THE ROLE OF SECOND TRIMESTER UTERINE ARTERY DOPPLER ULTRASOUND,INHIBIN-A AND PLACENTAL GROWTH FACTOR IN PREDICTION OF PREECLAMPSIA.

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ABSTRACT

Objective: to evaluate the role of second trimester uterine artery Doppler ultrasound velocimetry (UADV), maternal serum Inhibin-A andplacental growth factor (PLGF) concentrations and as predictors of preeclampsia.
Study design: Cohort study.
Methodology: This prospective study was conducted at Obstetrics & Gynecology department, Alazhar University hospitals, Cairo, Egypt between May 2012 and May 2014. Ninety low risk normotensive singleton pregnant women were recruited for the study. Blood samples were collected at 16- 18 weeks, centrifuged to extract the serum then stored at – 80 °C until tested for inhibin-A and free PLGF levels. At 22-24 weeks, bilateral uterine artery Doppler velocimetry were recorded. The primary outcome was preeclampsia defined as hypertension with proteinuria after 20 weeks gestation. Women who developed preeclampsia were compared against normotensive control group.**Statistical analysis:** Receiver operating characteristics (ROC) curves were used for detection of the sensitivity and specificity and cut off value for each predictor using SPSS version 21 for analysis (IBM Inc., Chicago, Illinois, USA).
Results: Eight cases out of 90 had developed preeclampsia. Women who developed preeclampsia had significantly higher median uterine artery resistance index (UARI) (0.645 ± 0.3 vs. 0.485 ± 0.16, P = 0.009) and significantly lower median uterine artery pulsitility index (UAPI) (0.625 ± 0.24 vs. 1.0 ± 0.68 with P= 0.001) than normotensive control group.Maternal serum inhibin-A level was significantly higher in women with preeclampsia than the normotensive control group (1375 ± 1431 vs. 540 ± 1900 with P = 0.016) while level of PLGF was non-significantly lower in women with preeclampsia than the normotensive control group (275.5 ± 369 vs. 390 ± 583 with P = 0.156). ROC curves were analysed for cases and control groups, areas under the curve (AUC) was 0.709 (95% CI, 0.527– 0.891, P = 0.016) for UARI with sensitivity of 87.5%, specificity 74% at cut off value ≥ 0.5650, AUC was 0.848 (95% CI, 0.742– 0.955, P = 0.001) for UAPI with sensitivity of 80%, specificity 74% at cut off value ≤ 0.735, AUC was 0.758 (95% CI, 0.566 - 0.951, P = 0.016) for Inhibin-A with a sensitivity of 75%, specificity of 80% at cut off value of ≥ 832.5 pg/ml and finally AUC was 0.652 (95% CI, 0.447 – 0.858 and P = 0.165) for PLGF with a sensitivity of 74% and specificity of 50% at cut off value of ≤ 205 pg/ml.
Conclusion: second-trimester uterine artery Doppler indices, serum Inhibin-A and PLGF and may be helpful as a predicting markers for preeclampsia.
Key words: Inhibin-A, placental growth factor, Doppler indices, uterine artery, Preeclampsia.

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INTRODUCTION

Preeclampsia (PE) is a pregnancy-specific syndrome characterized by the onset of hypertension and proteinuria after the 20th week of gestation. Preeclampsia 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 [1]. 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 [2, 3].

Despite of decades of research, the etiology of PE remains unclear, numerous pathophysiological scenarios, alone or in combination, have been postulated to be responsible for the development of the disease. Abnormal vascular remodeling at the maternal fetal interface early in pregnancy due to an altered immune response to paternal antigens leads to relative placental hypoperfusion which leads to anexponential release of multiple cytokine and growth factors into the maternal circulation as a result of oxidative stress. This may cause excessive systemic inflammatory response and generalized maternal endothelial dysfunction, contributing to the maternal clinical features of preeclampsia modulated by genetic and environmental parameters [4- 6].

Until recently, preeclampsia still lacks a safe and effective therapy or prophylaxis as well as a reliable method for prediction raising the concerns about the importance of continuing research in order to identify a predictive biomarker to improve the management of women who distend to have PE [7, 8].

