Preeclampsia (PE) features de novo pregnancy-induced hypertension and proteinuria that can progress to eclampsia, which is a convulsive, life-threatening condition **(Kusuma et al., 2022)**. PE is an obstetrical syndrome associated with deleterious short-and long-term maternal, fetal, and neonatal consequences, especially in early-onset PE **(Vikraman and Elayedatt, 2022)**.

Uterine stromal cell decidualization is an essential process for fetal implantation and local adaptation, growth, and maintenance of the placenta **(Sahu et al., 2019)**. Defective decidualization and abnormal function of trophoblast cells are important causes of adverse pregnancy outcomes including PE **(Li et al., 2022)**.

Placental growth factors (PLGF-1 and -2) are predominantly expressed in the placenta and are secreted during pregnancy in a strongly correlated manner (Frang et al., 2019). PLGF is the key protein for placental angiogenesis and vasculogenesis, especially during embryogenesis **(Reijnders et al., 2019)**. Soluble fms-like tyrosine kinase 1 (sFlt-1) is an anti-angiogenic factor that is largely produced in the placenta, and binds to and antagonizes PLGF **(Rowson et al., 2022)**.

Placental protein 13 (PP13) is a member of the galectin family with a conserved carbohydrate recognition domain, which is synthesized in the syncytio-trophoblast and is involved in the early placentation process **(Advedissian et al., 2015)**. PP13 was found in microvesicles and exosomes that are released from the syncytio-trophoblast into the maternal circulation, contain high amounts of PP13 and so can be detected in maternal blood **(Sammar et al., 2018)**. This study tried to evaluate the role of early estimation of serum PP13 in prediction of gestational hypertensive disorders (GHD) in newly pregnant women.

Patients and methods

***Setting*:** Departments of Obstetrics & Gynecology and Medical Biochemistry, Faculty of Medicine, Benha University

***Design*:** Prospective observational comparative study

***Ethical consideration*:** The study protocol was preliminary approved by the Local Ethical Committee at Benha Faculty of Medicine at 12-3-2020 and after completion of case collection and obtaining the results, it was finally approved by the number; RC: 5-6-2022 and was registered by ClinicalTrials.gov Identifier: NCT05517512.

***Study protocol*:** All women attending the Antenatal Care clinic of Benha University Hospital with one missed period during the study duration underwent demographic data, estimation of the baseline systolic (SBP) and diastolic blood pressure (DBP) and gave urine samples for estimation of the level of proteinuria using a dipstick test.

***Exclusion criteria*:** History of essential hypertension (HTN), renal, hepatic, or cardiac diseases, metabolic syndrome, body mass index (BMI) >35 kg/ m2, manifest diabetes, or endocrinopathy are the exclusion criteria. Women who attended the clinic after more than one missed period, refused to sign the written consent or missed during pregnancy were also excluded.

***Inclusion criteria*:** Normotensive newly pregnant women who attended the clinic with a one-missed period (T0), free of exclusion criteria, and attend all follow-up visits.

***Diagnosis and categorization of GHD*:** Gestational hypertension (GHTN) was defined according to the American Society of Hypertension

(Lindheimer et al., 2009) and PE was defined and categorized according to severity and time of onset according to guidelines of the American College of Obstetricians and Gynecologists **(Bernhard et al., 2014; Von Dadelszen et al., 2003)**.

Study groups

1. GHTN group included women who developed GHTN but did not progress to PE.
2. PE group included pregnant women who developed PE during pregnancy.
3. NT group included a number of women who completed their pregnancy free of GHTN equal to number of PE women and of cross-matched number, age and BMI to PE women.
4. Non-pregnant women (n=20) of cross-matched age and BMI as a control for serum levels of studied biomarkers; <50 pg/ml for a placental growth factor **(Krauss el al., 2004)**, and ~ 150 pg/ml for soluble fms-like tyrosine kinase-1 **(Chen and Khalil 2017)**, while serum PP 13 was documented to be undetectable in non-pregnant women **(Burger et al., 2004)**.

