Determination of Serum Inhibin-A Levels in Differentiating Pre-Eclampsia and Gestational Hypertension

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ABSTRACT

Objective: To determine the association of serum Inhibin-A levels in differentiating Pre-eclampsia and gestational hypertension.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, in collaboration with the Gynaecology and Obstetrics Department at Combined Military Hospital (CMH), Rawalpindi Pakistan, from Jul 2020 to Jun 2021.

Methodology: Nulliparous women with single pregnancy in the third trimester admitted to evaluate high blood pressure in the Department of Gynaecology and obstetrics CMH Rawalpindi were selected after getting their informed written consent. Forty women with pre-eclampsia, forty with gestational hypertension, and 40 normotensive pregnant healthy women receiving routine antenatal care were matched for parity, age, and gestational age.

Results: The concentration of inhibin A [Median (IQR)] in women having preeclampsia were significantly higher [1745 pg/ml (1601.2–1821.7)] than subjects with gestational hypertension [895 pg/ml (834.5-977.5)] and normotensive pregnant women [637pg/ml (589-684.7)] with *p*-value 0.001. Further, Women with severe preeclampsia had a considerably greater serum concentration of inhibin A [Median (IQR)] [1895 pg/ml (1827.2-1955.7)] than those with mild preeclampsia [1686 pg/ml (1537.5-1762.7)].

Conclusion: As per our study, median serum Inhibin A concentrations were higher in pre-eclampsia subjects than in gestational hypertension. In clinical situations, serum Inhibin A levels may be used to differentiate between pre-eclampsia and gestational hypertension.

Keyword: Gestational hypertension, Inhibin-A, Pre-eclampsia.

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INTRODUCTION

Hypertension is one of the foremost reasons for maternal and neonatal deaths during pregnancy worldwide.[1] For hypertension in pregnancy, the International Society for Study Hypertension in pregnancy categorizes it into chronic hypertension, gestational hypertension, pre-eclampsia–de novo or superimposed on chronic hypertension, and white coat hypertension. "Gestational hypertension refers to hypertension without proteinuria or other signs and symptoms of pre-eclampsia-related end-organ dysfunction that develops after 20 weeks of gestation". During pregnancy, it is the most common cause of hypertension.[2] Pakistan has an incidence of gestational hypertension of approximately 6.5%.[3] Prior history of pre-eclampsia, multiple gestations, and obesity contribute to the highest incidence.[4] Women with gestational hypertension may develop pre-eclampsia in 10 to 50 % of cases.[5]

"Pre-eclampsia refers to the new onset of hypertension and proteinuria or the new onset of hypertension and significant end-organ dysfunction (with or without proteinuria)". It affects 4.6% of pregnancies worldwide.[6] Incidence of pre-eclampsia in Pakistan is approximately 3 (2.1%). It is diagnosed clinically in patients having systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mmHg occurring after 20 weeks of gestation in a woman whose blood pressure has previously been normal along with proteinuria, i.e., 0.3 g or more of protein in a 24-hour urine specimen or protein to creatinine ratio of >30 mg/mol in spot urine. Subjects having pre-eclampsia are at risk of fetal growth retardation, placental abruption, preterm birth, eclampsia, cardiovascular disease and HELLP syndrome.

The prognosis and management of gestational hypertension and pre-eclampsia are different. An accurate diagnosis, when possible, can help make

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management decisions regarding timing of delivery, need for anti-seizure prophylaxis and assessing maternal prognosis for risk for progression in the current pregnancy, recurrence risk in future pregnancies, and long-term maternal health risks. As urine protein is raised in various clinical conditions, it is not specific for the pre-eclampsia diagnosis. Therefore, there is a need for a stable biochemical marker that can differentiate between these entities and predict the patients who require a higher degree of medical intervention. There have been numerous studies indicating that pregnant patients with pre-eclampsia have elevated levels of in hibin-A. It is a heterodiameric glyco-protein hormone produced by placental trophoblasts in pregnancy.[7,8] It has a biphasic pattern during pregnancy. It remains constant during 14-30 weeks of gestation, with peak levels around the eighth-week gestation and at term.[8] It is commonly used for a combined chromosomal abnormality screening in the second trimester. Research has concluded that an increase in serum Inhibin A levels in the mother is associated with intra-uterine growth restriction. Inhibin A is widely studied in pre-eclampsia, but only a few studies have compared and established its role in gestational hypertension. Our study aimed to determine the serum Inhibin A concentration in preeclamptic, gestational hypertension and normotensive pregnancies in their third trimester.

METHODOLOGY

This cross-sectional study was carried out after approval by the Institute Ethical Committee (IRB/20 /1057) at the Department of Chemical Pathology and Endocrinology, AFIP in association with the Department of Gynaecology and Obstetrics CMH, Rawalpindi, from July 2020 to June 2021. World Health Organization calculator was used to calculate the sample size with a 2.5% incidence of pre-eclampsia (National level) at a 95% confidence interval3.9 Non-probability consecutive sampling technique was employed for sampling.

