Original Research Article

Maternal Mid-Trimester Placental Growth Factor and Uterine Artery Doppler Velocimetry in Evolution of Pre-Eclampsia

Abstract

Background: Early detection of pre-eclampsia (PE) has become one of the fundamental goals of perinatal medicine. Although clinical symptoms of PE emerge after 20th week of gestation, trophoblast invasion that is responsible for pathogenesis occurs in the first trimester. This study aimed to evaluate the clinical utility of Placental Growth Factor and uterine artery Doppler velocimetry in evolution of preeclampsia.

Methods: This was a cohort prospective study that was carried out on sixty pregnant women who were classified into 3 equal groups of pregnant women who had gestational age ranged from 20 to 26weeks: first group included pregnant women with normal pregnancy, second group included pregnant women with mild preeclampsia, and the third group included cases with severe preeclampsia.

Results: The mean of gestational age at delivery was significantly lower in patients who had severe preeclampsia than those with mild preeclampsia and controls. In contrast, there was no significant difference in the mean of gestational age at delivery of patients with mild preeclampsia and controls. As for serum glutamic-oxaloacetic transaminase (SGOT), the concentration increased significantly in cases with severe preeclampsia compared to that of mild preeclampsia and controls. However, there were non-significance differences between controls and cases with mild preeclampsia. Receiver Operating Characteristic (ROC) curve showed that perfusion index (PI) had significantly higher diagnostic accuracy than other indices in predicting outcome of pregnancy and uterine artery pulsatility index had

significantly higher diagnostic accuracy than both placental growth factor (PlGF) and uterine artery resistance index in predicting the severity of preeclampsia.

Conclusions: Uterine artery Doppler screening is simple and noninvasive and there is a clear association of elevated mean uterine artery PI, respiratory index (RI) and also serum PlGF in the second trimester, with the occurrence of PE. Uterine artery pulsatility index (UAPI) was significantly associated with a high incidence of PE. The sensitivity or prediction is increased by adding serum marker PlGF and combinations provide an increase in the ability of prediction and determination compared to the tests used alone.

Keywords: Maternal, Mid-Trimester, Placental Growth Factor, Uterine artery Doppler velocimetry, Pre-Eclampsia.

Introduction:

Preeclampsia (PE) is a major cause of maternal and fetal morbidity and mortality worldwide affecting 2 to 8% of all deliveries, with a trend towards an increase in recent years. It is responsible for approximately 18% of all maternal deaths globally (70,000 deaths annually), mostly in low and middle income countries and its management and consequences are responsible for considerable health care expenditure [1].

One-quarter of medically indicated preterm deliveries are linked to PE. In the past decades, a progressively increasing prevalence of PE has been attributed to multiple risk factors including a significant rise in maternal obesity and diabetes, delayed childbearing and to an increased rate of multiple gestations. Women who develop PE are at significant risk of early cardiovascular disease, hypertension, cerebrovascular accidents, and death [2].

Furthermore, there is growing evidence that offspring born to preeclamptic women are at increased risk for early onset of cardiovascular disease and stroke during adulthood. That risk seems to be more substantial when PE is diagnosed before 34 weeks of gestation [3].

Many pathological mechanisms have been asserted in order to explain preeclampsia. It was reported in the literature that defective trophoblastic invasion at first trimester caused preeclampsia [4].

There is growing evidence that an imbalance in these factors released from the placenta and maternal endothelium, including PlGF (placental growth factor), sFlt-1 (soluble Fms-like tyrosine kinase 1), and sEng (soluble Endoglin), are associated with the onset of the disorder [5].

Placental growth factor is thought to induce nonbranching angiogenesis leading to a low-resistance placental vascular network. In a healthy pregnancy, it increases with gestation in maternal circulation, with concentrations peaking at 26 to 30 weeks and declining towards term [6].

In pregnancies complicated by preeclampsia, limited angiogenesis early in pregnancy with shallow vascular invasion of maternal spiral arteries leads to subsequent placental hypoperfusion. The angiogenic imbalance between PlGF and sFlt-1 has a pivotal role in the pathogenesis the disease, and their ratio is, thus, a useful tool in its prediction. PlGF is abnormally low in women with preeclampsia as compared to their gestational age-matched controls. The decrease in PlGF level is evident as early as the beginning of the second trimester of pregnancy, before the development of signs and symptoms of the disease [7].

After evaluation through prospective cohort studies, and a randomized controlled trial, PlGF-based testing is now recommended as a diagnostic adjunct in women with suspected preeclampsia, with PlGF and sFlt-1 testing to be fast-tracked for clinical use in the United Kingdom in 2020 [8].

Moreover, Doppler ultrasound is an crucial tool in the management of high-risk pregnancies. Direct assessment of trophoblast invasion in human pregnancy is not possible; however, the use of Doppler imaging permits noninvasive evaluation of the uteroplacental circulation by comparing systolic and diastolic waveforms [9]. The aim of this study was to evaluate the clinical utility of Placental Growth Factor and uterine artery Doppler velocimetry in evolution of preeclampsia.