Blood sampling

Blood samples (5 ml) were obtained at women's 1st attendance to the clinic (T0), at the 6th, 24th, 32nd, and 36th GW. Blood samples were divided into two parts, one for immediate estimation of random blood glucose (RBG) and the other part was allowed to clot, centrifuged at 3000 rpm for 10 minutes to separate serum that was collected in a sterile Eppendorf tube and stores at -20°C till be assayed.

Laboratory investigations

Serum biomarkers' levels were measured according to the manufacturer's instructions using ELISA kit (Abcam Inc., Cambridge, USA; Catalogue No: ab100629; ab119613 & ab100553, respectively) for human PLGF **(Fischeret al., 2008)**, sFlt-1 **(Rebecca et al., 2008)** and PP13 **(Stefanovic et al., 2015)**. Readings were obtained using a 96 well microplate ELISA reader (Dynatech. MR 7000)

Follow-up

At time of the 1st attendance (T0), and at the 6th, 24th, 32nd, and 36th gestational week (GW) BP was measured and blood samples were obtained.

Study outcomes

The study outcome is the ability of estimation of serum PP13 for prediction of development of GHD and categorization of PE.

Statistical analysis

Statistical analyses were conducted using the paired t-, one-way ANOVA and Chi-square tests through IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) for Windows statistical package. According to previous literature, the documented date for the development of early-onset PE was at or after the 24th GW **(Von Dadelszen et al., 2003; Xu et al., 2018)**, so such BP measures were evaluated as the dependent variate versus T0 data as independent variate to assess their ability for early prediction of early-onset PE using the Receiver Operating Characteristic Curve and Regression analyses. P-value <0.05 was considered statistically significant.

Results

During the study duration, 584 women were eligible for evaluation, 63 were excluded and 81 were missed during follow-up, and 440 women were enrolled in the study. During pregnancy, 54 women (12.3%) developed GHTN (GHTN group) and 42 women (9.5%) progressed to PE (PE group), and 42 women of the 346 women who completed their pregnancy

free of hypertensive manifestations were included in the NT group (**Fig. 1**). The enrollment data of the studied women showed non-significant differences as shown in (**Table 1**).

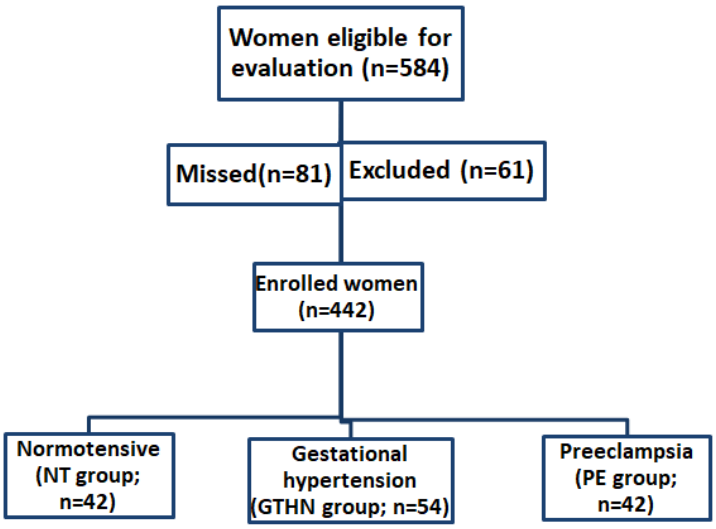

Fig.1. Patients' flowchart

Table 1. Patients' enrolment data

Variables\ Groups	NT (n=42)	GHTN (n=54)	PE (n=42)
Age (years)	26.5±5.6	28.5±4.1	26.4±4.7
Weight (kg)	81.5±8.4	80.8±8.2	82.2±8.4
Height (cm)	167.4±4.2	166.4±4	165.8±4.6
Body mass index (kg/m^2)	29.4±2.8	29.4±2.4	29.7±2.5
Primigravida	33 (78.6%)	43 (79.6%)	27 (64.3%)
Random blood glucose (mg/dl)	86.8±8.1	90.4±7.7	89.1±8.6

Mean, standard deviation, numbers, and percentages

The mean level of BP measures obtained at T0 showed non-significant differences between enrolled women. However, BP measures of women of the GHTN and PE groups were increased progressively till later in pregnancy with significant difference in comparison to BP measures of women of the NT group and significantly higher measures in women of PE than women of GHTN groups (**Table 2**). The incidence of early-onset PE was 28.6% and of severe PE was 21.4% among women of PE group.

