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Mid Trimester Beta-HCG and AFP Level and it's Correlation with Development of Preeclampsia

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Abstract

Background: Preeclampsia affects 2-8% of pregnancies and is a major cause of maternal and perinatal morbidity and mortality. Without intervention, the mother is at substantial risk for seizures (eclampsia), renal and liver failure, pulmonary edema, stroke, and death. Preeclampsia is also recognized as a major risk factor for cardiovascular disease later in life for both the woman and her child. HCG has many important functions during pregnancy including promotion of progesterone production, implantation and decidualization, angiogenesis, cytotrophoblast differentiation and immune cell regulation. AFP serum levels during pregnancy also have been used as an ancillary aid in the diagnosis of placental abnormalities, fetal death, growth restriction/retardation, and preterm labor. *Aims and Objective:* 1. To evaluate maternal serum mid-trimester human chorionic gonadotropin (HCG) and alfa-fetoprotein levels (AFP). 2. To look for correlation between maternal serum mid-trimester human chorionic gonadotropin and alpha-fetoprotein levels with development of preeclampsia. *Material and Methods:* Study Design: Descriptive and observational prospective study. Study Area: The study was conducted in department of obstetrics and Gynaecology, at Tertiary care center. Statistical Analysis : Data was analyzed using SPSS Ver 23. Frequencies, Descriptive, chi square test were done. *Results:* The mean AFP who developed severe preeclampsia was 58.84 ± 7.01 ng/ml compared to 42.85 ± 15.27 ng/ml who developed mild preeclampsia compared to 32.42 ± 8.17ng/ml who remained unaffected during study ($p < 0.05$). The mean β HCG who developed severe preeclampsia was 58510.00 ± 9065.68 mIU /ml compared to 42398.13 ± 13615.98 mIU /ml who developed mild preeclampsia compared to 29154.24 ± 12330.26 mIU /ml who remained unaffected during study ($p < 0.05$). *Conculsion:* Elevated levels of maternal serum AFP & β hCG measured between 14 – 24 weeks of gestation have significant positive correlation with pre-eclampsia & adverse obstetric outcome. Second trimester markers (AFP & β hCG) may be used as predictor of preeclampsia.

Keywords: Preeclampsia, Alfa feto protine (AFP), Human chorionic gonadotropin (HCG)

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Introduction

Preeclampsia affects 2–8% of pregnancies and is a major cause of maternal and perinatal morbidity and mortality.[1]

Without intervention in preeclampsia, the mother is at substantial risk for seizures (eclampsia), renal and liver failure, pulmonary edema, stroke, and death. Preeclampsia is also recognized as a major risk factor for cardiovascular disease later in life for both the woman and her child.[2]

Despite considerable research, the cause of preeclampsia remain unclear, though pathophysiology research suggests that preeclampsia is a disease of the placenta characterized by shallow trophoblastic invasion and endothelial dysfunction.[2]

The pathophysiology of pre-eclampsia is not precisely known but a two-step model that is widely accepted has been described.[2]

Preeclampsia is defined as a systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on 2 occasions at least 4 hrs a part after 20 weeks gestation in women with a previously normal blood pressure or ≥160 mm Hg systolic or ≥ 110 mm Hg diastolic, and proteinuria ≥ 300 mg/ 24 hrs or a protein/creatinine ratio ≥0.3 mg/dl or a dipstick reading of ≥1+.[2]

hCG (human chorionic gonadotropin) is a glycoprotein composed of 244 amino acids that is produced by the syncytiotrophoblast and maintains pregnancy by stimulating progesterone synthesis by the corpus luteum.[3]

Since hCG is of importance both for the invasion of trophoblasts and angiogenesis, which, in pregnancies complicated by pre-eclampsia and other disorders due to placental dysfunctional, are known to be insufficient, several studies have investigated the potential of hCG to predict these disorders.[3]

Elevated hCG levels in the second trimester could be due to a reduced production of hCG in early pregnancy, which results in an insufficient trophoblast migration into the spiral arteries, with a subsequent placental hypoxia that stimulates secretion of the pro-angiogenic hCG as a compensatory mechanism.[3]

