Original article

Role of maternal serum inhibin A and insulin – like growth factor 1 levels as predictors of fetal outcome in high risk pregnancies.

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Abstract

Background: Timely prediction of adverse pregnancy and perinatal outcome has been the endeavor of clinicians since a long time. Identification and validation of a serum based biomarker is the need of the hour and many tentative molecules have been studied with variable results. Two such molecules are inhibins and insulin like growth factors. This study was planned to assess the utility of inhibin A and IGF-1 as predictors of adverse pregnancy and fetal outcomes.

Methods: The study population comprised of all pregnant women presenting to the ante natal clinic in SSK hospital between gestation periods of 24-28 weeks fulfilling the inclusion criteria. The criteria adopted for inclusion in the study included primi-gravida with singleton pregnancy or multi-gravida with high risk factors in the previous pregnancy such as gestational hypertension, preeclampsia, eclampsia, placental abruption, intrauterine growth retardation, intrauterine fetal demise, preterm delivery and early pregnancy loss.

Results: The mean levels of inhibin A were significantly higher in women with overall adverse pregnancy outcome such as preeclampsia, gestational hypertension, and preterm delivery as compared to the normal counterparts. No significant difference in the level of IGF-1 was observed with various adverse outcomes. Inhibin A at the cutoff of $\geq$ 443pg/ml was found to be reliable marker of preeclampsia and preterm delivery.

Conclusion: We may hypothesize from our findings that second trimester inhibin A levels may be further evaluated through large prospective studies as an reliable biomarker of adverse pregnancy outcome.

Keywords: *Preeclampsia, IGF-1, Inhibin A, SGA*

Introduction

Preeclampsia is a common hypertensive disorder affecting 7 -10 % of pregnancies with profound implications for both maternal and fetal well being (1). The in depth understanding of the various molecular pathways that predispose certain women to develop this condition is still vague. According to the present understanding, preeclampsia develops as a two stage process with poor placentation in the first half of pregnancy followed by maternal response in the second half of pregnancy(2).

Although medical science has made great progress in the recent past, accurate prediction of adverse pregnancy outcomes such as preeclampsia, preterm deliveries, intrauterine growth retardation, still births etc still elude clinicians. They contribute greatly to maternal and fetal morbidity and mortality. Identification and validation of a serum

based biomarker is the need of the hour and many tentative molecules have been studied with variable results. Two such molecules are inhibins and insulin like growth factors.

Inhibins are dimeric glycoproteins which are members of the transforming growth factor β superfamily(3). Inhibin A is produced by the placenta and has been shown by some researchers to be raised as early as 10-15 weeks of pregnancy in women who subsequently develop preeclampsia (4). Insulin like growth factor-1 (IGF-1) is a mitogenic polypeptide that is thought to play a critical role in abnormal placentation- a hallmark of preeclampsia (5).

This study was planned to assess the utility of inhibin A and IGF-1 as predictors of adverse pregnancy and fetal outcomes.

Method

The study was conducted by the collaboration between the departments of biochemistry and obstetrics and gynecology, Lady Hardinge Medical College and associated SSK hospital, New Delhi. The study was commenced after procuring due clearance from the institutional ethical committee. Bilingual written informed consent was taken from all eligible subjects with detailed explanation of methodology, purpose and implicit risks if any. A total of 60 subject were enrolled in the study.

The study population comprised of all pregnant women presenting to the ante natal clinic in SSK hospital between gestation periods of 24-28 weeks fulfilling the inclusion criteria. The criteria adopted for inclusion in the study included primi-gravida with singleton pregnancy or multi-gravida with high risk factors in the previous pregnancy such as gestational hypertension, preeclampsia, eclampsia, placental abruption, intrauterine growth retardation, intrauterine fetal demise, preterm delivery and early pregnancy loss. Subjects with chronic hypertension, renal failure, Diabetes Mellitus, thyroid disorders, multiple pregnancies, epilepsy and fetal congenital malformations were excluded from the study.