Patients and Methods:

This was a cohort prospective study that was carried out on sixty pregnant women who were classified into 3 groups of pregnant women who had gestational age ranged from 20 to 26 weeks: first group included 20 pregnant women with normal pregnancy, second group included 20 pregnant women with mild preeclampsia, and the third group included 20 cases with severe preeclampsia after approval by the Ethical Committee, faculty of medicine, Tanta University. Each case participated in the present study was fully informed concerning the nature of the disease and the diagnostic procedures. All the sixty pregnant women attended

the antenatal care in outpatient clinic and or inpatient department of Obstetrics & Gynecology at Tanta University Hospitals during the period from December 2019 till December 2020. The patients that failed to continue their follow up were replaced throughout the study. All cases signed a well-informed written consent to declare their agreement to be enrolled in this study, as agreed upon by the ethical committee.

Women aged between 20-35 years, with singleton pregnancy, gestational age (20-26) weeks and BMI (≥18 & ≤30) kg/m^2 were included in the study. While Women with chronic diseases like chronic hypertension, diabetes mellitus, renal diseases, liver diseases, women with placental abruption, in labor or preterm labor and eclamptic pregnant women were excluded from the study.

When we started our study, it was carried out on 60 normal pregnant women. It wasn't possible at that time to predict the outcome of our study as regards development of pre-eclampsia and its timing, as defined according to the American College of Obstetrics and Gynecology (ACOG). so, grouping of patients weren't done. During the course of the study till its end we were able to divide our patients into the following groups: Group A: Those who remained normotensive throughout the entire pregnancy. Group B: Those who subsequently developed mild Pre-eclampsia (systolic ≥140 and <160 mm Hg, diastolic≥90 and 110 mm Hg). Group C: Those who subsequently developed severe Pre-eclampsia (systolic ≥ 160 mm Hg, diastolic ≥ 110 mm Hg).

All cases were subjected to the following:

History taking: including personal data, obstetric history, medical & surgical history: to exclude general and systemic diseases as diabetes mellitus, hypertension and thyroid dysfunction. family history: of preeclampsia, diabetes mellitus and hypertension.

General examination: including vital signs as pulse, temperature and respiratory rate. Arterial blood pressure measurement: Blood pressure measurement was done by the

auscultatory method using a stethoscope and a mercury manometer. In a semi-sitting position with left lateral tilt, the cuff was placed around the right upper arm, roughly at the same vertical height as the heart, attached to a mercury manometer. A cuff of appropriate size was fitted smoothly and then inflated manually until the artery is completely occluded. Listening with the stethoscope to the brachial artery at the elbow, then the pressure in the cuff was slowly released by 2-3 mm/second. When blood just started to flow in the artery, the turbulent flow created the first Korotkoff sound; the pressure at which this sound was first heard is the systolic blood pressure. The cuff pressure was further released until no sound could be heard (fifth Korotkoff sound), at this point the diastolic arterial pressure was measured. If the measured arterial blood pressure was ≥ 140/90; it was repeated after 6 hours to confirm hypertension. BMI: is calculated by dividing a person's weight in kilograms by the square of their height in meters. Underweight = 18.5 kg/m2, Normal weight= 18.5-24.5 kg/m2, Overweight = 25-29.5 kg/m2 and Obese= BMI ≥30 kg/m2.

Head and neck, chest, heart and lower limb examination.

Ultrasonographic examination: The ultrasonographic examinations were performed with Samsung UGEO H60, Korean manufacturer with imaging facility to assess fetal measurements, estimate date of delivery, detect multiple pregnancies or to exclude vesicular mole. Doppler ultrasound of the uterine arteries is performed using Real-time ultrasound color-pulsed Doppler equipment with a 5-MHz curvilinear probe. Measurement of Doppler indices of uterine artery on both sides to detect any abnormality. The pulsatility index: (Peak systolic velocity - End diastolic velocity / Time averaged maximum velocity) of the right and left uterine arteries were measured, and the calculation of the mean measurement of the two vessels. The resistance index: (Peak systolic velocity - End diastolic velocity) / Peak systolic velocity) of the right and left uterine arteries were measured, and the mean measurement of the two vessels was calculated.

Investigations: Investigations were done at Clinical Pathology Department, Faculty of Medicine; Tanta University including complete blood picture (CBC), serum albumin, blood urea nitrogen (BUN), serum creatinine, serum ALT & AST, complete urine analysis for proteinuria: Proteinuria is diagnosed when ≥ ±1proteinuria by dipstick analysis on two consecutive occasions at least 6 hours apart but not more than one week apart and measurement of serum PlGF: Serum placental growth factor analyzed between 20 and 26 weeks of gestation to detect if there was a significant statistical difference between the study groups. Three ml of maternal venous blood were collected aseptically in red topped vacutainer. After collection of the whole blood, the blood was allowed to clot by leaving it undisturbed at room temperature for 15-30 minutes. The clot was removed by centrifugation and the resulting supernatant (serum) was immediately transferred into Eppendorf tubes using a Pasteur pipette. If the serum was not analyzed immediately, the serum was apportioned into 0.5 ml aliquots and stored at –20°C.