AFP (alpha feto protein) is a 69-kDa single-polypeptide chain that contains 3%–5% carbohydrate and is produced in the yolk sac and fetal liver.[4]

Although the exact cause for an unexplained elevation is not completely understood, placental pathology studies suggest that it is associated with chorionic villitis and placental vascular lesions. These lesions allow leakage of the AFP from the high concentration fetal circulation to the low concentration maternal circulation, thereby elevating the maternal serum AFP.[4]

AFP serum levels during pregnancy also have been used as an ancillary aid in the diagnosis of neural tube defects and brain/spinal cord malformations, pregnancy-related hematologic disorders, placental abnormalities, fetal death, growth restriction/retardation, and preterm labor.[4]

Aims and Objective

1. To evaluate maternal serum mid-trimester human chorionic gonadotropin and alfa-fetoprotein levels.
2. To look for correlation between maternal serum mid-trimester human chorionic gonadotropin and alpha-fetoprotein levels with development of preeclampsia.

Materials and Methods

Study Design: Descriptive and observational prospective study.

Study Area: The study was carried out in the Department of Obstetrics and Gynaecology at tertiary care centre.

Study Population: The pregnant women attending OPD who fulfil selection criteria were randomly selected.

Sample size: The sample size is calculated at 95% confidence level assuming standard deviation of β HCG of 1.80 MoM in severe preeclampsia as found in the study of M Tosun *et al*.[5]

At the precision of 0.35 MoM, minimum 102 patients are required as sample size. It was further enhanced rounded of 120 patients as final sample size required for present study expecting 20% drop out/ attrition.

Inclusion Criteria

- Age >18 years and < 35 years
- Primigravida

- POG between 14 to 24 weeks

Exclusion Criteria

- Multiple gestations
- Hydatidiform mole
- Hydrops fetalis
- Family history of preeclampsia
- Obesity (BMI > 35 kg/m2 or more)
- Smoker
- Preexisting medical disorders (Hypertension, Diabetes Mellitus, Renal Disease, Vascular Disease, Autoimmune Disease, Thrombophilias)

Methodology

After taking written and informed consent and fulfilling the inclusion criteria, patients will include into the study. Detailed antenatal history was elicited from the patient, then they were clinically examined and subjected to ultrasonography.

Specimen Collection & Preparation

Patients venous blood was withdrawn in strict aseptic condition, and maternal serum determination of hCG and AFP levels were calculated. All these patients were followed till delivery.

Principle of β-hCG estimation:

The VITROS Total β-hCG II test is performed using the VITROS Total β-hCG II Reagent Pack and the VITROS Total β hCG II Calibrators on the VITROS ECi/ECIQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System using Intellicheck® Technology (Fig. 1 and Table 1).

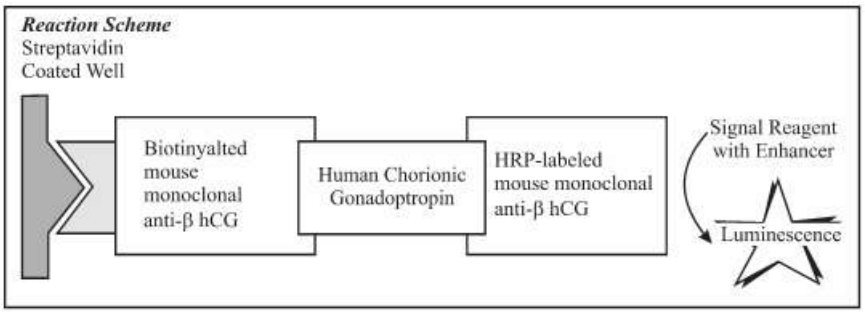

Fig. 1:

