Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2015; 3(7B):2586-2590 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2015.v03i07.030

Research Article

Study on Elevated Maternal Serum Alpha-Fetoprotein in Second Trimester and Pregnancy Outcome

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Abstract: This study was a prospective observational study sponsored by DBT (Department of Biotechnology), New Delhi. It was carried out in Gauhati Medical College and Hospital during the period 2014-15 with a total of 202 women whose maternal serum alpha-fetoprotein (MSAFP) was measured between 16 and 20 weeks of gestation. The objective of the study was to determine whether elevated MSAFP level between 16 and 20 weeks of gestation is associated with increased risk of adverse pregnancy outcome including preterm birth, preeclampsia/ eclampsia, SGA, Stillbirth/Neonatal death. MSAFP level was determined by using an immunometric immunoassay technique. Women with MSAFP level ≥ 2.5MoM were defined as elevated MSAFP. Results showed women with elevated MSAFP were significantly more likely to have subsequent adverse pregnancy outcome (70%) compared to women with MSAFP < 2.5 MoM (18.6%) ($p<0.0001$) with a relative risk of 3.76 (95% confidence interval 2.5 -5.5).It was thus concluded from the study that there is a strong association between elevated MSAFP in the second trimester and a subsequent adverse pregnancy outcome including preterm birth, preeclampsia/eclampsia, SGA (Small for gestational age) and Stillbirth/ neonatal death.
Keywords: maternal serum alpha-fetoprotein (MSAFP), preterm birth, preeclampsia, Stillbirth

INTRODUCTION

Alpha fetoprotein (AFP) is a glycoprotein which is synthesized early in gestation by the fetal yolk sac and Later by the fetal gastrointestinal tract and liver. It is the major serum protein in the embryo –fetus and is analogous to albumin. Its concentration increases steadily in both fetal serum and amniotic fluid until 13 weeks, after which, levels rapidly decrease. Conversely, AFP is found in steadily increasing quantities in maternal serum after 12 weeks and reaches a peak between 28 and 32 weeks. The abnormal concentration gradient between fetal plasma and maternal serum is on the order of 50,000:1[1, 2]. Fetal body wall defects uncovered by integument, such as NTDs and ventral wall defects, permit AFP to leak into the amniotic fluid, resulting in maternal serum AFP levels that may be dramatically increased. Thus, it has been used as a marker of open neural tube defects [3, 4]. In addition to NTDs, many other types of birth defects and placental abnormalities are associated with AFP elevation. When no fetal or placental abnormality is detected after a specialized sonographic evaluation, with or without amniocentesis, the AFP elevation is considered unexplained. These pregnancies are at increased risk for a variety of subsequent adverse pregnancy outcomes. Some include a fetal anomaly not detectable prenatally, fetal-growth restriction, oligohydramnios, placental abruption, preterm membrane rupture, preterm birth, and even fetal death [5, 6, 7].

MATERIAL AND METHODS

The type of study was prospective observational study conducted in GMCH over a period of 1 year from 2014-2015. A proforma for the study was prepared and details of each case were recorded for proper evaluation and analysis. Pregnant women attending Antenatal OPD in GMCH who were between 16 weeks to 20weeks were counseled about MSAFP test and were enrolled in the study after obtaining written and informed consent. The study protocol was approved by the ethical committee of the institution and was sponsored by Department of Biotechnology (DBT), New Delhi. Gestational age in pregnant women was determined from her last menstrual period and confirmed by ultrasonography. Fresh blood sample (3-5ml) was collected from the participants between 16 to 20 weeks of gestation and the serum was then separated by centrifugation. Serum AFP levels were then measured. The VITROS AFP test was performed using the VITROS AFP Reagent Pack and the VITROS AFP Calibrators on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600

Immunodiagnostic System and the VITROS 5600 Integrated System using Intelli check Technology. An immunometric immunoassay technique was used. The value of MSAFP was expressed in terms of Multiples of Median (MOM). As the sample in this study, within the limited period of time, was small for the purpose of calculating the median value for unaffected pregnancies according to gestational age, the reference median value for gestation wise MSAFP was taken from a study that was conducted in North west India from 5420 pregnant women by Gurjit Kaur and associates, Genetic Centre, Government Medical College & Hospital, Chandigarh, India [8]. The MOM was calculated for rounded weeks (16th rounded week includes gestation from 15+4 to 16+3).

MSAFP level ≥ 2.5 MoM were considered abnormal. As the association between low MSAFP level and Down syndrome is well established, pregnancies with MSAFP level below 0.5 MoM were excluded from the study.

All the pregnancies were followed up until delivery for maternal and fetal outcome by contacting over the telephone number provided by the participant in the proforma. Data were analyzed using Microsoft Excel 2010 (Microsoft Corporation, USA).