Measurement of serum PlGF antigen by ELISA: Measurement of Placental growth factor by DEMEDITEC ELISA Kit: That ELISA Kit is a solid phase enzyme linked immunosorbent assay (ELISA) based on the sandwich technique. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site of the PLGF molecule. An aliquot of patient sample containing endogenous PLGF is incubated in the coated well. After a washing step a biotin-linked polyclonal antibody specific for PLGF is added to the wells. Following a wash to remove any unbound antibody a streptavidin HRP enzyme complex is added to the wells. After incubation the unbound enzyme complex is washed off. The amount of bound peroxidase is proportional to the concentration of PLGF in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of PLGF in the patient sample.

Reagents: Microtiter wells: 12*8 (break apart) strips, 96 wells; wells coated with anti-PLGF anti-body (monoclonal). Zero standard, 1 vial, 1 ml, ready to use. Concentration: 0 pg/ml. contains non-mercury preservative. Standard (standard 1-5), 5 vials, 1 ml, ready to use; Concentrations: 25, 50, 125, 500, 1000 pg/ml. contain non-mercury preservative. Control low & high, 2 vials, 1 ml each, ready to use; for control values and ranges please refer to vial label or QC Data sheet. Contain non-mercury preservative. Assay Buffer: 1 vial, 30 ml, ready to use. Contain non-mercury preservative. Enzyme conjugate: 1 vial, 14 ml, ready to use, biotinylated goat-anti-human PLGF antibody. Contain non-mercury preservative. Enzyme complex:1 vial, 14 ml, ready to use, contains streptavidin horseradish peroxidase. Contains non-mercury preservative. Substrate solution: 1 vial, 14 ml, ready to use, tetramethylbenzidine (TMB). Stop solution: 1 vial, 14 ml, ready to use, contains 0.5 M H2SO4, avoid contact with the stop solution. It may cause skin irritations and burn. Wash solution: 1 vial, 30 ml (40* concentrated), see preparation of the reagent.

Reagent preparation: All reagents were brought and required number of strips to room temperature prior to use. Wash solution: deionized water was added to the 40*concentrated wash solution. 30 ml of concentrated wash solution was diluted with 1170 ml deionized water to a final volume of 1200 ml. The diluted wash solution is stable for 2 weeks at room temperature.

Assay procedure:

General remarks: All reagents and specimens were allowed to come to room temperature before use. All reagents were mixed without foaming. Once the test has been started, all steps were completed without interruption. New disposal plastic pipette tips were used for each standard, control or sample in order to avoid cross contamination. As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Test procedure: All standards, samples, and controls were run in duplicate. All standards, samples and controls were run concurrently so that all conditions of testing are the same. The desired number of microtiters well were secured in the holder. 25 µl of each standard was dispensed, controls and sample with new disposable tips into appropriate wells. 250 µl of assay buffer were dispensed into appropriate wells. The reaction was incubated for 30 minutes at room temperature (without covering the plate). The content of the wells was shaked out and the wells were rinsed the wells 3 times with diluted wash solution (400 µl per well). The wells were striked sharply on absorbent paper to remove residual droplets. 100 µl Enzyme Conjugate were dispensed into each well. The reaction was incubated for 30 minutes at room temperature (without covering the plate). The content of the wells was briskly shaked out. The wells were rinsed the wells 3 times with diluted wash solution (400 µl per well). the wells were striked sharply on absorbent paper to remove residual droplets. 100 µl Enzyme Complex were dispensed into each well. The plate was incubated for 30 minutes at room temperature. The content of the wells was briskly shaked out and the wells were rinsed with diluted wash solution (400 µl per well), strike the wells sharply on absorbent paper to remove residual droplets. 100 µl of Substrate Solution were added to each well. The reaction was incubated for 30 minutes at room temperature. The enzymatic reaction was stopped by adding 100 µl of Stop Solution to each well. The absorbance of each well at 450±10 nm was determined with microtiter plate reader.

Statistical analysis

Statistical analysis of data was carried out using SPSS version 23. Shapiro –Wilks test was used to test normal distribution of variables. Numerical data were expressed as mean ± standard deviation or median and range. Categorical data were summarized as percentages. The significance for the difference between groups was determined by using two-tailed

Student's t test and one way ANOVA (analysis of variance) test or for quantitative data as appropriate. Also Qualitative variables were assessed by chi-squared $\chi 2$test.

Results:

There was no significant difference in maternal ages between the three groups compared and also regarding to BMI. **[Table 1]**

Table 1: Distribution of the study cases according to age &BMI.