Inadequate placental perfusion has led to the use of Doppler ultrasonography to assess the velocity of the blood flow in the uterine arteries. Pregnancies associated with an abnormal uterine Doppler after 24 weeks of gestation are associated with a more than six fold increase in the rate of preeclampsia [9].

Inhibin is a dimeric glycoprotein hormone belonging to the transforming growth factor-b (TGF-β) superfamily, released by the fetoplacental unit during pregnancy. It is reported to be increased in women who develop PE [10, 11].

Recent advances in understanding preeclampsia and fetal growth restriction have elucidated important biological roles for placentally derived angiogenic factors. Placental growth factor (PLGF) is a member of the vascular endothelial growth factor (VEGF) family and is implicated in angiogenesis and trophoblastic invasion of the maternal spiral arteries. It is reported to be increased in women who develop PE [12- 14].

Methodology:

The current study was a prospective cohort study of singleton pregnant ladies who had their routine antenatal care at Alazhar University Hospitals between May 2012 and May 2014. Ninety ladies were included in the study; all were primigravida, non-smokers, non-diabetics, had no history of chronic hypertension, had normal renal and liver function and taking only routine vitamins and iron supplementations. Women younger than 18 years, had multiple pregnancy, had operative interference within the last 12 months, received any type of blood products during the current pregnancy or had pre-existing renal impairment were excluded.

Duration of pregnancy was estimated by measuring the crown–rump length in the late first trimester (11 to 13+6 weeks' gestation). Blood samples were collected at 16- 18 weeks, centrifuged to extract the serum then stored at –80 °C untill tested for maternal serum Inhibin-A and free PLGF levels using quantitative sandwich enzyme-linked immunosorbent assay technique (ELISA) (R&D Systems, Inc., 614 McKinley Place NE, Minneapolis, MN 55413, USA). At 22- 24 weeks uterine artery, Doppler velocimetry were performed using trans-abdominal ultrasonography with color flow mapping, According to Fetal Medicine Foundation (FMF) guidelines **[15]**. The transducer was placed in the lower lateral quadrant, angled medially and color Doppler was used to identify each uterine artery. Three consecutive wave forms were recorded for both right and left uterine arteries, resistance index (RI) and pulsitility index (PI) were measured and the median RI and PI for both uterine arteries were estimated.

The primary outcome was pre-eclampsia defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or both on at least two occasions four hours apart, developing after 20 weeks of gestation in previously normotensive women with proteinuria of 300 mg or more in 24 hour, or two readings of at least + 1 on dipstick analysis of midstream or catheter urine specimens if 24-hour urine collection was not available [4].

Statistical analysis:

Statistical analysis was performed using SPSS version 21 (Statistical package for social studies) (IBM Inc., Chicago, Illinois, USA). For quantitative variables; minimum, maximum, range, mean, standard deviation, median and interquartile range (IQR) were calculated. For analytical statistics; student t-test, Mann-Whitney and Chi-square tests were used to assess the statistical significance of the difference between the two population means in a study involving independent samples. Correlation coefficient (Pearson- r) measures the association between variables. Receiver operating characteristic (ROC) curve used to illustrate the diagnostic properties of a test on a numerical scale. The sensitivity and specificity values of the test were used to estimate the ability of the studied variables to predict development of Preeclampsia. P value considered significant if < 0.05.

RESULTS

One hundred and seven cases were enrolled at the beginning of the study, 17 cases were dropped due to inability to continue their ante natal care. So ninety women were followed up until the end of the study, 8 cases developed preeclampsia (8.9%). Table 1 showed the basic characteristics of the patients, both groups are comparable as regard maternal age; women who developed preeclampsia had higher BMI than normotensive control group.