Table 1: Reference range

Sample type	Number of samples	Units = mIU/mL (IU/L)				
		Mean	Min	Max	2.5th Percentile	97.5th Percentile
Pregnant gestational age 1-10 weeks	112	31,142	44.71	256,740	63.7	150,854
Pregnant gestational age 11-15 weeks	43	55,425	11556	265,380	11795	151,996
Pregnant gestational age 16-22 weeks	50	27,023	7480.8	111,954	9383.8	61,410
Pregnant gestational age 23-40 weeks	45	24,031	1531.1	101,566	1737.2	98,576

Principle of AFP estimation

The Vitros AFP assay is performed using the Vitros AFP Reagent Pack and Vitros Immunodiagnostic products AFP Calibrators on the Vitros ECi Immunodiagnostic System with Intellicheck™ (Fig. 2 and Table 2).

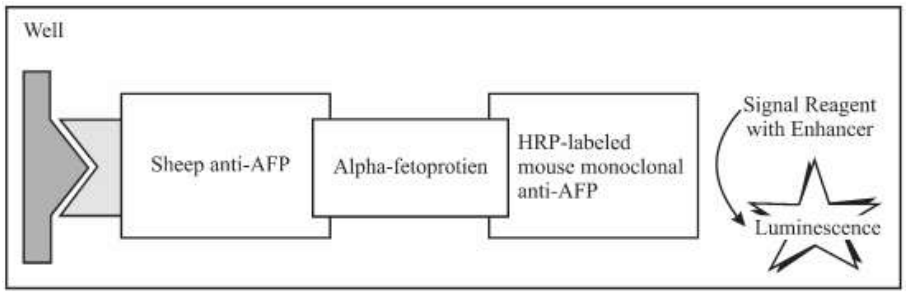

Fig. 2:

Table 2: Reference range

Weeks of Gestation	AFP Medians (ng/mL)
14	27.20
15	32.01
16	37.67
17	44.33
18	52.16
19	61.38
20	72.33
21	85.00
22	100.02

Statistical Analysis

The patients were divided into two groups according to outcome; normotensive group and pre-eclampsia group. These data were entered in Microsoft Office Excel shits to prepare master chart.

The analysis between two groups and continuous variables were summarized as mean & standard deviation and were analyzed by using unpaired 't' test & one way ANOVA as per indication.

- Nominal/Categorical variables were summarized as proportions (%) and were analyzed by using chi square test.
- '*p*' value < 0.05 was taken as significant.
- SPSS IBM Version 21.0 software was used for all statistical calculations.

Results

- In present study, mean age of participants who developed preeclampsia was 25.85 ± 2.34 years while participants who remained unaffected was 26.90 ± 3.01 years. ($p > 0.05$)
- In this study, maximum number of participants from both groups were from lower middle class according to Modified Kuppuswami scale. 46.15% of participants who developed preeclampsia & 61.22% of participants who remained unaffected during study.
- In this study, 53.85% of participants who developed preeclampsia were delivered by LSCS as compared to 14.29% in unaffected participants. ($p < 0.05$)
- In present study indications of LSCS in participants were NPOL (33.33%), Failure of induction (19.05%), Breech presentation (14.29%), Fetal distress (14.29%), Severe oligohydramnios (9.52%), IUGR (4.76%), Abnormal Doppler study (4.76%).
- The mean birth weight of neonates of participants who developed pre-eclampsia was 2.00 ± 0.30 kg and in neonates of participants who remained unaffected was 2.89 ± 0.42 kg. ($p < 0.05$)
- Mean gestational age at delivery of participants who developed pre-eclampsia was 35.24 ± 2.19 weeks while of participants who remained unaffected it was 38.15 ± 1.06 weeks. ($p < 0.05$)
- The mean AFP in participants who developed severe preeclampsia was 58.84 ± 7.01 ng/ml compared to 42.85 ± 15.27 ng/ml who

developed mild preeclampsia compared to 32.42 ± 8.17 ng/ml who remained unaffected during study. (p<0.05)

- The mean β HCG in participants who developed severe preeclampsia was 58510.00 ± 9065.68 mIU/ml compared to 42398.13 ± 13615.98 mIU/ml who developed mild preeclampsia compared to 29154.24 ± 12330.26 mIU /ml who remained unaffected during study. (p<0.05)
- Elevated levels of maternal serum AFP & β hCG measured between 14–24 weeks of gestation have significant positive correlation with pre-eclampsia & adverse obstetric outcome.