Age		**Control N=20**	**Mild preeclampsia N=20**	**Severe preeclampsia N=20**	**P-Value**
17-25	N	6	8	7	0.921
	%	30%	40%	35%	
26-32	N	12	9	10	
	%	60%	45%	50%	
33-43	N	2	3	3	
	%	10%	15%	15%	
Total	N	20	20	20	
	%	100%	100%	100%	
BMI					
Normal weight	N	6	9	5	0.676
	%	30%	45%	25%	
Over-weight	N	13	9	13	
	%	65%	45%	65%	
Obese	N	1	2	2	
	%	5%	10%	10%	
Mean ± SD		27.23 ± 2.8	25.89 ± 2.9	27.19±3.04	0.264

SD: standard deviation

There was statistically significant decrease in the mean numbers of gravidity among cases with either mild or sever preeclampsia compared to controls. There were statistically significant differences between the studied groups regarding parity. there was no significant difference in the mean of gestational age at delivery of patients with mild preeclampsia and controls. [**Table 2**]

Table 2: Distribution of the study subjects according to gravidity, parity, &gestational age at delivery.

Gravidity		**Control N=20**	**Mild preeclampsia N=20**	**Severe preeclampsia N=20**	**P-value**
Gravidity	PG	7(35%)	10(50%)	13(65%)	0.272
	≤3	7(35%)	8(40%)	5(25%)	

	>3	6(30%)	2(10%)	2(10%)	
Mean ± SD		2.7 ± 1.69	1.85± 1.14[a*]	1.7 ± 1.1 [a*]	**0.045**[*]
Parity					
Zero	**N**	9	13	18	0.01
	%	45%	65%	90%	
1	**N**	6	7	1	
	%	30%	35%	5%	
2	**N**	3	0	0	
	%	15%	0%	0%	
3	**N**	1	0	1	
	%	5%	0%	5%	
4	**N**	1	0	0	
	%	5%	0%	0%	
Total	**N**	20	20	20	
	%	100.0%	100.0%	100.0%	
Gestational age at delivery (weeks)					
Mean ± SD		36.3±2.05	35.4±1.35	33.3 ± 2.1	**0.001**[**]

SD: standard deviation, *: statistically significant as p value < 0.05

The current study showed that preeclampsia is usually accompanied by new-onset proteinuria, massive proteinuria is found to be consistently associated with developing of severe preeclampsia. **[Figure 1]**

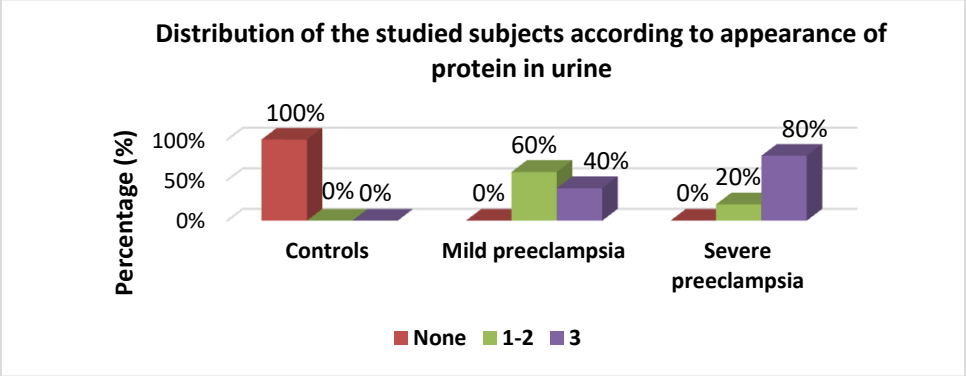


Figure 1: Distribution of the study subjects according to appearance of protein in urine.

There was a significant difference between the three studied groups with regards to blood pressure both systolic (SBP) and diastolic (DBP) which increased with the occurrence and development of preeclampsia from mild feature to severe. **[Figure 2]**

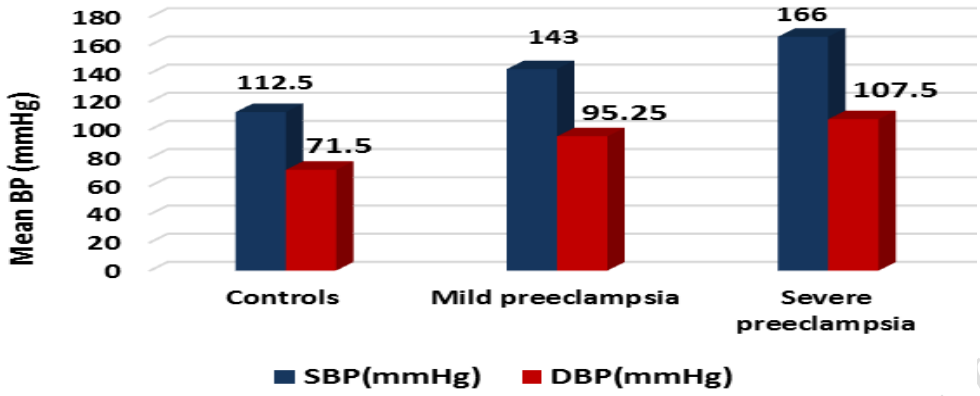


Figure 2: Mean of blood pressure among patients and control groups

There was statistically significant difference in the hemoglobin levels between cases with mild and those with severe preeclampsia and also , there was no significant difference in the mean value of RBCs ,and WBCs between studied groups. [**Table 3**]