RESULTS

The incidence of adverse pregnancy outcome was more in the group with elevated second trimester MSAFP level (70%) compared to the group with MSAFP < 2.5MoM (18.6%). Relative risk was 3.7625 (95% CI = 2.5458 to 5.560) P< 0.0001 which is significant

The incidence of preterm delivery in the group with elevated MSAFP (≥ 2.5 MOM) was 20% . Whereas the incidence of preterm delivery in the group with MSAFP level < 2.5MOM was 5.23%. P value is 0.0124, considered significant. Relative risk = 3.822 (95% Confidence Interval: 1.467 to 9.959).

The incidence of Pre eclampsia/ Eclampsia in the group with elevated MSAFP Level (≥ 2.5MOM) was 23.33 %. Whereas the incidence of Pre eclampsia / Eclampsia in the group with MSAFP level < 2.5 MOM was 3.49 % . P value is 0.0007, considered extremely significant. Relative risk = 6.689 (95% Confidence Interval: 2.413 to 18.53).

The incidence of SGA in the group with elevated MSAFP level (≥ 2.5 MOM) was 23.3 % . Whereas the incidence of SGA in the group with MSAFP level < 2.5 MOM was 6.7%. The P value is 0.0111, considered significant. Relative risk = 3.344 (95% Confidence Interval: 1.433 to 7.808).

The incidence of stillbirth / neonatal death in the group with elevated MSAFP level (≥ 2.5 MOM) was 20% . Whereas the incidence of it in the group with MSAFP level <2.5 MOM was 1.16 % . The P value is 0.0002, considered extremely significant. Relative risk = 17.200 (95% Confidence Interval: 3.640 to 81.280).

Table-1: Summary of results for each outcome

Outcome	Total n (total)= 202 (%)	<2.5 MOM n=172(%)	≥ 2.5 MOM n=30(%)	P	RR (95% CI)
Preterm Delivery	15(7%)	9(4%)	6(3%)	0.0124	3.82(1.4-9.9)
Pre eclampsia/ Eclampsia	13(6%)	6(3%)	7(3%)	0.0007	6.6(2.4-18.5)
SGA	19(9%)	12(6%)	7(3%)	0.01	3.3(1.4-7.8)
Stillbirth / Neonatal Death	8(4%)	2(1%)	6(3%)	0.0002	17.2(3.6-81.2)
All Complications	53(26%)	32(16%)	21(10.3%)	0.0001	3.76(2.5-5.5)

DISCUSSION

In the present study, it has been found that there is a strong association between second trimester elevated MSAFP and adverse pregnancy outcome which includes preterm birth, preeclampsia/eclampsia, and small for gestational age, stillbirth or neonatal death. The p value < 0.0001 (by Fisher's Exact Test), which is statistically extremely significant. The relative risk was 3.7. Rebecca Allen *et al.;* [9] in a retrospective study also found that the rate of adverse pregnancy outcome (Preeclampsia, preterm delivery, abruption, SGA, Stillbirth/ Neonatal death) in the group with MSAFP level > 2MOM in the second trimester was 52% compared to 12 % in the group with MSAFP ≤ 2.0 MOM. The Odds ratio was 7.9 (95%CI 4.3-14.4) and p value < 0.0001, which was significant.

It was observed in the present study that the incidence of preterm delivery in the group with MSAFP ≥ 2.5 MOM was 20 % as opposed to 5.23 % in the group with < 2.5 MOM. The p value was 0.0124 (by Fisher's exact test) which is considered significant. The relative risk was 3.82 (95% CI 1.46 9.95).Davis RO et al[10] found that preterm delivery rate increased from 8 % at levels less than 0.5 MOM to 18.1% (p less than 0.001) at levels greater than or equal to 2.5 MOM in group 1 (predominantly white) and 28.1 % (p = 0.01) in group 2 (predominantly black). Waller et

al[11]observed in a study that among women with high levels of MSAFP (at least 2.5 MOM), 24.3% had preterm births, compared with 3.8 % of women with low MSAFP levels (≤0.81MOM). Beta *et al.;* [12] performed a case control study measuring AFP in the first trimester which showed significantly higher median AFP MOM (1.33 Vs. 0.97, p = 0.006) in those delivering preterm. Dehgani- Firouzabadi *et al.;* [13] and Smith *et al.;* [13] also confirmed a raised MSAFP level was associated with preterm labour (p = 0.021 and p = 0.001 respectively). Rebecca et al[8][8] reported that 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 a odds ratio of 2.9 (95 % CI 1.3 – 6.4). Puntachai P *et al.;* [15] in a retrospective cohort study found that the rate of preterm birth was significantly higher in women with high MSAFP (11.7 vs. 6.6 %) with a relative risk of 1.76.

It has been found in the present study that the incidence of pre eclampsia /eclampsia in the group with elevated MSAFP (≥ 2.5 MOM) was 23.33% compared to 3.49% in the group with MSAFP level < 2.5 MOM. The p value was 0.0007 (by Fisher's Exact Test) which is considered extremely significant. Walters et al[15] reported that 13% of women with elevated MSAFP level developed preeclampsia compared to 1 % of the women with normal MSAFP. Williams et al[17] compared 201 women with unexplained elevated MSAFP (≥ 2.0 MOM) with 211 women with normal MSAFP. A significant association was found between elevated MSAFP and pre eclampsia, adjusted risk ratio being 3.8. Several other studies have also confirmed these findings. Rebecca *et al.;* [9] found that unexplained elevated MSAFP in the second trimester was strongly associated with a subsequent risk of pre eclampsia. However, Kang *et al.;* [18], Wald *et al.;* [19], Cho *et al.;* [20] and Davidson *et al.;* [21] did not find any significant increase in AFP in those that developed preeclampsia compared to controls.

