Original Article

Second-trimester maternal serum alpha-fetoprotein elevation and its association with adverse maternal/fetal outcome: ten years experience

Salvatore Anfuso, Emanuele Soncini, Patrizia Bonelli[1], Giovanni Piantelli, Dandolo Gramellini

Department of Gynecology, Obstetrics and Neonatology, University of Parma, Italy; [1] Department of Laboratory Diagnostics, Parma Hospital, Italy

Abstract. *Background:* Alpha-fetoprotein (AFP) is the major serum protein in the embryonic stage and in the early fetal stage. The aim of this study was to determine any possible association between an "unexplained" elevation of maternal serum alpha-fetoprotein (MSAFP) levels in the second trimester of pregnancy and adverse maternal/fetal outcome. *Methods:* A retrospective cohort study, was carried out in the University of Parma, by reviewing all triple tests that had been found positive for neural tube defect screening, showing an "unexplained" MSAFP elevation (≥2.5 multiples of the median [MoM]), which could not be ascribed to any apparent reason. These results were compared with those of negative controls (MSAFP <2.5 MoM) in order to evaluate the course and outcome of pregnancy. Statistical analysis was performed by chi-square test, Fisher's exact test, Student's t-test, and odds ratio calculation. *Results:* We reviewed 16,747 tests: 143 tests with high MSAFP levels were found, including 105 data already available. Out of them 21 tests were excluded from the study because of the presence of fetal malformations, chromosomal diseases, or late miscarriage. Among the 84 remaining pregnancies, 43 were significantly associated with increased rates of pregnancy pathology compared with the control group of 199 patients, with 25 complicated pregnancies. In addition, high MSAFP levels were correlated with a less favorable neonatal outcome in terms of low birth weight, Apgar score, and transfer to a neonatal intensive care unit. *Conclusions:* Unexplained elevation of MSAFP levels in the second trimester of pregnancy is associated with an adverse maternal/fetal outcome, possibly suggesting the need for a more strict management of pregnancies. (www.actabiomedica.it)

Key words: Alpha-fetoprotein, screening, pregnancy, perinatal, outcome, morbidity

Introduction

AFP is a glycoprotein that is normally produced in early pregnancy by the fetal yolk sac, liver, and gastrointestinal tract. Moreover, a considerable amount of AFP is synthesized by the choroid plexuses and is released into the cerebrospinal fluid. Although its function is still unclear, AFP is the major serum protein in the embryonic stage and in the early fetal stage.

MSAFP assay in the second trimester of pregnancy (15 to 20 weeks of gestation) is part of the triple test for biochemical screening of chromosomal diseases, which also includes measurements of beta-human chorionic gonadotropin (βhCG) and unconjugated estriol (unE_3). Elevated MSAFP levels >2-2.5 MoM also enable the screening of patients at risk for fetal malformations, in particular those with abdominal wall or neural tube closure defects (NTD).

Furthermore, an important finding has emerged in the last few decades indicating a correlation between

second-trimester high MSAFP levels (>2-2.5 MoM) that are "unexplained" – i.e. they cannot be explained by any possible causes of AFP elevation – and poor maternal/fetal outcome. Specifically, the conditions that may occur in the presence of elevated MSAFP levels are, among others: spontaneous abortion, gestational hypertension, preeclampsia (PE), chronic hypertension, preterm delivery (PD), intrauterine growth restriction (IUGR), and preterm premature rupture of membranes (PPROM).

Katz et al. (1) in a review of a large case series of pregnancies with "unexplained" MSAFP elevation, confirmed its association with poor maternal/fetal outcome. The authors reported that the MSAFP elevation is probably a consequence of placental injury.

Simpson (2) confirmed the association between elevated MSAFP levels and poor maternal/fetal outcome, but only when the elevation occurred in the second trimester and not in the third trimester.

The purpose of our study was to demonstrate whether, in our population, "unexplained" high MSAFP levels in the second trimester were correlated or not with a poor maternal/fetal outcome.