Table 2. Blood pressure measures of the studied women throughout pregnancy

Variables\ Groups		NT (n=42)	GHTN (n=54)	PE (n=42)
SBP (mmHg)	T0	119.6±3.2	119±4.2	120±3.3
	6 GW	120.3±4.3	136.9±3.5*	124±4.5*†
	24 GW	120.3±3.3	142.4±1.6*	147.1±9.4*†
	32 GW	120±2.9	146.1±2.1*	148.3±10*
	36 GW	119.2±3.6	149.3±2.1*	151.8±7.1*†
DBP (mmHg)	T0	83.5±2.5	83.5±2.5	84±2.1
	6 GW	83.8±3.4	92.1±1.8*	86.9±2.6*†
	24 GW	83.8±3.5	93.4±1.5*	94.5±5.8*
	32 GW	83.4±3.7	94.5±1.3*	98.6±6.8*†

	36 GW	83±3.2	95.2±1.3*	104.5±5.2*†

*Mean and standard deviation, * indicates significant difference versus NT group; † indicates significant difference versus GHTN group.*

Serum PP13 levels were undetectable in non-pregnant women, while were detected in T0 samples of all pregnant women and were significantly lower in women of the PE group compared to the levels estimated in women of NT ($p<0.001$) and GHTN ($p=0.032$) groups. During pregnancy, serum PP13 levels continued to increase in all pregnant women with significantly ($P<0.001$) higher levels in NT than women of other groups. Serum PP13 levels at the 6th GW were significantly ($P=0.001$) higher in GHTN than PE women, but thereafter the difference was non-significant. On contrary, serum levels of sFlt-1 and PLGF in T0 samples were comparable between pregnant and non-pregnant women with non-significant differences between women of the three groups. Serum levels of both markers increased gradually in all pregnant women, but serum levels of sFlt-1 were significantly higher and serum PLGF levels were significantly lower in women of the PE group than women of the other groups with significant differences between women of the GHTN and NT groups (**Table 3**).

Table 3. Serum biomarkers' levels estimated throughout the pregnancy in samples of women of the three groups.

Variables\ Groups		**Non-pregnant**	**NT (n=42)**	**GHTN (n=54)**	**PE (n=42)**
PP13 (ng/ml)	**T0**	Nil	132.4±6.9	129.6±7.1	125.5±8.9*†
	6 GW		152±9.1	141.5±7.5*	133.5±9.6*†
	24 GW		198±11.4	156.5±10.3*	162±14.6*
	32 GW		213.5±11.2	191.7±14*	186±16.4*
	36 GW		289.5±19.3	240.5±23.3*	244±28.8*
sFlt-1 (pg/ml)	**T0**	137.9±14.7	139±14.6	142.4±15	142±12.8
	6 GW		503.5±130	554±147	670±138.5*†
	24 GW		565±93	728±186*	841.5±212*†
	32 GW		965.8±251	1108±172*	1435±645*†
	36 GW		1718±344	1120±216*	2401±622*†
PLGF (pg/ml)	**T0**	31.8±6.4	31.5±6.4	30±5.9	31.2±6.1
	6 GW		189±25.5	91.3±12.4*	75±15.2
	24 GW		306±43.4	115.4±18.7*	83.5±10.5*†
	32 GW		518±90.8	157.6±23.9*	99±13.6*†
	36		582.7±75.3	200±30*	127±21.4*†

	GW				
sFlt-1/PLGF ratio	**T0**	0.234±0.05	0.228±0.05	0.21±0.04	0.22±0.04
	6 GW		2.7±0.6	6.1±1.6*	9.06±2.2*†
	24 GW		1.9±0.4	6.4±1.95*	10.22±2.8*†
	32 GW		2.25±0.5	6.3±1.5*	14.9±7.4*†
	36 GW		2.97±0.55	5.67±1.2*	19.2±5.45*†

*Mean and standard deviation, * indicates significant difference versus NT group; † indicates significant difference versus GHTN group.*

Linear regression analysis defined low serum PP13 levels as a significant predictor for elevated SBP (β=-0.251, p=0.003) and DPB (β=-0.311, p<0.001) measures at the 24th GW and ROC curve analysis showed that serum PP13 at the cutoff point of 122 ng/ml in the T0 sample could identify cases liable to develop early-onset (<34 GW) PE women with AUC of 0.811 (p=0.019; 95%CI: 0.652-0.969; **Fig. 2**).