Table 3: Hematological Parameters for Different Studied Groups (Healthy Controls and patients Groups)

		Control N=20	Mild preeclampsia N=20	Severe preeclampsia N=20	P-Value
Hb (g/dL)	Range	9.5-13.4	9.9–14.4	8–13.6	**0.001*****
	Mean ± S. D	12.01 ± 1.05	12.03 ± 1.09	10.7 ± 1.5^{ab***}	
WBCs count 10^3/ µL)×(	Range	7-16.7	7.8–14.300	8.950–19.330	0.089
	Mean ± S. D	11.059 ± 2.966	11.342 ± 1.738	12.780 ± 2.902^{a*}	
10^3/ ×PLT (µL)	Range	157-400	96-465	67-333	**0.023***
	Mean ± S. D	271.3 ± 64.937	228.187 ± 75.618	205.571 ± 80.208^{a**}	
RBCs count 10^6/ µL)×(	Range	3.7-4.7	3–5.4	3–5.4	0.74
	Mean ± S. D	4.08 ± 0.303	3.96 ± 0.622	4.1 ± 0.775	

SD: standard deviation, *: statistically significant as p value < 0.05

There was statistically significant difference in the mean level of both ALT and AST among different studied groups. As regards albumin, results revealed that patients with severe preeclampsia had statistically significant lower albumin level compared to that of both control subjects and patients with mild preeclampsia. In addition, the present study showed that cases with preeclampsia either severe or mild had higher creatinine level compared to controls **[Table 4]**

Table 4: Comparison between the serum ALT, AST, albumin, and creatinine among control and preeclampsia groups

Groups Variables	**Control N=20**	**Mild preeclampsia N=20**	**Severe preeclampsia N=20**	**P-Value**
ALT *(U/L)*				**0.001*****
Mean ± SD	27.95±11.28	27.05±5.53	38.58±12.24 ^{a**,b***}	
AST *(U/L)*				**0.007****
Mean ± SD	32.85±7.46	31.85±5.85	39.68±10.25 ^{a**,b**}	
Albumin *(g/dL)*				**<0.001*****
Mean ± SD	3.67±0.29	3.19±0.39 ^{a***}	2.94±0.45 ^{a***,b*}	
Creatinine (*mg/dl)*				**<0.001*****
Mean ± SD	0.82±0.18	1.92±0.27 ^{a***}	1.94±0.23 ^{a***}	

SD: standard deviation, *: statistically significant as p value < 0.05, ALT: alanine transaminase, AST: aspartate transaminase.

Neonatal birth weight, NICU admission, Neonatal outcomes and maternal outcomes were discussed in the following table. **[Table 5]**

Table 5: Maternal, fetal &neonatal outcomes in all studied cases

Birth Weight (gm)		**Control N=20**	**Mild preeclampsia N=20**	**Severe preeclampsia N=20**	**P-Value**
1000-1499		0(0%)	2(10%)	6(31.5%)	**<0.001*****
1500-2499		0(0%)	10(50%)	13(68.5%)	
≥2500		20(100%)	8(40%)	0(5%)	
Mean ± SD		3320.5±360.15	2286±497.5^{ab***}	1714±387.54^{ab***}	**<0.001*****
Admitting to the NICU					
No	N	16	9	0	**<0.001*****
	%	80%	45%	0%	
Yes	N	4	11	19	
	%	20%	55%	100%	
Total	N	20	20	19 (1 Excluded IUFD)	
	%	100%	100%	100%	
Fetal &neonatal Complications					
None	N	16	14	0	**<0.001****
	%	80%	70%	0%	
IUGR	N	4	6	16	
	%	20%	30%	80%	
IUFD	N	0	0	1	
	%	0%	0%	5%	
Neonatal death	N	0	0	3	
	%	0%	0%	15%	
Total	N	20	20	20	
	%	100%	100%	100%	
Maternal Complications					
None	N	16	20	15	**0.002*****
	%	80%	100%	75%	
Preterm		4	0	0	
		20%	0%	0%	

Eclampsia	N	0	0	3	
	%	0%	0%	15%	
HELLP	N	0	0	1	
	%	0%	0%	5%	
Death	N	0	0	1	
	%	0%	0%	5%	
Total	N	20	20	20	
	%	100%	100%	100%	

SD: standard deviation, *: statistically significant as p value < 0.05, NICU: Neonatal Intensive Care Unit, IUGR: intra uterine growth retardation, IUFD: intra uterine fetal death.