Table 3: Distribution of study participants according to preeclampsia & age

Age (Years)	Control		Pre-eclampsia		Total	
	No.	%	No.	%	No.	%
20-25	34	34.69	8	61.54	42	37.84
26-30	54	55.10	5	38.46	59	53.15
>30	10	10.20	0	0.00	10	9.01
Total	**98**	**100.00**	**13**	**100.00**	**111**	**100.00**

Chi-square = 4.110 with 2 degrees of freedom; p=0.128

Table 4: Distribution of study participants according to preeclampsia & socio-economic status

Socioeconomic status	Control		Pre-eclampsia		Total	
	No.	%	No.	%	No.	%
Lower	17	17.35	3	23.08	20	18.02
Lower middle	60	61.22	6	46.15	66	59.46
Upper lower	7	7.14	2	15.38	9	8.11
Upper middle	14	14.29	2	15.38	16	14.41
Total	**98**	**100.00**	**13**	**100.00**	**111**	**100.00**

Chi-square = 1.619 with 3 degrees of freedom; p=0.897

Table 5: Distribution of study participants according to preeclampsia & mode of delivery

Mode of Delivery	Control		Preeclampsia		Total	
	No.	%	No.	%	No.	%
LSCS	14	14.29	7	53.85	21	18.92
Vaginal Delivery	84	85.71	6	46.15	90	81.08
Total	**98**	**100.00**	**13**	**100.00**	**111**	**100.00**

Chi-square = 9.273 with 1 degree of freedom; p=0.002

Table 6: Distribution of study participants according to preeclampsia indications of LSCS

Indication for LSCS	Control		Preeclampsia		Total	
	No.	%	No.	%	No.	%
NPOL	7	50.00	0	0.00	7	33.33
Failure of induction	0	0.00	4	57.14	4	19.05
Breech	3	21.43	0	0.00	3	14.29
Fetal Distress	3	21.43	0	0.00	3	14.29
Severe Oligohydramnios	1	7.14	1	14.29	2	9.52
IUGR	0	0.00	1	14.29	1	4.76
Abnormal Doppler study	0	0.00	1	14.29	1	4.76
Total	**14**	**100.00**	**7**	**100.00**	**21**	**100.00**

Chi-square = 18.750 with 6 degrees of freedom

Table 7: Comparison between groups according to mean Birth Weight (kg)

Groups	N	Mean Birth Weight	Std. Deviation	'p' Value*
Control	98	2.89	0.42	<0.001
Preeclampsia	13	2.00	0.30	

*Unpaired 't' test

Table 8: Comparison between groups according to Gestational Age

Groups	N	Mean Gestational Age (weeks)	Std. Deviation	'p' Value*
Control	98	38.15	1.06	0.002
Preeclampsia	13	35.24	2.19	

*Unpaired 't'test

Table 9: Comparison between groups according to AFP (ng/mL)

Groups	N	Mean AFP	Std. Deviation	'p' Value*
Control	98	32.42	8.17	0.002
Preeclampsia	13	49.00	14.76	

*Unpaired 't' test

Table 10: Comparison between groups according to β HCG (mIU/ml)

Groups	N	Mean β HCG	Std. Deviation	'p' Value*
Control	98	29154.24	12330.26	<0.001
Preeclampsia	13	48595.00	14216.35	

*Unpaired 't' test

Table 11: Comparison between groups according to AFP (ng/mL) & Severity of Preeclampsia

Groups	N	Mean AFP	Std. Deviation	'p' Value*
Control	98	32.42	8.17	
Mild Preeclampsia	8	42.85	15.27	<0.001
Severe Preeclampsia	5	58.84	7.01	

*ANOVA

Table 12: Comparison between groups according to mean β hCG (mIU/ml) & Severity of Preeclampsia