After detailed clinical examination and routine investigations, the subjects were called for regular follow up visits. Blood was obtained by venepuncture and the serum stored at -20 degree Celsius for special investigations. Serum inhibin A and IGF-1 levels were assayed by commercially available ELISA kits supplied by DRG international, Germany. The inter-assay and intra-assay precision of Inhibin A was 7.3% and 3.6 % respectively. While the same values for IGF-1 were 7.2% and 4.2% respectively.

The maternal outcome was assessed by the development of gestational hypertension, placental abruption, preeclampsia and eclampsia while the fetal outcome was gauged by the mode of delivery, period of gestation at the time of delivery, birth weight, APGAR score at 5 min, need for neonatal resuscitation or admission to neonatal ICU, small for gestational age babies and intrauterine fetal death or early neonatal death.

Statistical analysis

All data analysis was done using SPSS package version 19 (SPSS Inc., Chicago, IL, USA). Data were presented as mean ± standard deviation (standard error of means) or number of subjects and percentage. Chi- square test or student's t test was used for comparison of means between parametric data. The Mann Whitney test was used to compare the means in case of non parametric data. The sensitivity and specificity of various markers ass determinants of risk were determined. P value <0.05 was considered significant.

Results

The demographic profile of the study population is tabulated in table no 1. The mean age of the subjects was 26.38 ±4.82 yrs with the maximum number (29/60) participants were in the 21-25 yrs age group. The mean BMI of the study population at the time of enrollment was 23.23 ± 4.00 kg/$m^{2.}$ The mean gravid status of the subjects was 2.5 ±1.3. Approximately 20% of the population were primigravida, 45% were of gravid 2, 23.3% were of gravid 3 and 11.7% were of gravida ≥ 4. The mean period of gestation at the time of enrollment was 23.5 ± 1.35 weeks.

Parameter	Mean ± SD
Age (yrs)	26.38 ±4.82
BMI (Kg/m^2)	23.23 ± 4.00
Gravida	2.5 ±1.3
Period of gestation (weeks)	23.5 ± 1.35

Table 2: The distribution of risk factors is depicted in table

Risk factor	Number	Percentage
Primigravida	12	20%
Previous history of		
Preeclampsia	16	26.7%
Eclampsia	02	3.3%
Gestational Hypertension	09	15%
Placenta Abruption	02	3.3%
Intrauterine Death	03	5%
Intrauterine Growth restriction	09	15%
Preterm Delivery	11	18.3%
Early Pregnancy Loss	22	36.7%

Primigravida comprised of 20 % of the study population whereas preeclampsia was present in 27 % of the previous pregnancies of the multi-parous women.

The maternal outcome pattern was also studied. Normal maternal outcome was observed in 52 subjects whereas adverse maternal outcome in the form of gestational hypertension, preeclampsia, eclampsia, placental abruption was observed in 8 subjects. Vaginal delivery occurred in 51 pregnancies whereas LSCS was conducted in 9 pregnancies in the study population.

The fetal outcome is illustrated in table no 3. The mean birth weight was 2.58 ± 0.55 kg while the mean gestational age was 37.50 ± 1.97 weeks. The other parameters which were assessed include SGA, Apgar score, neonatal resuscitation and admission to neonatal ICU.

Fetal outcome	Number	Percentage (%)
Birth Weight (Kg)		
< 1.5 Kg	1	1.7
1.5 – 1.9 Kg	5	8.3
2.0 – 2.4 Kg	15	25
> 2.5 Kg	39	65
Gestational Age at Delivery		
< 34	3	5
34 - 36	13	21.7
> 36	44	73.3
SGA		
< 10^{th} centile	8	13.3
10 -90^{th} centile	52	86.7
>90^{th} centile	0	
APGAR score at 5 minutes		
<7	5	8.3
>7	55	91.7
Neonatal Resuscitation	1	1.7
Admission to neonatal ICU	2	3.3

The serum levels of inhibin A and IGF-1 in pregnancies with normal and adverse outcomes are compared in tables 4a and 4b respectively.

The mean levels of inhibin A were significantly higher in women with overall adverse pregnancy outcome such as preeclampsia, gestational hypertension, and preterm delivery as compared to the normal counterparts.