The mean PLGF level was significantly lower for women who experienced severe preeclampsia compared with healthy women. **[Figure 3]**

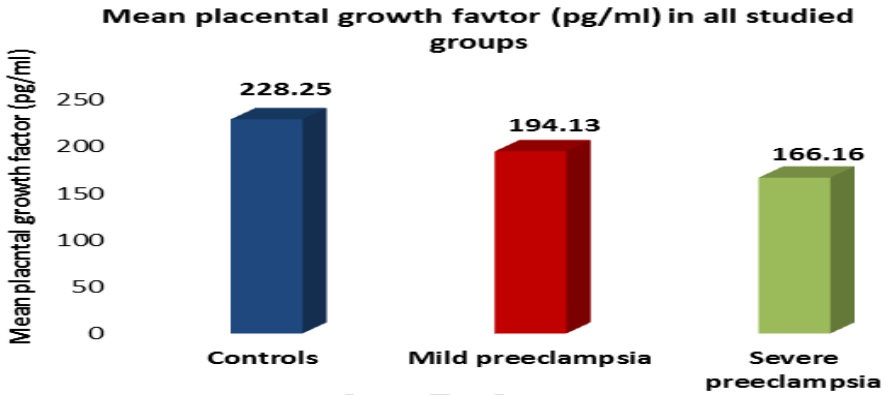


Figure 3: Comparison between all studied groups regarding serum PLGF level

The values of uterine artery PI and RI were significantly higher in patients with preeclampsia either mild or severe than in normal women. **[Figure 4]**

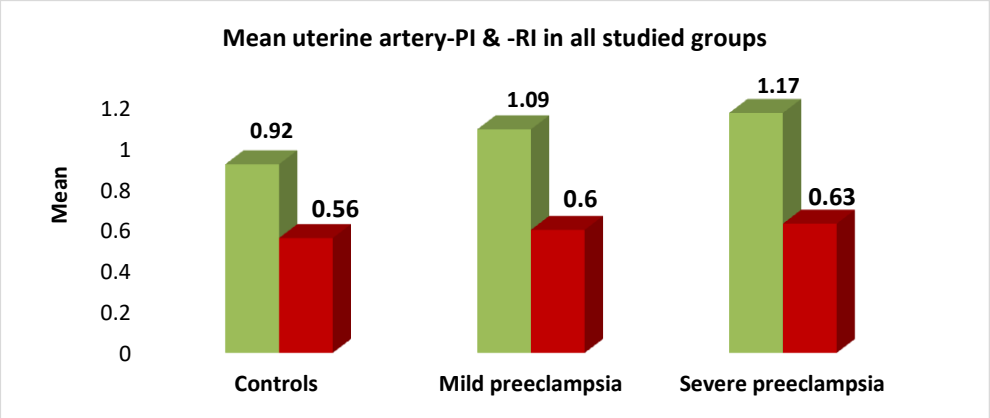
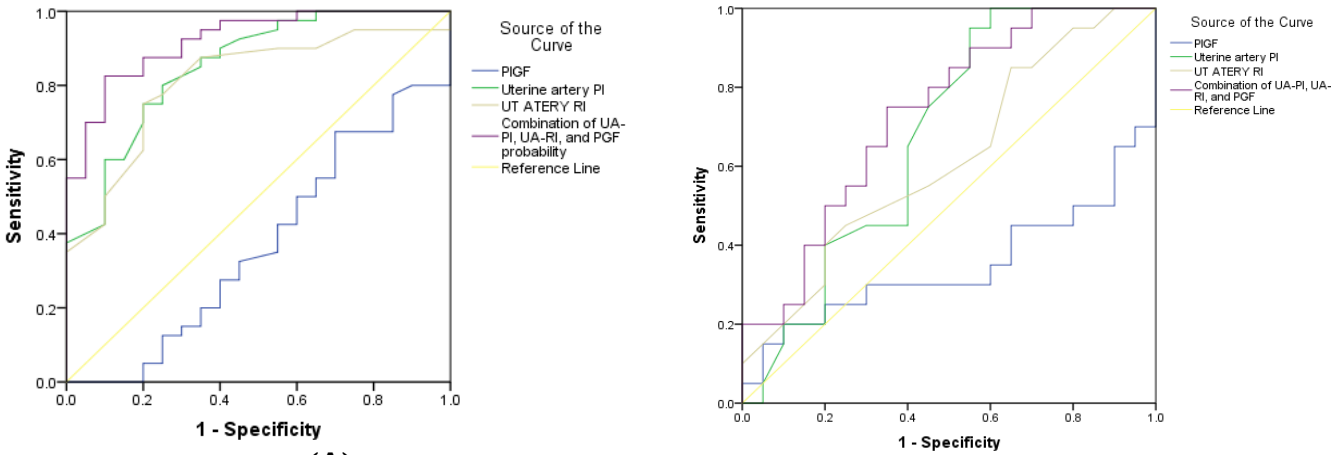


Figure 4: uterine artery-PI, and-RI for Different Studied Groups (Healthy Controls and patients Groups)

ROC analysis showed that combined testing of Doppler indices UA-PI and UA-RI, and PLGF increased the diagnostic accuracy for Preeclampsia compared with either test alone.

Compared with single detection, combined measurement of the Doppler indices, and PLGF markers showed 75% sensitivity and 60% specificity, 65.2% PPV and 70.6% NPV for distinguish patients with severe preeclampsia from those with mild preeclampsia. [**Figure 5**]



(A) **(B)**

Figure 5: ROC curve of PLGF, Doppler indices, and their combination to differentiate normotensive pregnant women from preeclamptic pregnant women (A) and patients with mild Preeclampsia from those with severe Preeclampsia (B)

Discussion

Preeclampsia (PE) is a major cause of maternal and fetal morbidity and mortality worldwide affecting 2 to 8% of all deliveries, with a trend towards an increase in recent years. It is responsible for approximately 18% of all maternal deaths globally (70,000 deaths annually), mostly in low and middle income countries and its management and consequences are responsible for considerable health care expenditure [1].

