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Maternal Serum Alpha-Fetoprotein Screening In High-Risk Patients And Pregnancy Outcome.

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ABSTRACT

Alpha-fetoprotein (AFP) is the major serum protein in the embryonic stage and the early fetal stage. Maternal serum alpha-fetoprotein (AFP) levels during the first and or second trimester of pregnancy are altered in pregnancies with aneuploidy, neural tube defects, and adverse pregnancy outcomes, including fetal death, pre-eclampsia (PE), fetal growth restriction, and preterm birth this study aimed to measure maternal serum AFP levels in second trimester between 15-20 weeks of gestation and to determine whether unexplained elevated MSAFP levels is an effective predictor of adverse pregnancy outcome among Indian population. This study was a prospective observational study, carried out on 75 pregnant women. Maternal serum alpha-fetoprotein (MSAFP) was measured between 15 and 20 weeks of gestation after excluding congenital malformation or birth defects. MSAFP level was determined by using the radio-immunoassay technique. Women with MSAFP levels>2.0 MoM were considered abnormal while MSAFP levels ≤ 2.0 MoM were considered normal. All women were followed up till delivery and pregnancy outcomes were noted and compared between the two groups. Women with elevated MSAFP had significantly higher adverse pregnancy outcomes (75.4%) compared to women with MSAFP ≤2.0 MoM (26.1%) ($p<0.0001$ with the relative risk of 2.89,95% confidence interval 2.276 -3.667). Unexplained elevated MSAFP has high sensitivity, specificity, positive predictive value, and negative predictive value in predicting adverse pregnancy outcomes. It would, therefore be worthwhile screening pregnant women in the second trimester for maternal serum alpha-fetoprotein levels as it would help to identify high-risk pregnancies and allow close antenatal surveillance for better pregnancy outcomes.

Keywords: Maternal serum alpha-fetoprotein, Maternal and fetal outcome, Pregnancy

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INTRODUCTION

Congenital abnormalities have a major impact on neonatal morbidity/mortality as well as a heavy emotional burden on the family. Identifying them prenatally is an essential task of the obstetrician, who is involved in the care of the pregnant women [1]. Prenatal diagnosis is the art and science of identifying structural and functional abnormalities which includes screening methods and definitive diagnostic procedures. Screening identifies individuals whose risk is high enough that they could benefit from further evaluation [2]. Screening methods include assessment of serum markers like AFP, hCG, UE_3, inhibin A, PAPP-A, and USG assessment of congenital anomalies [3]. Definitive diagnostic procedures include amniocentesis, CVS, fetal blood sampling, and Preimplantation genetic diagnosis which allows analysis ofembryonal and fetal cells or tissues for chromosomal, genetic, andbiochemical abnormalities. Maternal serum alpha-fetoprotein is a simple and cost-effective screening method. [4]. Though, initially discovered to identify neural tube defects three decades ago, studies have documented that the values of MSAFP estimation extend well beyond the detection of NTD in the fetus [5]. Abnormally elevated and low levels of MSAFP are an indication of high-risk pregnancy and sub- optimal outcome of the pregnancy.[6] Before screening, the patients should receive counseling which includes the purpose of the tests, the risks involved, the limitations of the screening tests, and the patient's options.[7]

MATERIALS AND METHODS

In this study, we enrolled a total of 75 women in the year 2022-2023 at, the Department of Obstetrics & Gynecology, Government Kilpauk Medical College, Chennai, Tamil Nadu, India. The study group comprised 75 cases of pregnant patients at GA 15 to 22 weeks who attended our antenatal OPD with any one of the following high-risk factors

- Age above 35 years
- Previous H/O early pregnancy loss
- Previous H/O congenital anomalies
- Previous H/O neural tube defects
- Previous H/O baby with Down Syndrome
- Family H/O congenital anomalies/ chromosomal disorder
- Known epileptic patient on treatment
- Anemia complicating pregnancy
- Fetuses exposed to any teratogen.

Most of the patients had regular menstrual cycles, were not on any oral contraceptives and they knew their LMP correctly. For patients with irregular cycles/ unreliable dates, gestational age was determined by a dating scan. A detailed workup of each patient was carried out according to a well-designed proforma. A detailed history was taken and a thorough physical examination was performed. Routine investigations included Hb, urine analysis, Blood Grouping/ Typing, and VDRL. For the subjects in the study group, blood 3cc was collected by the venipuncture in a sterile test tube and sent to the laboratory where MSAFP measurement was done. The blood was allowed to clot and the serum was separated by centrifugation at room temperature and stored in -20ºC deep freezer.