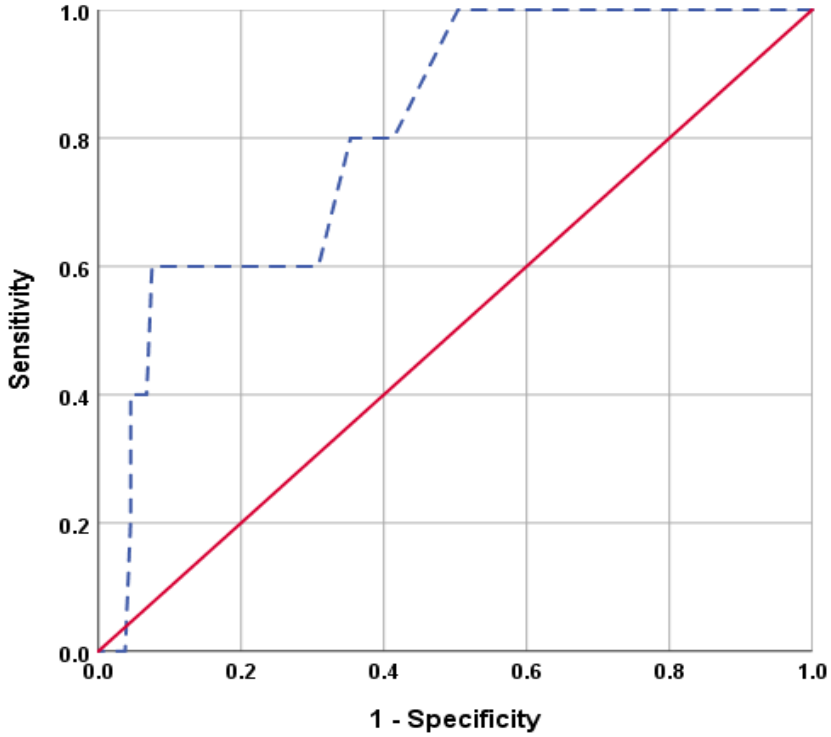

Fig. 2. ROC curve analysis for the serum PP13 level at a cutoff value of 122 ng/ml as a discriminator for women who will develop early-onset PE

Linear regression analysis defined lower serum levels of PPI3 and high sFlt-1/PLGF in the 6th GW sample as independent positive predictors of getting high SBP and DBP measures at and/or after the 24th GW, while high serum levels of sFlt-1 as the independent predictors of getting high DBP only (**Table 4**).

Table 4. Univariate and multivariate analyses of serum biomarkers' levels estimated in the 6th GW samples as independent predictors for getting high SBP and DBP at the 24th GW

Variables	The 6th GW serum level of	Univariate analysis		Multivariate analysis	
		Standardized coefficient (β)	P-value	Standardized coefficient (β)	P-value
Systolic BP (mmHg)	PP13	-0.267	0.003	-0.231	0.008
	sFlt-1	0.006	0.286	-	-
	PLGF	-0.119	0.058	-	-
	sFlt-1/PLGF ratio	1.708	<0.001	1.552	0.001
Diastolic BP (mmHg)	PP13	-0.223	<0.001	-0.176	0.001
	sFlt-1	0.011	0.006	0.020	<0.001
	PLGF	-0.054	0.204	-	-
	sFlt-1/PLGF ratio	0.858	0.009	1.645	<0.001

P<0.05 indicates significant value

Discussion

Pregnancy protein 13 was undetectable in the sera of non-pregnant women, while was detectable in that of the newly pregnant women, while PLGF and sFlt-1 were measurable in sera of non-pregnant and newly pregnant women; a finding illustrating the specificity of PP13 for pregnancy, indicating its early secretion with the onset of pregnancy and supported the previous studies that documented secretion of PP13 from an early stage of pregnancy, so that it can be detected in the maternal serum as early as the 5th GW (**Huppertz et al., 2008; Orendi et al., 2011**).