	Adverse outcome		Normal outcome			P value
	Mean inhibin A		Mean inhibin A			
	Pg/ml	MoM		Pg/ml	MoM	
Overall adverse outcome (N=24)	443.94± 57.74	1.99 ± 0.26	Overall normal outcome (N=36)	189.42± 24.8	0.85± 0.11	<0.001
Preeclampsia (N= 4)	632.7 ± 143.48	2.84 ± 0.64	Normotensive (N=52)	235.74± 27.07	1.06 ± 0.12	0.006
Gestational hypertension (N=4)	395.98 ± 72.3	1.78 ± 0.33	Normotensive (N=52)	235.74± 27.07	1.06 ± 0.12	0.065
Preterm Delivery (N=16)	440.49 ± 70.12	1.98 ± 0.31	Term delivery (N=44)	211.95 ±24.21	0.95 ±0.11	0.003
SGA (N=8)	287.81 ± 85.99	1.29 ± 0.38	AGA (N=52)	270.60 ± 30.62	1.22 ±0.14	0.948
Low APGAR score (N=5)	203.23 ± 58.73	0.91 ± 0.26	Normal APGAR (N=55)	279.23 ±30.75	1.26± 0.14	0.748

	Adverse outcome	Normal outcome		P value
	Mean IGF-1	Mean IGF-1		
	ng/ml		ng/ml	
Overall adverse outcome (N=24)	167.99± 18.77	Overall normal outcome (N=36)	216.72± 16.98	0.063
Preeclampsia (N= 4)	139.18 ±27.38	Normotensive (N=52)	201.96±13.6	0.192
Gestational hypertension (N=4)	193.80±80.23	Normotensive (N=52)	201.96±13.6	0.738
Preterm Delivery (N=16)	160.73 ±16.49	Term delivery (N=44)	210.51 ±16.21	0.090
SGA (N=8)	154.78±32.29	AGA (N=52)	203.76±13.96	0.207
Low APGAR score (N=5)	159.68±28.84	Normal APGAR (N=55)	200.64 13.83±	0.422

The mean IGF-1 levels in the cases that developed adverse outcome were not significantly lower than those with normal pregnancy outcome. No

significant difference in the level of IGF-1 was observed with various adverse outcomes.

Table no 5 is the comparative tabulation of inhibin A and IGF-1 as predictors of adverse pregnancy outcomes. The cut off for Inhibin A was taken as 443 pg/ml while that for IGF-1 was fixed at 168ng/ml.

outcome	Number of patients With the cut off inhibin A levels		Sensitivity	Specificity	P value
Normal(n=52)	< 443	43	75	82.7	0.028
	≥ 443	9			
Preeclampsia(n=4)	< 443	1			
	≥ 443	3			
Normal(n=52)	< 443	43	50	80.4	0.169
	≥ 443	9			
Gestational hypertension (n=4)	< 443	2			
	≥ 443	2			
Preterm babies(n=16)	< 443	9	43.8	84.1	0.038
	≥ 443	7			
Term babies (n=44)	< 443	7			
	≥ 443	37			
SGA(n=8)	< 443	6	25	76.9	1.000
	≥ 443	2			
AGA (n=52)	< 443	40			
	≥ 443	12			
Low APGAR score babies (n=5)	< 443	4	20	76.4	1.000
	≥ 443	1			
Normal APGAR score babies (n=55)	< 443	42			
	≥ 443	13			

Inhibin A at the cutoff of ≥ 443pg/ml was found to be reliable marker of preeclampsia and preterm delivery. However it was not significant for gestational hypertension, SGA and low APGAR score.

outcome	Number of patients With the cut off IGF-1		Sensitivity	Specificity	P value
Normal(n=52)	≤ 168	22	75	57.7	0.314
	> 168	30			
Preeclampsia(n=4)	≤ 168	3			
	> 168	1			
Normal(n=52)	≤ 168	22	50	78.6	1.000
	> 168	30			
Gestational hypertension (n=4)	≤ 168	2			
	> 168	2			
Preterm babies(n=16)	≤ 168	10	62.5	61.4	0.144
	> 168	6			
Term babies (n=44)	≤ 168	17			