Inclusion Criteria: Eighty Nulliparous women with single pregnancy in the third trimester admitted for the evaluation of high blood pressure in the Department of Gynaecology and obstetrics CMH Rawalpindi were included in the study.

Exclusion Criteria: Women having chronic hypertension, gestational diabetes, renal disease, cardiovascular disorders, twin pregnancies and multiparous were exclude from the study.

Forty normotensive pregnant healthy women receiving routine antenatal care were matched for parity, age, and gestational age. Gestational hypertension was included in our analysis until the completion of the puerperal period was ended in our study. Women ≥20th week of gestation having a systolic blood pressure of above 140 mmHg or diastolic blood pressure of 90 mmHg with protein to creatinine <30 mg/mmol and absence of signs of organ involvement were grouped as Gestational hypertension omen having high blood pressure (systolic pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on two or more occasions) and urine protein to creatinine ratio of >30mg/mmol or signs of organ dysfunction after 20 weeks of gestation were classified as pre-eclamptic.[10,11] Women with high blood pressure ≥160 mm Hg or diastolic blood pressure ≥110 mm Hg, headache, and proteinuria (urine protein concentration >5 grams per day) were classified as severe pre-eclampsia. At the same time, the rest were categorized as mild pre-eclampsia.

Subjects from gestational hypertension and the normotensive group were communicated through-out their gestation and during the follow-up by phone call to exclude women who had developed any of the following: severe hypertension, proteinuria, decreased the number of platelets (100,000/mL), or elevated liver enzymes (over the 2 SD for our laboratory standards), They did not meet any of the above criteria and delivered a healthy, full-term baby.

About 5 ml venous blood specimen was taken before administering any antihypertensive drugs for serum inhibin A and ALT in a gel tube. At the same time, the sample for platelets was collected in EDTA container. Serum was separated and stored at -21°C till analysis for Inhibin A. Spot urine was taken in a urine container for protein and creatinine. Serum ALT was analyzed by UV kinetic with the P5P method. In contrast, urine creatinine was analyzed by Jeffe kinetic (2nd generation) at Siemens Advia 1800 on the same day and urinary protein while Platelets were analyzed at Sysmex XP 100.

Samples for Inhibin-A were analyzed in batch after taking all pre-analytic variables into account by Enzyme-linked immunosorbent assay (Ansh Lab, Webster, USA). An interassay coefficient of variation of the test kit was less than 10%. Test reliability was assessed by internal quality control through certified control material.

All subjects calculated the protein to creatinine ratio (mg/mmol). In addition, twenty-four-hour urine for protein was also collected in those subjects with

PCR >30mg/mmol, and Urinary protein was analyzed by immunoturbidimetric method at ADVIA 1800.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Frequency and percentages were calculated for qualitative variables. Shapiro Wilk test determined the normality of distribution. Data being nonparametric, quantitative variables were expressed as median and IQR. Kruskal Willis test was applied to compare values of serum inhibin A among study groups with a *p*-value ≤0.05 that were statistically significant (95% confidence interval). We used the Mann Whitney U test to determine if mild and severe preeclampsia differed significantly with a *p*-value ≤0.05.

RESULTS

Initially, 144 subjects were screened, out of which 80 subjects were finally selected based on inclusion criteria, and 24 were excluded because of concurrent medical conditions (17 developed gestational diabetes, four chronic hypertension, and one cardiovascular disease). Forty women with pre-eclampsia and forty women with gestational hypertension were matched with 40 healthy normotensive pregnant subjects. Demographics and disease characteristics were given in the Table-I.

Table-I: Comparison of disease characteristics among the study population.

Characteristics	Pre-eclampsia Group (n=40)%	Gestational Hypertension (n=40)%	Healthy Pregnant Group (n=40)%	*p*-value
	Median (IQR)	Median (IQR)	Median (IQR)	
Maternal Age (years)	26 (4)	26 (7)	25 (3)	0.049
Gestational (weeks)	34 (4)	34 (9)	34 (3)	0.977
Baseline Mean Arterial Pressure (mm Hg)	123 (9.75)	118 (6.5)	87.5 (6.4)	<0.001
Protein to Creatinine ratio (mg/mmol)	42 (13.75)	7 (7.75)	8 (5)	<0.001
ALT (IU	32 (100.5)	28 (8.75)	29 (9.7)	0.039
Platelets (1000 cells/mm^3)	155.5 (88)	364.5 (115)	315.5 (149)	<0.001

This revealed significant differences (0.001) between the three groups in mean arterial pressure, protein to creatinine ratio, and platelet count. However, no significant difference was found between gestational age and ALT.

Kruskal Willis test was applied to compare values of serum inhibin a between healthy normotensive women, pre-eclampsia, and gestational hypertension groups. The results as given in Table-II indicated that women with pre-eclampsia had significantly high levels of serum Inhibin A concentration compared to those with gestational hypertension (p<0.001).