RESULTS

The study group consisted of 75 patients at GA 15 to 22 weeks who had any of the risk factors cited above. MSAFP screening was done for these patients and the value of MSAFP was converted from ng/ml to MOM by dividing the patient's value with the mean value for the particular GA. Values above 2.5 MOM were considered elevated and < 0.5 MOM were considered low. All patients were followed till delivery and the pregnancy outcome was noted The relation between abnormal MSAFP value and adverse pregnancy outcome was correlated.

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Table 1: Distribution of Age Group

Age	Number of patients	Percentage
16-20	4	5.33%
20-25	45	60%
26-30	16	21.33%
31-35	8	10.66%
> 35	2	2.66%
Observation	60 % of the patients lie in the 20-25 age group	

Table 2: Distribution of Gravidity

Gravidity	Number	Percentage
Primi	14	18.66%
G2	28	37.33%
G3	18	24%
G4	9	12%
G5	6	8%
Observation	Most of the patients are second gravida	

Table 3: Distribution of Patients according to Gestational Age (GA)

GA	Number	Percentage
15	9	12%
16	12	16%
17	9	12%
18	7	9.33%
19	6	8%
20	11	14.66%
21	3	4%
22	18	24%
Observation	Most of the patients screened were at 22 weeks, thoughthe screening was done between 15 to 22 weeks	

Table 4: Gravidity Distribution According to Age

Gravidity	Age				
	16-20	21-25	26-30	31-35	>35
1	9	3	1	2	0
2	2	14	5	1	1
3	1	12	5	4	0
4	0	7	1	0	1
>=5	0	1	3	2	0

Table 5: Laboratory Standard Value of MSAFP for Each Week ofGestation in MOM

GA (eeks)	Median value of MSAFP	Multiple of Median	
		2.5	0.5
15	11	27.5	5.5
16	14	35	7
17	20	50	10
18	27	67.5	13.5
19	35	87.5	17.5
20	42	105	21
21	50	125	25
22	60	150	30
	This table shows the median according to gestational age. This standardized mean was followed in the study.		

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Table 6: No of cases showing elevated MSAFP level according togestational age
(Total Number of Cases – 75)

GA	Total Number of Patients	Number of Patients with Elevated MSAFP	Percentage
15	9	5	55.55%
16	12	4	33.33%
17	9	2	22.22%
18	7	4	57.14%
19	6	3	50%
20	11	5	45.45%
21	3	0	0
22	18	5	27.77%
Observation	Out of the study group, 37.33% showed elevated MSAFP value. 57.14% in the 18 weeks gestation showed elevated MSAFP levels.		

Table 7: Patients according to high-risk factor

High Risk Factors	Number	Percentage
BOH	26	34.66%
Hypertension / BOH	5	6.66%
Known epileptic / BOH	2	2.66%
BOH/ Anemia	1	1.33%
Hypertension complicationpregnancy	3	4%
Positive family history ofHypertension	2	2.66%
Anemia / Hypertension	2	2.66%
Anemia	6	8%
Anemia / previous history ofanomalous baby	1	1.33%
Anemia / Positive family H/O	1	1.33%
Known epileptic on treatment	3	4%
Known epileptic on treatment / positive familyhistory	1	1.33%
Fever	3	4%
Previous history of anomalousbaby	6	8%
Drug intake	5	6.66%
Positive family history	6	8%
Elderly Gravida	2	2.66%
Observation	The maximum number of cases screened waswith history of BOH- 34.66%	

Table 8: Causes of Elevated MSAFP

Causes	Number of Cases	Percentage
Anomalies	3	10.71%
Early pregnancyloss	2	7.14%
IUD	2	7.14%
Preterm	7	25%
IUGR	1	3.57%
LBW	5	17.85%
Neonatal complications	3	10.71%
Normal	5	17.85%

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Table 9: Type Of Congenital Anomalies In Elevated Msafp

Total no of congenital anomalies	- 3
CNS anomalies	- 2
Other anomalies	- 1

Sl. No	Anomaly	Number
1	Anencephaly	1
2	Spina Bifida	1
3	Exomphalos	1

Table 10: Association of Elevated MSAFP Level and CongenitalAnomalies

Diagnosis	MSAFP		
	Normal	Elevated (≥ 2.5 MOM)	Low (≤ 0.5 MOM)
CongenitalAnomalies	Nil	3	Nil
Observation	1. MSAFP levels are elevated in all the 3 cases ofpregnancies with anomalous babies.		