Table (1)	**Patient's characteristics and differences between preeclampsia and normotensive control groups.**		
Parameters	Cases no. =8	Control no. =82	P- Value
Age (years)	23-34 (29 ± 2.2)	19-32 (27 ± 3.8)	<0.07
BMI (Kg/m²)	27-31 (29.2 ± 2.9)	24- 29 (26.1 ± 3.1)	<0.004*
Systolic blood pressure	145- 165 (152 ± 4.8)	90- 125 (117 ± 7.9)	≤ 0.001*
Diastolic blood pressure	90- 120 (106.5 ± 7.742)	60- 85 (72.82 ± 6.647)	≤0.001*
Proteinuria (Frequency and %)			
Nil	0 (0%)	80 (97.5%)	
1+	5 (62.5%)	2 (2.5)	
2+	2 (25%)	0 (0%)	≤ 0.001*
3+	1 (12.5%)	0 (0%)	

***Significant at P-value ≤ 0.05. Differences were estimated using student t-test and Chi-square test**

Table (2)	**Comparison between both groups as regard inhibin-A levels,PLGF levels, UARI and UAPI. All were presented by the minimum, the maximum and the median ± IQR.**		
Parameters	Cases no. =8	Control no. =82	P- Value
Serum Inhibin-A (pg/ ml)	345-2315 (1375 ± 1431)	104 -2004 (540 ± 1900)	0.016*
Serum PLGF (pg/ ml)	37- 533 (275.5 ± 369)	37-620 (390 ± 583)	0.156
UARI	0.43 ± 1.0 (0.645±0.3)	0.38-1.1 (0.485 ± 0.16)	0.009*
UAPI	0.53-0.93 (0.625±0.24)	0.55-1.84 (1.0 ±0.68)	0.001*

***Significant at P-value ≤ 0.05. Differences were estimated using Mann-Whitney test and Chi-square test.**

Table 2 showed differences between both groups as regard the studied predictors; Women with preeclampsia had abnormal uterine artery Doppler indices, higher serum inhibin-A level, lower serum level of PLGF concentrations than normotensive control group.

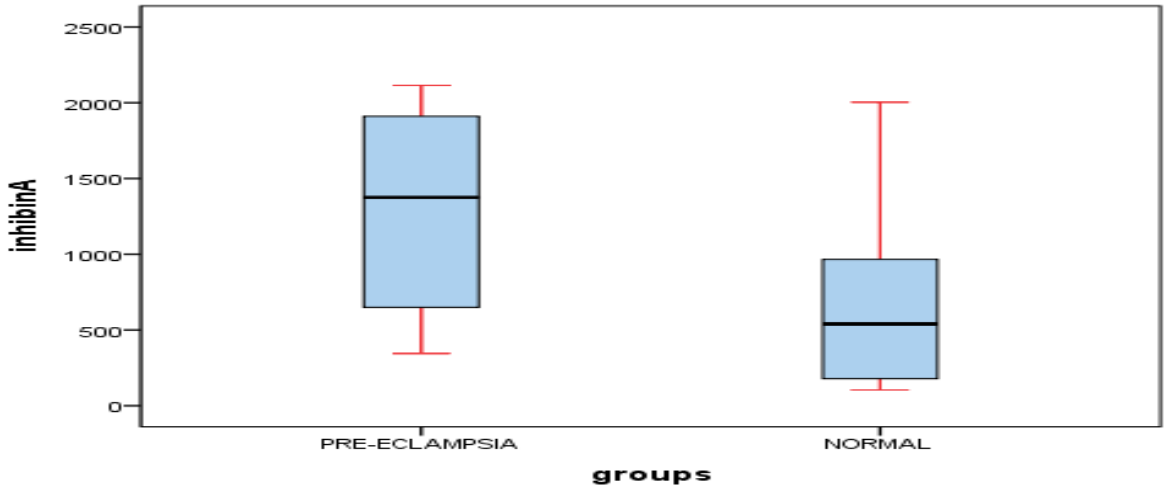

Figure (1): Box plotsshow the medians and ranges for serum inhibin A levels in both groups.