In the present study, the incidence of Small for gestational age (SGA) in the group with elevated MSAFP (≥ 2.5 MOM) was 23.3 % compared to 6.7% in the group with MSAFP level < 2.5 MOM. The p value was 0.0111 (by Fisher's exact test), which is considered significant. The relative risk was 3.344 (95% CI 1.433 to 7.808). Haddow *et al.;* [22] reported from a study that in singletons, MSAFP level of ≥ 3.0 MOM indicated 7 fold increased risk for birth weight under 2500g. Wald *et al.;* [19][18] showed that mean birth weight of infants born with unexplained MSAFP elevations > 3.0 MOM, was 344g less than the birth weight of infants born to women with MSAFP values between 0.75 and 1.49 MOM. Brock *et al.;* [3] reported that among women who unexplained elevated MSAFP values > 2.3 MOM, 10.7% had delivered infants weighing < 2500g compared with 4.2% of women with normal MSAFP values. Burton *et al.;* [23] found that amongst women who had elevated MSAFP values > 2.5 MOM, 15 % delivered SGA infants compared to only 7.2% among women with normal MSAFP values. Katz *et al.;* [5] observed that there was 2 to 4 fold increase in LBW resulting from preterm delivery and IUGR associated with an elevated MSAFP. Zarzour *et al.;* [24] reported that birth weight <2500g was present less frequently with low or normal MSAFP (7.7%) than in elevated MSAFP (14.6%) (Odds ratio 2.04, p=0.0005). Krause *et al.;* [25] found in a study that compared with women with MSAFP levels at 0.75 – 1.24 MOM , those with MSAFP levels ≥ 2.5 MOM had an increased risk of SGA (RR 2.8, 95% CI 2.4, 3.2) and LBW (RR 5.8; 95% CI 5.0, 6.6). However, studies by Davis RO *et al.;* [10], Smith *et al.;* [14], McPherson *et al.;* [26] showed no significant increase in IUGR with an elevated AFP.

It was observed in the present study that the incidence of stillbirth / neonatal death in the group with elevated MSAFP (≥ 2.5 MOM) was 20% compared to 1.16 % in the group with MSAFP level < 2.5 MOM. The p value was 0.0002, which is considered extremely significant. Relative risk was 17.20 (95% CI 3.6 to 81.2). Waller et al found that women with MSAFP level ≥ 3.0 MOM had a very high risk of fetal death (Odds ratio 10.4; 95% CI 4.9 to 22.0) as compared to women with normal levels of MSAFP. MSAFP level 2.0 to 2.9 MOM were also associated with an elevated risk of fetal death (Odds ratio 2.4; 95% CI 17 to 3.4). Haddow *et al.;* [22] reported that in singletons, MSAFP ≥ 3.0 MOM indicated 30 fold increased risk for fetal death. Robinson et al found that in women with MSAFP values 2.5 – 2.9 MOM, there is a 6% risk of fetal death, in women with MSAFP values 3.0 to 3.9 MOM, there is a 9% risk of fetal death and for values > 4.0 MOM the risk of fetal death is 24%, and there is a relative risk of 4.7 for neonatal death in cases of unexplained, elevated MSAFP. Krause et al[25] also found that compared with MSAFP levels at 0.75 to 1.24 MOM, those with MSAFP levels ≥ 2.5 MOM had an increased risk of infant death (Relative risk of 1.9; 95% CI 1.2, 2.8). Puntachai P *et al.;* [15] in a retrospective cohort study found that the rate of fetal death was significantly higher in women with high MSAFP level (15.8 Vs 6.7%), with relative risk of 2.35. Simpson *et al.;* [6] reported that although unexplained elevated MSAFP is significantly associated with increased risk for fetal / neonatal loss, it does not translate in to an effective screening test.

CONCLUSION

Pregnant women with Maternal Serum Alpha Fetoprotein level ≥ 2.5 MOM measured between 16 weeks to 20 weeks of gestation do have an increased risk of adverse pregnancy outcome (both maternal and fetal) compared to women with MSAFP level < 2.5

MOM. The adverse outcome includes preterm delivery, pre eclampsia, and eclampsia, small for gestational age, stillbirth and neonatal death. The results are not only statistically, but also clinically significant. It would therefore be worthwhile testing pregnant women at 16 to 20 weeks gestation for maternal serum alpha fetoprotein as it would help classify women as high risk pregnancy and allow greater antenatal surveillance for any abnormality to be detected at the earliest so that timely intervention can be taken for a better pregnancy outcome.

Acknowledgement:

The authors extend sincere thanks to the Department of Biotechnology (DBT), New Delhi for providing financial assistance in conducting the study.

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