Table-II: Comparison of serum inhibin A between healthy normotensive women, preeclampsia, and gestational hypertension groups.

Groups	n	Serum Inhibin A (pg/ml) Median (IQR)	*p*-value
Healthy Normotensive Pregnant	40	637 (589-684.7)	<0.001
Gestational Hypertension	40	895 (834-977.5)	
Preeclampsia	40	1745 (1601.2-1821.7)	

In pre-eclamptic patients, 8 out of 40 (20%) had severe pre-eclampsia. Compared to subjects with mild pre-eclampsia, subjects with severe pre-eclampsia had significantly higher concentrations of inhibin A (Table-III).

Table-III: Comparison of serum inhibin A in mild versus severe preeclampsia.

Groups	Serum Inhibin A (pg/ml) Median (IQR)	*p*-value
Mild Preeclampsia (n=32)	1686 (1537.5- 1762.7)	<0.001
Severe preeclampsia (n=8)	1895 (1827.2- 1955.7)	

DISCUSSION

Pregnancy-related hypertension is one of the common causes of maternal and perinatal mortality worldwide.[9] Pre-eclampsia complicates the majority of pregnancies.[10] Several pregnancy complications are associated with preeclampsia, including high blood pressure and damage to other organs, especially the liver and kidneys. At the same time, Gestational hypertension is a mild entity clinically presented by high blood pressure with no urine protein or other organ damage during pregnancy.

Our first study is from Pakistan, and we recruited pregnant women who developed hypertension after >20th week of gestation. Results based on our analysis found that the Median levels of Inhibin A were significantly elevated in pre-eclampsia, and this finding is consistent with Musttukrishna *et al*.[8] They reported comparable findings in 20 pregnant women with PE, with 20 healthy normotensive pregnant women matched for gestational age. Inhibin A levels were around eight times higher in pre-eclamptic women than in healthy normotensive pregnant women. Our observations support the hypothesis that Inhibin A has a role

in developing pre-eclampsia and may play a role in determining maternal response.[11] Inhibin A levels were substantially more significant in women whose pregnancies were complicated by pre-eclampsia than gestational hypertension, with no overlap in levels across groups when matched for gestation age and parity. It is consistent with the results of Silver *et al.*[12] Who studied Inhibin A concentration in 60 healthy pregnant women, 60 preeclampsia, and 51 non-proteinuric hypertension. He found a higher median concentration (1833 pg/mL) of inhibin A in preeclamptic women than non-proteinuric gestational hypertensive women (1104 pg/mL). Our study showed no overlap between concentrations of Inhibin A in preeclamptic and gestational hyperten-sive subjects, which contradicts Yingying Gratacós *et al.*[13] This study found an overlap in Inhibin A concentration between 4 out of 20 (20%) women with pre-eclampsia and 11 out of 20 (55%) women with gestational hypertension. As 24 hr urine collection is cumbersome for patients, protein to creatinine ratio is sensitive and specific for the diagnosis of preeclam-psia.[14] Therefore we used spot urine protein to crea-tinine ratio to segregate women with preeclampsia from gestational hypertension; 30 mg/mmol was taken as cut off for diagnosis of Preeclampsia. The median serum of Inhibin A was raised in the severe PE group 1895 pg/mL compared to mild PE 1686 pg/mL, p=0.000). Therefore, Inhibin A concentration can be useful to differentiate severe from mild pre-eclampsia; our findings are in line with the findings of Phupong *et al,*[15] they recruited 52 pregnant women with pre-eclampsia, 23 women had mild pre-eclampsia remaining 29 developed severe pre-eclampsia, with comparable parity and the total number of prenatal care visits. Alghazali *et al,*[16] found similar results in a casecontrol study comparing maternal Inhibin A concentration in pregnant women with pre-eclampsia to the control group. Serum inhibin A could be used as a non-invasive biochemical marker in diagnosing hyperten-sion during pregnancy, separating patients with gestational hypertension from those with preeclampsia and predicting the development of preeclampsia in patients being evaluated for pre-eclampsia. A benefit of this could be identifying patients in need of additional maternal and fetal surveillance earlier; conversely, repeated testing for patients who are not at risk would be eliminated.[17,18]

CONCLUSION

As per our study, median serum Inhibin A concentration was elevated in pre-eclamptic women than in those with gestational hypertension. Serum Inhibin A levels may serve as an effective marker to differentiate between pre-eclampsia and gestational hypertension and predict which patients will require a higher level of medical intervention.

Conflict of Interest: None.

Authors' Contribution

SS: Idea conception, sampling, analysis, statistical analysis, discussion, literature review, M: Idea conception, data analysis, discussion, supervision, ZHH: Discussion, literature review, AB: Review of article, correction, RA: Gynecological consultation and discussion, UBK: Statistical analysis and verification.

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