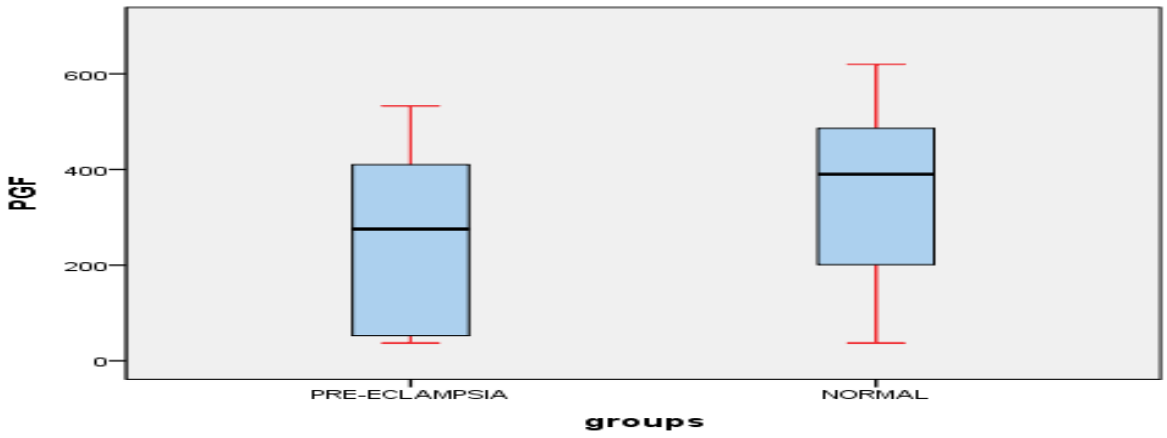

Figure (2): Box plots show the medians and ranges for serum PLGF levels in both groups.

Table (3):	**Receiver-operating characteristic (ROC) curve analysis for prediction of preeclampsia using, inhibinA level, PLGF level, UARI and UAPI.**					
ROC index	AUC (area under the curve)	95% CI	P-Value	Best Cut off Value (prediction probability)	Sensitivity of the test	Specificity of the test
Serum inhibin A	0.758	0.566 - 0.951	0.016	≥ 832.5 pg/ml	75%	80%
Serum PLGF	0.652	0.447 – 0.858	0.165	≤ 205pg/ml	74%	50%
UARI	0.709	0.527– 0.891	0.016	≥ 0.565	87.5%	74%
UAPI	0.848	0.742– 0.955	0.001	≤ 0.735	80%	74%

Table 3 showed that the areas under the curves (AUC) in ROC curve for serum inhibin-A,UARI and UAPI are statistically significant,all these parameters can be used as good predictors of preeclampsia with cut off values≥832.5pg/ml for serum Inhibin-A ,≥0.565for UARI and≤ 0.735 for UAPI.

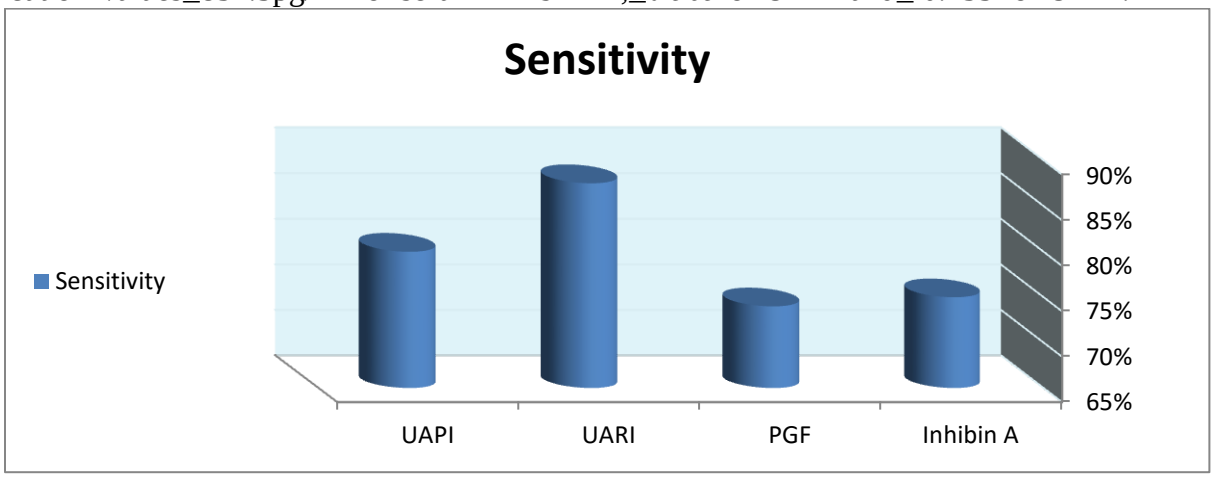

Figure (3): bar chart shows the sensitivity of all predictors.

In figure 3, UARI showed the highest sensitivity (the ability of the test to diagnose the disease)while serum Inhibin-A showed highest specificity (the ability of the test to exclude the disease).