The early secretion of PP13 could be attributed to its exclusive expression in the amniotic membranes and the placental fetal syncytiotrophoblast (**Than et al., 2009**) while other fetal and maternal tissues show very little to no expression of PP13 (**Than et al., 2014**). This early expression and secretion of PP13 was for its multitask functions through contribution for the mother's immune tolerance to pregnancy by induction of white blood cell apoptosis (**Sammar et al., 2019**), regulation of the activity of plasma neutrophils by polarizing them toward a placental-growth-permissive phenotype (**Vokalova et al., 2020**), induction of uterine vascular remodeling to increase the uteroplacental blood flow (**Drobnjak et al., 2019**) with structural stabilization of the expanding vessels by binding to sugar residues of extracellular and connective tissue molecules through its carbohydrate recognition domain (**Sammar et al., 2019**).

Serum PP13 levels were significantly higher in T0 samples of NT women than in samples of other women and despite the progressively increasing levels with pregnancy progress, as previously documented (**Sammar et al., 2011**), the difference is still significantly higher in favor of NT women. Low serum levels of PP13 in 6th GW sample could high BP measures at the 24th GW and in T0 samples could identify women liable to develop early-onset PE. These findings indicated the possible applicability of serum PP13 to classify newly pregnant

women according to their possible hypertensive status during pregnancy.

These findings supported the previously documented that serum PP13 level assay and PP13 mRNA expression levels are reliable markers for early detection of PE during the first trimester **(El Sherbiny et al., 2012; Chang et al., 2017)**, and go in hand with the meta-analysis that recommended PP13 to be used as an effective biomarker for the screening of PE **(Wu et al., 2021)**. Moreover, another study found combined estimation of serum PP13 and performing uterine artery Doppler improved the predictability for PE **(Soongsatitanon and Phupong 2020)**.

The detected significantly lower serum PLGF levels and higher sFlt-1 levels with high PLGF/sFlt-1 ratio throughout pregnancy in women who developed GHD in comparison to NT women and PE women than in GHTN women go in hand with that previously detected **(Shinohara et al., 2021; Rowson et al., 2022)**. Further, the statistically detected value of high PLGF/sFlt-1 ratio to discriminate between GHTN and PE was in hand with the study that assured the role of elevated sFlt-1/PLGF ratio in differentiation between PE and GHTN **(Yang et al., 2022)**.

To our knowledge, no previous trial for early prediction of GHD was performed and despite the detected higher ability of the sFlt-1/PLGF ratio over serum PP13 in samples of the 6th GW for prediction of PE, but, the diagnostic ability of PP13 the ratio at an earlier date (T0 sample) is advantageous. Thus, combined estimation of PP13 in the T0 sample and sFlt-1/PLGF ratio in the 6th GW sample is worth implementing in clinical screening for GHD among newly pregnant women since the 1st missed period to allow time-space to undertake preventive measures especially reduction of gestational weight gain which increases the risk for GHD **(Kyozuka et al., 2022)**.

Conclusion

Estimation of serum PP13 once there is a missed period can predict women liable to develop GHD especially early-onset PE and its coupling with the determination of sFlt-1/PLGF ratio in serum samples obtained at the 6^{th} GW will assure the suspicion.

Study's limitations

Postpartum follow-up was a limitation of this study to evaluate the ability of these markers to predict postpartum hypertension.

Acknowledgment

The authors provide great thanks to staff members of the Central Lab, Benha University Hospital for their provision of a great share in the used ELISA kits.

References

- **Advedissian T, Deshayes F, Poirier F, Grandjean C, Viguier M. (2015)**. Galectins, a class of unconventional lectins. Med Sci., 31(5): 499–505.
- **Bernhard KA, Siddiqui DS,** Leonard KM, Chauhan SP (2014). American college of obstetricians and gynecologists practice bulletins: ascertaining their citation, influence, and utilization. Am J Perinatol., 31(5): 373-82.
- **Burger O, Pick E, Zwickel J, Klayman M, Meiri H, Slotky R, et al (2004)**: Placental protein 13 (PP-13): effects on cultured trophoblasts, and its detection in human body fluids in normal and pathological pregnancies. Placenta., 25(7): 608-22.