Our results indicated that there was statistically significant difference between all studied groups according to the mean gravidity as (P=0.045). Seven cases in control group (35%) and ten cases (50%) with mild preeclampsia were primigravida compared to 13 in severe preeclampsia group. Moreover, the mean BMI of control cases was 27.23 ± 2.8 kg/m^2 while the mean BMI of cases in mild and severe preeclampsia group was 25.89 ± 2.9 kg/m^2, and 27.19±3.04 kg/m^2; respectively. There were no statistically significant differences between

all studied groups regarding to BMI (P=0.264)**.** It is noteworthy to mention that most of our preeclampsia cases (65% in the severe group and 45% in the mild one) were overweight while 10%in each group were obese. ***Motedayen et al, 2019's*** [10]results in accordance to ours stated that the mean BMI was 25.13% in the healthy group and 26.33% in the (severely) affected PE group with no statistically significant differences .

El-Shourbagy and his co- workers in 2017 [11]in a study on three groups of controls, mild and severe PE reported that mean systolic blood pressure among mild preeclampsia group was 145.31+ 4.81mm Hg, in severe preeclampsia group 167. 50+ 7.56 mmHg, compared to control group100.50 + 8.26 mm Hg, with statistically high significant difference between pre-eclampsia cases and control.

Kamel et al., in 2020 [12]found also statistically significant increase in maternal liver and renal functions together with complications in severe group compared to the mild one

Regarding fetal and maternal outcomes, there was statistically significant decrease of neonatal birth weight in severe group when compared with mild preeclampsia group as well as control group. NICU admission rate was greater in the severe preeclampsia group, (100%) *vs.* 55% in the mild preeclampsia group and 20% in the control group. A highly significant increase of fetal growth restriction in severe cases (80%) than that in mild preeclampsia group (30%) and control group (20%) was noted. Three neonatal deaths observed during the study were in the severe preeclampsia group. Also, one case had IUFD. Complications were observed in mothers who suffered from severe preeclampsia. Three cases developed eclampsia, 1 developed HELLP syndrome, and maternal death was detected in one case with severe preeclampsia. ***Omani-Samani et al., 2017*** [13]***, and McKenzie and Trotman, 2019*** [14] also found that patients with preeclampsia had significantly lower mean birth weights for the neonates compared to controls. ***El-Shourbagy et al., 2017*** [11] also found that the most common complication associated with pre-eclampsia in thier study was accidental

hemorrhage 6(15%). Other complications associated were pulmonary edema 4 (10%), HELLP 3 (7.5%), eclampsia 2(5%) and Acute renal failure (ARF) 1 (2.5) with no cases of maternal mortality. with the most common neonatal complications met with in the present study of pre-eclampsia cases were IUGR 12 (30%), preterm 5 (12.5%) IUFD 1 (2.5). Such findings were comparable with those of ***Aabidha et al., in 2015*** [15]who showed in their study that the most common complication in preeclampsia was antepartum hemorrhage (13.97%), followed by post-partum hemorrhage (10.75%) and eclampsia (5.37%), with no maternal mortality. And, pre-maturity (23.65%) was the most common neonatal complications followed by low birth weight (7.52%) and intra uterine growth restriction (9.67%) in their studied pre-eclampsia cases were the most common fetal complications. ***Belay et al., 2020*** [16] reported also that preterm birth was the commonest perinatal complication observed in this study accounting for 18.2%., Intrauterine growth restriction occurred in 12% of the cases, stillbirth occurred in about 1.7% and 2.27% of newborn ended up in early neonatal death.

Kamel and his co-workers, [12]in 2020 there was statistically significant increase of preterm birth in severe group when compared with mild cases associated with highly statistical increased of fetal growth restriction in severe cases. Neonatal outcomes were worse in the SHP group in comparison with MHP as it shows a highly significant difference in FGR (P= < 0.001) and a significant difference in preterm delivery (P=0.035).

Regarding results of our markers, the current study showed that the mean concentration of serum PlGF in control group, mild preeclampsia group, and severe preeclampsia group was 228.25 ± 86.83 (pg/ml), 194.13± 34.36(pg/mL), and 166.16± 61.79 (pg/mL); respectively. The mean PlGF level was significantly lower for women who experienced severe preeclampsia compared with healthy women. Our results were powered by ***Thadhani et al., 2004 [17], Atakul et al.,2019 [18], and Pant et al., 2019 [19]*** who reported that serum levels of PlGF were found to be higher in healthy pregnant women than PE cases especially severe

ones which is in keeping with previously published findings. Also, ***Li et al., 2016*** [20]found that the PlGF level was decreased in the early second-trimester in women who developed preeclampsia.