- Among the study group, 3 (4%) had congenital anomalies.
- Among the congenital anomalies, 100% had elevated MSAFP
- 10.71% of patients with elevated MSAFP had congenital anomalies.

Table 11: Association of MSAFP Level and Preterm deliveries

Diagnosis	Number of cases	MSAFP		
		Normal	Elevated (2.5 MOM)	Low (0.5 MOM)
Preterm deliveries	8	1	7	Nil
Observation	1. Among the study group, 8(10.66%) went into preterm labor. 2. Out of 8 cases 7 had elevated MSAFP (87.5%) 3. 1 patient had normal MSAFP (12.5%) 4. 25% of patients with elevated MSAFP levels had pretermdeliveries.			

Table 12: Association of MSAFP level and IUGR

Diagnosis	Number of cases	MSAFP		
		Normal	Elevated (2.5 MOM)	Low (0.5 MOM)
IMAGE	1	Nil	1	Nil
Observation	1. Among the study group, 1(1.33%) had IUGR. 2. Hence 100% of patients with IUGR had elevated MSAFP. 3. 3.75% of patients with elevated MSAFP had IUGR.			

Table 13: Association of MSAFP Level and LBW

Diagnosis	Number of cases	MSAFP		
		Normal	Elevated (2.5 MOM)	Low (0.5 MOM)
LBW excluding preterm	5	2	3	Nil
Observation	1. Among the study group, 5(6.66%) had LBW. 2. Among the LBW 60% had elevated MSAFP. 3. 40% had normal MSAFP. 4. 10.71% of patients with elevated MSAFP had LBW.			

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Table 14: Association of Early Pregnancy Loss and MSAFP

Diagnosis	Number of cases	MSAFP		
		Normal	Elevated (2.5 MOM)	Low (0.5 MOM)
Incompleteabortion	2	1	1	Nil
Completeabortion	1	Nil	1	Nil
Observation	1. Among the study group, 3 (4%) had early pregnancy loss 2. 66.66% had elevated MSAFP 3. 33.33% had normal MSAFP 4. 7.14% of patients with elevated MSAFP had earlypregnancy loss			

Table15: Fetal outcome in the study group (75 Patients)

Diagnosis	Number of cases		MSAFP Level					
			Normal		Elevated		Low	
	No	Percentage	No	%	No	%	No	%
Still Birth	2	2.66%	-	-	-	-	-	-
Fresh	1	1.33%	-	-	1	100	-	-
Macerated	1	1.33%	-	-	1	100	-	-
Neonatal death	1	1.33 %	1	100	-	-	-	-
Anomalies	3	4%	-	-	3	100	-	-
CNS	2	2.66%	-	-	2	100	-	-
Omphalocele	1	1.33%	-	-	1	100	-	-
Neonatal complications	3	4%	-	-	3	100	-	-

This study shows 2.66% of stillbirths in the study group. All of them showed elevated MSAFP levels. One of the stillbirths is a macerated IUD, delivered at 7 months by amother who had previous four abortions. The other stillbirth is a fresh IUD delivered at 6 months, by a motherwho was a case of severe PIH. One neonatal death occurred but the mother had normal MSAFP value. The baby died of respiratory distress 2 days after delivery. An autopsy was not done as the parents were not willing to subject the baby to autopsy.4% of the study group had anomalous babies out of which 66.66% had CNS anomalies (anencephaly and Spina Bifida) and 33.33% had ventral wall defect (exomphalos). Among the study group, 4% had neonatal complications and all had elevated MSAFP

Table 16: Pregnancy outcome in the study group of 75 patients

Outcome	Number	Percentage
Early Pregnancy Loss	3	4%
Congenital Anomalies	3	4%
IUD	2	2.66%
Preterm	8	10.66%
IMAGE	1	1.33%
LBW	6	8%
Neonatal Complications	3	4%
Neonatal Death	1	1.33%
Normal	48	64%

Table17: Adverse pregnancy outcome in patients with normalMSAFP includes

LBW	1	2.17%
Preterm	1	2.17%
Incomplete abortion	1	2.17%
Neonatal death	1	2.17%

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Table 18: Relation of MSAFP Value and Pregnancy outcome

MSAFP	Adverse PregnancyOutcome	Normal PregnancyOutcome
Abnormal MSAFP	23	6
Normal MSAFP	4	42

29 patients had abnormal MSAFP values out of which 28 patients had elevated MSAFP with an adverse pregnancy outcome in 23 cases and 1 patient had a low MSAFP but she had a normal pregnancy outcome.46 patients had normal MSAFP with an adverse pregnancy outcome in 4 cases. Sensitivity= 85.18%,Specificity = 87.5%,Positive predictive value =79.3% Negative predictive value = 91.3%.