- **Chang Y, Chen X, Cui H, Li X, Xu Y. (2017)**. New Predictive Model at 11 $^{+0}$ to 13 $^{+6}$ Gestational Weeks for Early-Onset Preeclampsia With Fetal Growth Restriction. Reprod Sci., 24(5): 783-789.
- **Chen J, Khalil R. (2017)**. Matrix Metalloproteinases in Normal Pregnancy and Preeclampsia. Prog Mol Biol Transl Sci., 148: 87-165.
- **Drobnjak T, Jónsdóttir A, Helgadóttir H, Runólfsdóttir M, Meiri H, Sammar M, et al. (2019)**: Placental protein 13 (PP13) stimulates rat uterine vessels after slow subcutaneous administration. Int J Women's Health., 11: 213-222.
- **El Sherbiny WS, Soliman A, Nasr A. (2012)**: Placental protein 13 as an early predictor in Egyptian patients with preeclampsia, correlation to risk, and association with outcome. J Investig Med., 60(5): 818-22.
- **Fischer C, Mazzone M, Jonckx B, Carmeliet P. (2008)**: FLT1 and its ligands VEGFB and PlGF: drug targets for anti-angiogenic therapy? Nat Rev Cancer., 8(12): 942–956
- **Frang H, Hurskainen P, Nicolaides K, Sairanen M. (2019)**. PlGF isoform 3 in maternal serum and placental tissue. Pregnancy Hypertens., 18: 9-13.
- **Huppertz B, Sammar M, Chefetz I, Neumaier-Wagner P, Bartz C, Meiri H. (2008)**. Longitudinal determination of serum placental protein 13 during the development of preeclampsia. Fetal Diagn Ther., 24: 230–6.
- **Krauss T, Pauer H, Augustin HG. (2004)**. Prospective analysis of placenta growth factor (PlGF) concentrations in the plasma of women with normal pregnancy and pregnancies complicated by preeclampsia. Hypertens Pregnancy, 23(1): 101-11.
- **Kusuma G, Georgiou H, Perkins A, Abumaree M, Brennecke S, Kalionis B. (2022)**. Mesenchymal Stem/Stromal Cells and Their Role in Oxidative Stress Associated with Preeclampsia. Yale J Biol Med., 95(1): 115-127.
- **Kyozuka H, Jin T, Fujimori M, Nomura S, Suzuki D, Fukuda T, et al. (2022)**. Effect of gestational weight gain on preeclampsia among underweight women: A single tertiary referral center study in Japanese women. J Obstet Gynaecol Res. 48(5): 1141-1148.
- **Li J, Han J, Zhao A, Zhang G. (2022)**: 3CircPAPPA Regulates the Proliferation, Migration, Invasion, Apoptosis, and Cell Cycle of Trophoblast Cells Through the miR-3127-5p/HOXA7 Axis. Reprod Sci., 29(4): 1215-1225.
- **Lindheimer MD, Taler SJ, Cunningham FG. (2009)**. American Society of Hypertension: ASH position paper: hypertension in pregnancy. J Clin Hypertens (Greenwich). 11(4): 214-25.
- **Orendi K, Kivity V, Sammar M, Grimpel Y, Gonen R, Meiri H, et al. (2011)**. Placental and trophoblastic in vitro models to study preventive and therapeutic agents for preeclampsia. Placenta, 32: S49-S54

- **Rebecca T, Braekke K, Harsem NK, Hyer M, Hoover RN, Staff AC. (2008)**. Blood pressure augmentation and maternal circulating concentrations of angiogenic factors at delivery in preeclamptic and uncomplicated pregnancies." Am J Obstet Gynecol., 199(6): 653.

- **Reijnders D, Olson KN, Liu C, Beckers KF, Ghosh S, Redman LM, et al. (2019)**. Dyslipidemia and the role of adipose tissue in early pregnancy in the BPH/5 mouse model for preeclampsia. Am J Physiol Regul Integr Comp Physiol., 317(1): R49-R58.

- **Rowson S, Reddy M, De Guingand D, Langston-Cox A, Marshall S, da Silva Costa F, et al. (2022)**. Comparison of circulating total sFLT-1 to placental-specific sFLT-1 e15a in women with suspected preeclampsia. Placenta. 120: 73-78.