	> 168	27			
SGA(n=8)	≤ 168	5	62.5	57.7	0.448
	> 168	3			
AGA (n=52)	≤ 168	22			
	> 168	30			
Low APGAR score babies (n=5)	≤ 168	4	80	58.2	0.164
	> 168	1			
Normal APGAR score babies (n=55)	≤ 168	23			
	> 168	32			

Discussion

The present study was carried out to assess the predictive value of second trimester Inhibin A and IGF-1 values as biomarkers for evaluation of risk for adverse outcome pregnancies. Adverse outcome was assessed by the appearance of gestational hypertension, pre eclampsia during pregnancy and preterm delivery, SGA and low APGAR score during parturition.

In the present study, 40% women developed adverse outcome with gestational hypertension and preeclampsia present in 5 and 6.7% of them. Similar figures for incidence of hypertensive disorders of pregnancy were reported by Aquilina et al (6) and Aye et al (7). Approximately 27% of the women later developed preterm delivery while 35% had low birth weight babies. Dane et al (8) and Stepan et al (9) found comparable demography in their study cohorts as well.

Inhibin A levels rises with gestational age during pregnancy. The increase has been attributed to the high prostaglandin and oxytocin levels during pregnancy (10). Studies have shown association with high inhibin A levels with incidence of trisomy 21(11). Furthermore many researchers have now also hinted towards the association of inhibin A >2.0 MoM with adverse pregnancy outcomes (12). Our study also illustrates the same finding.

Inhibin A levels were higher in gestational hypertension as well as in pre eclampsia but statistically significant difference was observed in preeclampsia only. Similar results were echoed in studies conducted by Spencer et al (13) and Florio et al (14). Aye et al found 3.26 MoM in women who developed preeclampsia and 0.99 in women who remained normotensive. Corresponding values form our study were 2.84 MoM and 1.11 MoM respectively(7).

A similar statistically significant difference was observed in case s with pre term delivery as compared to term delivery. Fitzgerald et al reported that higher inhibin A levels increase the risk for intra uterine growth retardation in the fetus(15). Our study also demonstrated higher values in SGA although the difference form AGA was not statistically significant. Dayal et al (16) demonstrated that a significant positive correlation can be discerned between second trimester serum inhibin A levels and the development of preeclampsia.

Insulin like growth factor-1 (IGF-1) is similar in structure to insulin. IGFs are important determinants of fetal growth and postnatal development (17). IGF-1 is a mitogenic polypeptide that stimulates cellular proliferation and differentiation. Hernandez- Valencia et al showed that lower IGF-1 levels during pregnancy are associated with intra uterine growth retardation (18). IGF system is also thought to play a role in abnormal placentation thereby highlighting its role in pre eclampsia (19).

In the present study, the IGF-1 levels were lower in women who developed preeclampsia as compared to nomotensive controls. However the decrease was not statistically significant. Our findings are concordant with observations reported by Giudice et al (19), Halhali et al (20). Similarly, lower IGF-1 levels were found in gestational hypertension in our study. Shang et al reported significantly lower IGF-1 levels in pregnancies complicated by hypertension (21). Statistically insignificant

reduction in IGF-1 levels was observed in pregnancies complicated by adverse fetal or perinatal outcomes. Chia et al also reported significantly reduced IGF-1 levels in IUGR pregnancies leading to SGA babies as compared to AGA babies (22). Lindsay et al concluded from their study on pregnant women with gestational Diabetes Mellitus that fetal IGF-1 levels were inversely associated with maternal diabetes (23).
Comparative assessment of the two markers as indicators of adverse pregnancy outcome revealed that inhibin levels higher than the cutoff of 443pg/ml significantly predicted the occurrence of preeclampsia and preterm delivery. However IGF-1 levels did not show any statistically significant role as an reliable predictor of adverse pregnancy and fetal outcome. We may hypothesize from our findings that second trimester inhibin A levels may be further evaluated through large prospective studies as an reliable biomarker of adverse pregnancy outcome.

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