DISCUSSION

Preeclampsia is a syndrome of unknown etiology that is associated with a considerable maternal morbidity and mortality. In spite of advancement in perinatal care, the incidence of preeclampsia has continued to increase worldwide [1].

At this time, PE still lacks a safe and effective therapy, as well as a reliable, early means of diagnosis or prediction. Many biophysical and biochemical markers have been used for prediction of women at high risk for developing preeclampsia, but many of them were unreliable, not specific or had a low predictive accuracy for clinical application.

In the current study, ninety of low risk normotensive women were followed up through their antenatal period, blood samples were collected in the second trimester and tested for Inhibin-A and PLGF biomarkers, Doppler ultrasound estimation for bilateral uterine artery velocimetry was done at 22-24 weeks gestation. All women were followed up until delivery; the 8 patients whom develop preeclampsia 8.9 % (case group) were compared against the 82 ladies who did not develop preeclampsia (normotensive control group).

There was no difference in the maternal age between the women who developed preeclampsia compared to the normotensive controls (29 ± 2.2 vs. 27 ± 3.8 years, $p < 0.07$) respectively. The BMI at recruitment was higher among women who developed preeclampsia (29.2 ± 2.9 vs. 26.1 ± 3.1 kg/m^2, $p < 0.04$), that is in agreement with **Dantas et al.** [16]who studied the relationship between BMI and preeclampsia and concluded that women with high BMI had an increased rate of developing preeclampsia.

Uterine artery Doppler analysis has the potential to predict pregnancy complications associated with uteroplacental insufficiency before the onset of clinical features. For almost 30 years, uterine artery Doppler studies have been utilized as a screening tool for uteroplacental insufficiency, mostly in the second trimester (from 18–24 gestation) [17].

In our study, abnormal uterine artery Doppler velocimetry either high median uterine artery resistance index (UARI) or low median uterine artery pulsatility index (UAPI) at 22- 24 weeks were significantly associated with a high incidence of PE where medians ± IQRs are (0.645 ± 0.3 vs. 0.485 ± 0.16, p = 0.009) and (0.625 ± 0.24 vs. 1.0 ± 0.68, p = 0.001) for RI

and PI respectively (**Table 2**). ROC curve analysis for the probability of median uterine artery RI and PI to predict PE showed that UARI can predict PE with sensitivity of 87.5 % and specificity of 74% at a cut off value ≥ 0.56 and also UAPI can predict PE with sensitivity of 80 % and specifity of 74% at a cut off value ≤ 0.74 (**Table 3**) (**Figure 4, 5**).

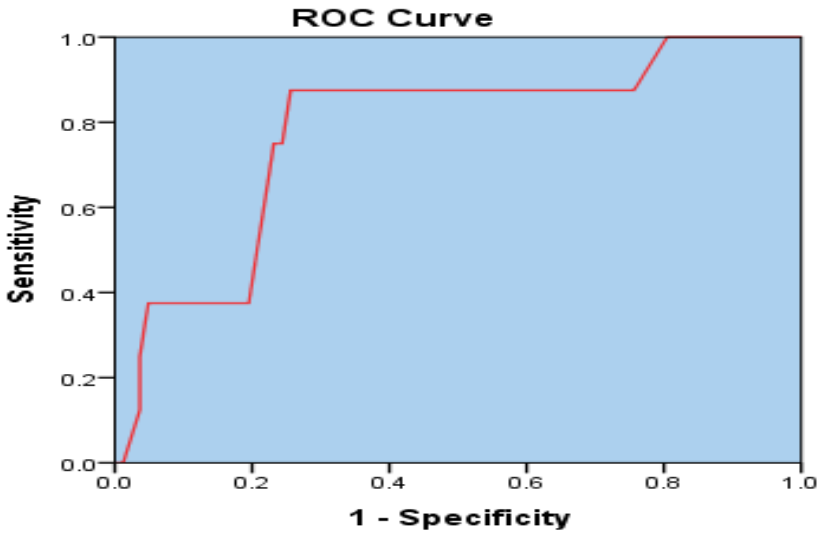

Figure (4): ROC curve analysis for UARI, AUC = 0.709 UARI can predict PE with sensitivity of 87.5%, specificity 74% at cut off value ≥ 0.565.