Groups	n	Mean β hCG	Std. Deviation	'p' Value*
Control	98	29154.24	12330.26	
Mild Preeclampsia	8	42398.13	13615.98	<0.001
Severe Preeclampsia	5	58510.00	9065.68	

*ANOVA

Discussion

Pathogenesis of preeclampsia is not exactly known yet. However, one of the most important factors is vasospasm, which causes vascular damage and local hypoxia. Due to unknown pathogenesis of preeclampsia, there is no specific screening test to predict preeclampsia. Preeclampsia is not a totally preventable disease but early diagnosis and treatment of this condition can reduce the intensity of adverse progression of the disease.[2]

Total of 120 subjects were recruited in present study, 9/120 (7.5%) subjects lost to follow-ups. Finally 111 subjects remained in the study for analysis. Following are some comparison between present study vs various studies parameters.

In this study, the normotensive group & the preeclampsia group were identical with respect to their socio economic status, residency & literacy. These demographic observations are similar with observations of Begum Z *et al.*[9] In present study, the caesarean rate in preeclampsia group was 53.85% and in normotensive group it was 14.29%. In study of Ozturk H *et al.*,[6] they found caesarean rate of 45.33% in preeclampsia group and 36% in normotensive group. In study of Dubey P *et al.*,[7] they found caesarean rate of 33.3% in preeclampsia group and 13.1% in normotensive group.

Table 13: Mean age of participants in both the group in various studies.

Different study groups	Normotensive group (years)	Preeclampsia group (years)	*p*-value
Present study	26.90 ± 3.01	25.85 ± 2.34	0.228
Ozturk H *et al*.[6]	26.2 ± 4.5	25.1 ± 4.5	0.275
Basirat Z *et al*.[11]	25.2 ± 5.0	24.88 ± 0.6	0.795
Begum Z *et al*.[9]	24.3 ± 5.2	23.3 ± 6.7	0.342
Ozdamar O *et al*.[10]	29.30 ± 5.72	28.12 ± 4.32	0.238

Majority of subjects were of 26–30 years (53.15%). The mean age of participants who developed preeclampsia was 25.85 ± 2.34 years while participants who remained unaffected was 26.90 ± 3.01 years. There was no statistical significant difference found between mean age of normal & preeclamptic group ($p > 0.05$).

Table 14: Gestational age at the time of delivery

Different study groups	Normotensive group	Preeclampsia group	*p*-value
Present study	38.15 ± 1.06 weeks	35.24 ± 2.19 weeks	0.002
Tosun M *et al*.[5]	38.02 ± 1.17 weeks	36.47 ± 2.77 weeks	<0.01
Dubey P *et al*.[7]	40.18 ± 1.29 weeks	Mild- 38.09 ± 1.87 weeks Severe- 33.78 ± 3.27 weeks	<0.001
Ozdamar O *et al*.[10]	39.02 ± 0.865	34.9 ± 3.61 weeks	<0.001
Tavor O *et al*. [12]	39.7 ± 2 weeks	38.85 ± 2.54 weeks	-

In study of Tavor O *et al*.,[12] they did not have statistically significant difference in gestational age at the time of delivery between both groups, It may be due to the confounding effect of other factors.

Table 15: Baby birth weight in different study

Different study groups	Normotensive group (kg)	Preeclampsia group (kg)		*p*-value
		Mild	Severe	
Present study	2.89 ± 0.42	2.08 ± 0.29	1.89 ± 0.31	<0.001
Tosun M *et al*.[5]	3.51 ± 0.46	3.42 ± 0.50	2.21 ± 0.68	<0.01
Dubey P *et al*.[7]	3.40 ± 0.46	3.19 ± 0.67	1.75 ± 0.79	<0.001
Tavor O *et al*.[12]	3.25 ± 0.54	3.04 ± 0.69		0.048
Ozdamar O *et al*.[10]	3.38 ± 0.35	2.38 ± 0.10		<0.001

In present study mean birth weight in normotensive group (2.89 ± 0.42 kg) was significantly higher than preeclampsia group (2.00 ± 0.3 kg). In the above studies there was statistically significant difference in birth weight between normotensive and pre-eclampsia groups ($p < 0.05$), this results are comparable with present study.