DISCUSSION

Maternal serum alpha-fetoprotein (AFP) levels during the first and or second trimester of pregnancy are altered in pregnancies with aneuploidy, neural tube defects, and adverse pregnancy outcomes, including fetal death, pre-eclampsia (PE), fetal growth restriction, and preterm birth [8]. We have proposed that the best approach to screening for PE is to use Bayes' theorem to combine the *a-priori* risk from maternal characteristics and medical history with the measurement of biomarkers. Our approach assumes that, if the pregnancy was to continue indefinitely, all women would develop PE and whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE [9]. The effect of maternal factors and biomarkers is to modify the mean of the distribution of gestational age at delivery with PE so that, in pregnancies at low risk of PE, the gestational age distribution is shifted to the right with the implication that in most pregnancies delivery will occur before development of PE [10]. In high-risk pregnancies, the distribution is shifted to the left, and the smaller the mean gestational age the higher the risk of PE. Maternal serum alpha-fetoprotein (AFP) levels during the first and or second trimester of pregnancy are altered in pregnancies with aneuploidy, neural tube defects, and adverse pregnancy outcomes, including fetal death, pre-eclampsia (PE), fetal growth restriction, and preterm birth [11]. We have proposed that the best approach to screening for PE is to use Bayes' theorem to combine the *a-priori* risk from maternal characteristics and medical history with the measurement of biomarkers [12]. Our approach assumes that, if the pregnancy was to continue indefinitely, all women would develop PE and whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE [13]. The effect of maternal factors and biomarkers is to modify the mean of the distribution of gestational age at delivery with PE so that, in pregnancies at low risk of PE, the gestational age distribution is shifted to the right with the implication that in most pregnancies' delivery will occur before development of PE [14]. In high-risk pregnancies, the distribution is shifted to the left, and the smaller the mean gestational age the higher the risk of PE. The rate of preterm delivery in pregnancies with MSAFP level > 2 MOM was 18% compared to 7% when MSAFP level is ≤ 2.0 MOM (p = 0.005) with an odds ratio of 2.9(95% CI-1.3 to 6.4) [15]. The rate of preterm birth was significant. higher in women with high MSAFP (20% vs. 5.23%) with a relative risk of 3.822 (95% CI-1.467 to 9.959 [16]. In our study, sensitivity, specificity, PPV, and NPV of the test was 64.17%, 66.87%,16.92%, and 94.78% respectively. In the present study, pre-eclampsia occurred in 20% of women in the group with elevated MSAFP (>2 MoM) compared to 6% of women in the group with MSAFP level ≤2 MoM [17]. the strong association between second-trimester elevated MSAFP levels and adverse pregnancy outcomes (preterm birth, preeclampsia, oligohydramnios, IUGR, placental-abruption, PPROM, IUFD, stillbirth, neonatal death) was found in our study (p-value <0.0001) [18-20].

CONCLUSION

Women with unexplained elevated Maternal Serum Alpha-Fetoprotein levels>2.0 MoM measured between 15 to 20 weeks of gestation do have an increased risk of adverse pregnancy outcome (both maternal and fetal) compared to women with MSAFP level ≤2.0 MoM. The results are not only statistically, but also clinically significant and agree with most reports published so far. It would therefore be worthwhile screening pregnant women in the second trimester for maternal serum alpha-fetoprotein levels as it would help to identify high-risk pregnancies and allow close antenatal surveillance for a better pregnancy outcome. The present study shows unexplained elevated MSAFP level has high sensitivity, specificity, positive predictive value, and negative predictive value in predicting adverse pregnancy outcomes. Its measurement is easily accessible and safe. Further, it is found to be one of the

cost-effective and non-invasive screening methods. But till now no definitive follow-up and treatment plans have been practiced for high-risk women. So, we recommend educating women about the signs and symptoms of complications and besides biochemical screening undergo more frequent antenatal checkups and testing by other modalities.

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