In addition, our results revealed that the mean PI and RI in control group was 0.92 and 0.56 respectively and it was 1.09 and 0.6 in patients with mild preeclampsia whereas it was 1.17 and 0.63 in cases with severe preeclampsia. The values of uterine artery PI and RI were significantly higher in patients with preeclampsia either mild or severe than in normal women. ***Chyad and her colleuges in 2018*** [21]reported highly statistically significant difference between preeclampsia and nonpreeclampsia according to level of uterine artery Doppler parameter (RI and PI value) between 14 to 20 weeks of pregnancy.

Bahlman et al. 2016 [22]showed that the diagnostic sensitivity of the combined use of the sFlt-1/PGF ratio and uterine artery pulsatility index (UAPI) was 74% for the diagnosis of preeclampsia. Also, our results are powered by ***Li et al., 2016*** [20]that found that uterine artery PI is an important marker for the prediction of preeclampsia. In thier study, the sensitivity was 76% with the specificity set at 80% and it dropped slightly to 57% for a specificity of 90%. ***AbdelgalilElsheikh et al., 2016*** [1]also reported that 0.848 UAPI can predict PE with sensitivity of 80% specificity 74% at cut off value ≤ 0.735 & = 0.709 UARI can predict PE with sensitivity of 87.5%, specificity 74% at cut off value ≥ 0.565.

Köpük and his co-workers in 2019 [23]also reported that Prediction of PE could be made by ROC analysis with a PI cut-off value of >2.23, with a sensitivity of 42.31% and a specificity of 82.10%. Those results were in agreement with ***Spencer et al., 2007*** [24]who highlighted the ability of second trimester UAPI to predict early onset PE with a sensitivity of 76% and specificity of 80%. ***Bhattacharyya et al., 2012*** [25]showed that abnormal uterine artery flow velocity was associated with an increased relative risk of preeclampsia both in high risk and low-risk women with sensitivity and specificity of increased uterine artery RI for prediction

of preeclampsia of 70 and 94.87 %, respectively and concluded that Doppler velocimetry of uterine artery at 24 weeks could be considered as a reliable screening test to predict PE in both low and high-risk women.

On the other hand, ***Myatt et al., 2012*** [26]reported that second trimester Doppler ultrasound indices showed a low sensitivity for detection of PE in low-risk nulliparous women and ***Pongrojpaw et al.*** [27]in 2010 reported that mid trimester uterine artery Doppler waveform analysis cannot be used as screening test for PE in higher risk women. However, women with normal uterine artery Doppler results are unlikely to develop preeclampsia.

Kleinrouweler et al., 2012 [28]conducted a systemic review and mata-analysis to investigate the capacity of circulating placental growth factor (PLGF), and other angiogenic factors to predict preeclampsia and reported that PLGF showed modest but significantly different concentrations before 30 weeks of gestation in women who developed preeclampsia but the test accuracy (32% sensitivity) was too poor for accurate prediction of pre-eclampsia in clinical practice. Also, ***McElrath et al., 2012*** [29]reported poor predictive ability of PLGF for prediction of PE (sensitivity 47% and specificity 62%).

Finally, ***Conde-Agudelo et al., 2015*** [30]**.** conducted a comprehensive review of different markers used to predict the development of preeclampsia. They reported that, at present there is no single test to predict PE but multivariable prediction models (based on combinations of maternal demographic characteristics and medical and obstetrical history with biophysical and biochemical tests performed either in the first trimester or early second trimesters) have shown a high predictive accuracy for early-onset PE in populations at low to moderate risk of developing this disorder. They postulated that, among women at a low to moderate risk to develop Preeclampsia, the predictive accuracy of angiogenic factors including PLGF was moderate to high when measured during the second trimester (sensitivities ranging from 17 to 100%, specificities from 51 to 97%).

The predictive value of an abnormal UADV between 22 and 26 weeks of gestation for the occurrence of preeclampsia and early onset preeclampsia is consistent also with a previous report by ***Espinoza et al., 2007*** [31]. They further demonstrate that the combination of abnormal UADV and a maternal plasma PlGF of <280pg/mL in the second trimester confers a much higher risk for preeclampsia and early onset or severe preeclampsia than abnormal UADV alone

YU et al., 2011 [32]reported that both serum inhibinA and activin A levels were increased, while the PLGF level was decreased, in the early second trimester in women who developed preeclampsia. If each marker were used alone, the sensitivity and specificity would be limited in a clinical setting. However, the combination of activin A, inhibin A and uterine artery PI or activin A, PlGF and uterine artery PI provided a test with high sensitivity and specificity that may be useful in predicting preeclampsia. Moreover, the combination of all three serum markers with uterine artery PI had an even higher prediction value.

Conclusions:

Uterine artery Doppler screening is simple and noninvasive and there is a clear association of elevated mean uterine artery PI, RI and also serum PlGF in the second trimester, with the occurrence of PE. Uterine artery pulsatility index (UAPI) was significantly associated with a high incidence of PE. The sensitivity or prediction is increased by adding serum marker PlGF and combinations provide an increase in the ability of prediction and determination compared to the tests used alone.

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