Table 16: Mean beta HCG & AFP level in various studies

Different study groups	β hCG		*p*-value	AFP		*p*-value
	Normotensive Group	Preeclampsia group		Normotensive group	Preeclampsia group	
Present study	29154.24 ± 12330.26 IU/L	48595 ± 14216.35 IU/L	<0.001	32.42 ± 8.17 ng/mL	49 ± 14.76 ng/mL	0.002
Öztürk H *et al*.[6]	0.957 ± 0.364 MoM	2.965 ± 1.006 MoM	0.001	0.942 ± 0.292 MoM	2.717 ± 0.951 MoM	0.001
Tosun M *et al*.[5]	0.78 ± 0.33 MoM	3.38 ± 1.80 MoM	<0.01	1.00 ± 0.42 MoM	1.09 ± 0.42 MoM	<0.01

Different study groups	β hCG		*p*-value	AFP		*p*-value
	Normotensive Group	Preeclampsia group		Normotensive group	Preeclampsia group	
Yadav V *et al*.[8]	8091.44 ± 1493.68 IU/L	19791.70 ± 987.02 IU/L	<0.001	52.50 ± 15.52 ng/mL	151.04 ± 7.2 ng/ mL	<0.001
Dubey P *et al*.[7]	25,365 ± 11,193 IU/L	71,222.22 ± 14,847 IU/L	<0.0001	36.33 ± 13.05 ng/mL	136.76 ± 17.14 ng/mL	<0.0001
Basirat Z *et al*.[11]	27460 ± 25862 IU/L	39840 ± 24630 IU/L	0.031			
Begum Z *et al*.[9]	4937.0 ± 526.1 IU/L	45439.6 ± 5003.6 IU/L	<0.001			

In present study, mean β hCG & AFP levels were higher in preeclampsia group than in control group. Difference between the groups was statistically significant in this study and above mentioned study.

Conclusion

In the present study we have observed that elevated levels of maternal serum AFP & β hCG measured between 14–24 weeks of gestation have significant positive correlation with preeclampsia.

Our finding suggests that second trimester markers may be used as predictor of preeclampsia and it may be useful in distinguishing women with preeclampsia from normal pregnancy. β hCG strongly correlates with adverse obstetric outcome as observed in this study.

Though there are no specific guidelines for the best screening test or at which gestational age to screen pregnant females for prediction of pre-eclampsia, the present study suggests that pregnant females with high level of β hCG & AFP, should be followed at frequent intervals and early intervention with counselling and, if needed, medications to alter the risk profile to prevent later development of the pre-eclampsia.

Limitations of the study

- There is variation in serum level of markers according to gestational age that may interfere in prediction of accurate risk.
- In present study the sample size was relatively small.
- Evaluation of multiple markers with doppler studies would have given better correlation with preeclampsia. So large scale multicentric randomized controlled trial will be needed to confirm the hypothesis.

Recommendations

1. Prediction of preeclampsia act as a primary prevention (before the development of disease). So, If there is any test to predict the preeclampsia with high sensitivity & specificity will help full to classify the patient as high risk for development of preeclampsia in future.
2. High level of β-hCG & AFP have a significant correlation with preeclampsia & adverse obstetric outcome.
3. Ideally prediction test should be combined to increase the detection rate & decrease the false positive rate for preeclampsia. Other test to combined are uterine artery Doppler, urinary calcium, fibronectin level, micro-albuminuria, PAPP-A level etc.
4. Till the date there is no single specific test available to predict the pre-eclampsia, there is high false positive & false negative rate, so there is need of further research to discover the specific test to predict preeclampsia.
5. At present scenario, along with various test for prediction of pre-eclampsia, there should be continue risk assessment during pregnancy at frequent interval to diagnose the high risk factors for preeclampsia.

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