- **Sahu M, Deepak V, Gonzales S, Rimawi B, Watkins K, Smith A, et al. (2019)**. Decidual cells from women with preeclampsia exhibit inadequate decidualization and reduced sFlt1 suppression. Pregnancy Hypertens., 15:64-71.

- **Sammar M, Dragovic R, Meiri H, Vatish M, Sharabi-Nov A, Sargent I, et al. (2018).** Reduced placental protein 13 (PP13) in placental derived syncytiotrophoblast extracellular vesicles in preeclampsia - a novel tool to study the impaired cargo transmission of the placenta to the maternal organs. Placenta, 66: 17-25.

- **Sammar M, Drobnjak T, Mandala M, Gizurarson S, Huppertz B, Meiri H. (2019)**. Galectin 13 (PP13) Facilitates Remodeling and Structural Stabilization of Maternal Vessels during Pregnancy. Int J Mol Sci., 20(13): 3192.

- **Sammar M, Nisemblat S, Fleischfarb Z, Golan A, Sadan O, Meiri H, et al .(2011)**. Placenta-bound and body fluid PP13 and its mRNA in normal pregnancy compared to preeclampsia, HELLP, and preterm delivery. Placenta, 32: S30-36

- **Shinohara S, Sunami R, Kasai M, Yasuda G, Uchida Y. (2021)**. Predictive value of the sFlt-1/PlGF ratio for preeclampsia in twin pregnancies: a retrospective study. Hypertens Pregnancy., 40(4): 330-335.

- **Soongsatitanon A, Phupong V. (2020)**. Prediction of preeclampsia using first-trimester placental protein 13 and uterine artery Doppler. J Matern Fetal Neonatal Med., 35(22): 4412-4417.

- **Stefanovic V, Cukuranovic R, Dolicanin Z, Cukuranovic J, Stojnev S, Bogdanovic D, et al (2015)**: Placental growth factor and placental protein 13 in patients with Balkan endemic nephropathy, a worldwide disease. Ren Fail., 37(7): 1145-8

- **Than N, Romero R, Goodman M, Weckle A, Xing J, Dong Z, et al. (2009)**. A primate subfamily of galectins expressed at the maternal-fetal interface that promote immune cell death. Proc. Natl. Acad. Sci. U. S. A., 106(24): 9731-6.

- **Than N, Romero R, Xu Y, Erez O, Xu Z, Bhatti G, et al .(2014)**. Evolutionary origins of the

placental expression of Chromosome 19 cluster galectins and their complex dysregulation in preeclampsia. Placenta, 35: 855-865

- **Vikraman SK, Elayedatt RA. (2022)**. Pre-eclampsia screening in the first trimester - preemptive action to prevent the peril. J Matern Fetal Neonatal Med., 35(9): 1808-1816.
- **Vokalova L, Balogh A, Toth E, Van Breda S, Schäfer G, Hoesli I, et al. (2020)**. Placental Protein 13 (Galectin-13) Polarizes Neutrophils Toward an Immune Regulatory Phenotype. Front Immunol., 11:145.
- **Von Dadelszen P, Magee LA, Roberts JM. (2003)**. Subclassification of preeclampsia. Hypertens Pregnancy. 22: 143–148.
- **Wu Y, Liu Y, Ding Y. (2021)**. Predictive Performance of Placental Protein 13 for Screening Preeclampsia in the First Trimester: A Systematic Review and Meta-Analysis. Front Med (Lausanne)., 8:756383.
- **Xu Z, Jin X, Cai W, Zhou M, Shao P, Yang Z, et al. (2018)**. Proteomics Analysis Reveals Abnormal Electron Transport and Excessive Oxidative Stress Cause Mitochondrial Dysfunction in Placental Tissues of Early-Onset Preeclampsia. Proteomics Clin Appl., 12(5): e1700165.
- **Yang H, Guo F, Guo Q, Wang Y, He P, Zhang H, et al .(2022)**. The clinical value of PlGF and the sFlt1/PlGF ratio in the management of hypertensive pregnancy disorders: A retrospective real-world study in China. Clin Chim Acta., 528: 90-97.