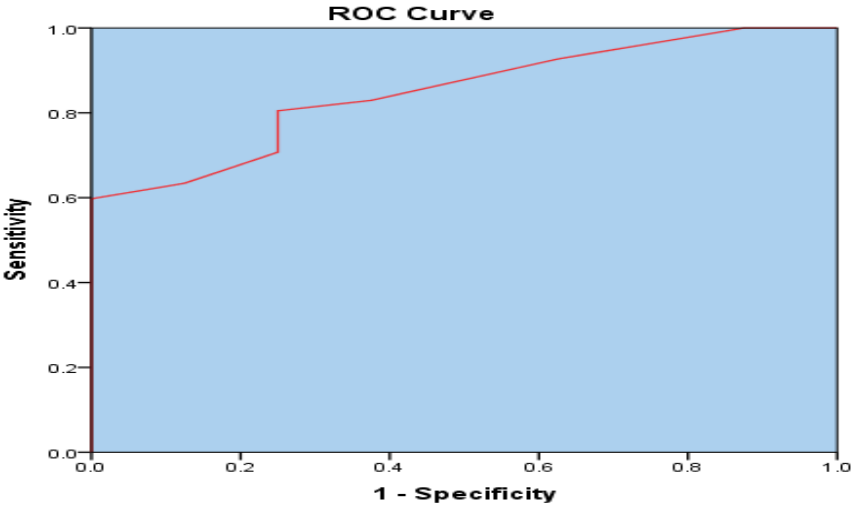

Figure (5): ROC curve analysis for UAPI, AUC = 0.848 UAPI can predict PE with sensitivity of 80%, specificity 74% at cut off value ≤ 0.735.

Those results were in agreement with **Coleman et al. [18]**, who reported that UARI of > 0.58 can predict PE with a sensitivity of 91% and specificity of 42% and with **Spencer et al**. [19] 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.** [20] showed that an

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.** [21] reported that second trimester Doppler ultrasound indices showed a low sensitivity for detection of PE in low risk nulliparous women and **Pongrojpaw et al.** [22]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.

The level of maternal serum inhibin-A during the second trimester of pregnancy was significantly higher among women who developed preeclampsia compared to the control group (1375 ± 1431 vs. 540 ± 1900, P = 0.016) (**Table 2, Figure 1**). ROC curve analysis for the probability of Inhibin-A to predict PE showed that Inhibin-A can predict PE with sensitivity of 75%, specificity 80% at cut off value ≥ 832.5 pg/ ml (Table 3, Figure 6).

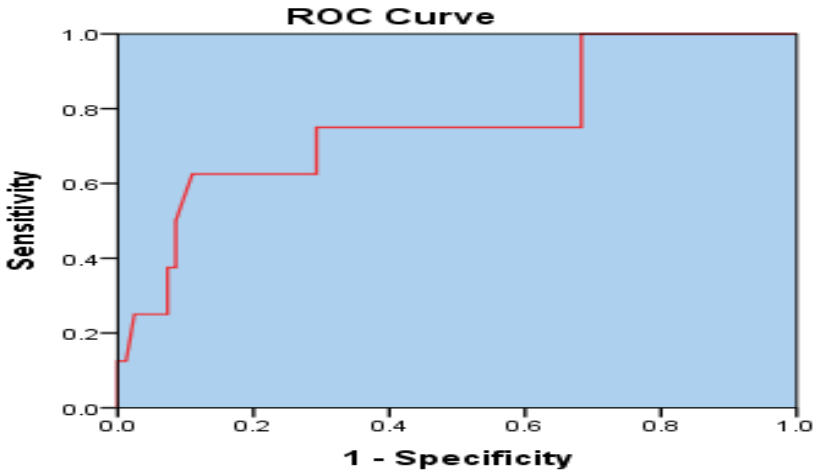

Figure (6): Receiver-operating characteristic (ROC) curve analysis for inhibin A level, AUC = 0.758. Inhibin-A can predict PE with sensitivity of 75%, specificity 80% at cut off value ≥ 832.5 pg/ml.

These results are in agreement with those of **Ree et al. [23]**who conducted a retrospective study of 4,764 subjects who underwent second-trimester multiple marker screening for Down syndrome to evaluate the role of serum inhibin-A and other serum markers in prediction of preeclampsia and reported that second trimester serum markers including inhibin-A may be helpful for prediction of PE and that inhibin-A seems to be the most important one among the other surveyed markers. Also with **Olsen et al.** [24]who conducted another retrospective study of 7767 subjects undergoing second-trimester serum aneuploidy screening and concluded that elevated second trimester Inhibin-A was associated with early onset preeclampsia.

On the other hands **Roes et al.** [25] **and Sibai et al.** [26] reported that inhibin-A alone has a poor sensitivity but if combined with other markers, it could have a useful role in prediction of preeclampsia.

The published data regarding PLGF in the prediction of preeclampsia are controversial. In our study, we found that level of second trimester PLGF was non-significantly decreased in the preeclampsia group when compared to the women in the control group (275.5 ± 369 vs. 390 ± 583, P = 0.156) (**Table 2, Figure 2**). ROC curve analysis for the probability of PLGF to predict PE revealed that PLGF can predict PE with sensitivity of

74%, specificity 50% at cut off value ≤ 205 pg/ ml (**Table 3, Figure 7**).

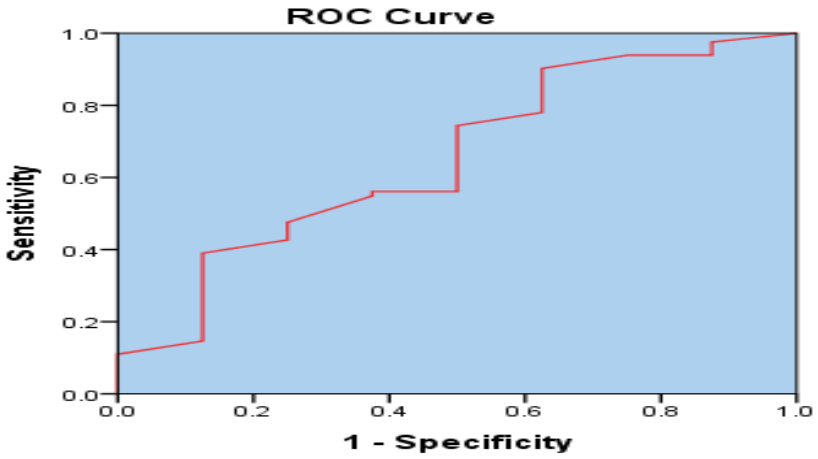

Figure (7): ROC curve analysis for PLGF level, AUC = 0.652. PLGF can predict PE with sensitivity of 74%, specificity 50% at cut off value ≤ 205pg/ml.

These results are in agreement with many authors who highlighted the poor predictive ability of PLGF to predict PE. **Kleinrouweler et al.** [27] 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.** [28] reported poor predictive ability of PLGF for prediction of PE (sensitivity 47% and specificity 62%).

On the other hand many authors showed that low level of maternal serum PLGF precede the clinical presentation of PE [29, 30] ,provide a good indicator of SGA and severe PE [31] and predict women who will develop early-onset preeclampsia [32].**Conde-Agudeloet al. [33]**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%).

YU et al., [34] reported that both serum inhibin-A and activin A levels were increased, while the PLGF level was decreased, in the early second trimester in women who developed pre-eclampsia. 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.

Finally Conde-Agudelo et al. [33] 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.Replication of such models to confirm their accuracy in the prediction of preeclampsia is needed. Maternal plasma/serum

concentrations of angiogenic and antiangiogenic factors in the second trimester of pregnancy coupled with uterine artery Doppler velocimetry appear to have a moderate to high predictive accuracy for early-onset preeclampsia.Discovery approaches have promise for the identification of potential biomarkers. Preeclampsia is a syndrome, and therefore it is unlikely that a single test (or a combination of tests) to identify a particular phenotype (e.g. early-onset disease, late-onset disease and HELLP syndrome) would be effective in predicting all cases of the disorder.

CONCLUSION

In conclusion, by using second trimester Doppler velocemetry of uterine artery, maternal serum level of Inhibin-A and PLGF, we may be able to identify women who are more prone to develop preeclampsia.

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