Materials and methods

At the University of Parma Department of Obstetrics, Gynecology and Neonatology, we reviewed the results of all triple tests that were performed in Parma Hospital between January 1st 1993 and March 31st 2003. Out of them, we selected all cases that were tested positive for NTD screening, i.e. with MSAFP levels ≥2.5 MoM, and compared them with a group of twice as many negative controls (MSAFP <2.5 MoM) that were randomized according to random number tables. For all pregnant women, we evaluated general characteristics such as age, weight, ethnicity, parity, and gestational age at the time of the triple test, as well as any pathologic conditions at test time, the number and type of congenital fetal defects, and the number of spontaneous abortions. In the group with elevated MSAFP levels, we excluded all pregnancies in which a specific cause for MASF elevation was present, i.e. NTDs, omphalocele, gastroschis, intrauterine fetal death (IUFD), etc. In the control group, we excluded all pregnancies with aborted or malformed fetuses. We then evaluated and compared the course and outcome of pregnancy in the two resulting groups. In particular, we considered the following maternal/fetal conditions observed during pregnancy: PE, according to the American College of Obstetricians and Gynecologist (ACOG) criteria (3); hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome, according to Sibai's criteria (4); PD, defined as the extraction or expulsion of the fetus before 37 completed weeks of gestation; PPROM, defined as the preterm rupture of the amniochorionic membranes of the fetal sac; and, IUGR, according to the ACOG criteria (5). In addition, we also evaluated neonatal outcome based on the presence or absence of low birth weight (LBW) and small-for-gestational-age (SGA) birth, rated according to Northern Italy's neonatal anthropometric standards in use at our center (6); Apgar score between 1 and 3 at 1 and 5 minutes, respectively; and, the need for transfer to a neonatal intensive care unit (NICU). Statistical analysis was performed by chi-square test, Fisher's exact test, Student's t-test, and odds ratio (OR) calculation.

Results

Over the considered period, we reviewed a total of 16,747 triple tests. Out of them, 143 were found positive for NTD screening, with MSAFP levels ≥2.5 MoM. Pregnancy data were available for 105 of the positive patients and for 201 of the 286 negative controls (with MSAFP levels <2.5 MoM).

The epidemiological characteristics of the two study groups did not show statistically significant differences (Table 1).

Out of the 105 patients with MSAFP levels ≥2.5 MoM, 21 were excluded from the study because of the presence of fetal malformations, chromosomal diseases or late miscarriage (between the 14th and the 20th week). In the control group, only 2 women presented second-trimester complications, namely spontaneous abortion at 17 weeks and a complex fetal heart malformation for which the patient agreed to have a therapeutic abortion (Table 2). We therefore evaluated

Table 1. Epidemiological characteristics of the two study groups

No. of women	MSAFP ≥2.5 MoM 105	MSAFP <2.5 MoM 201	P
MSAFP MoM (M ± SD)	3.75 ± 1.88	1.05 ± 0.37	<0.001
Age - yrs (M ± SD)	30 ± 4	29 ± 4	0.06
Weight – kg (M ± SD)	62 ± 11	62 ± 11	0.8
Ethnicity			
• White (%)	93 (89%)	187 (93%)	
• Black (%)	12 (11%)	13 (6%)	0.14
• Yellow (%)	0	1 (0.5%)	
Nulliparous (%)	68 (65%)	124 (62%)	0.61
Gestational age at test time - wks (M ± SD)	16 ± 0.5	16 ± 0.5	0.13

MoM = Multiples of the Median. M ± SD = Mean ± Standard Deviation

Table 2. Second-trimester complications and MSAFP levels

No. of women	MSAFP ≥2.5 MoM 105	MSAFP <2.5 MoM 201	P
Congenital malformations (%)	16 (15.2%)	1 (0.5%)	**<0.0001**
• NTDs (%)	13 (12.4%)	-	
• Omphalocele (%)	2 (1.9%)	-	
• Polymalformation syndrome (%)	1 (1%)	-	
• Heart disease (%)	-	1 (0.5%)	
Chromosomal disease (%)	1* (1%)	-	
Spontaneous abortion (%)	4† (3.8%)	1 (0.5%)	**0.03**

* = 1 case of trisomy 18 → spontaneous abortion
† = Without diagnosis of chromosomal disease or malformation

Table 3. Third-trimester complications and MSAFP levels

No. of women	MSAFP ≥2.5 MoM 84	MSAFP <2.5 MoM 199	OR (CI 5% - 95%)	p
Preeclampsia/HELLP syndrome	10 (11.9%)	10 (5%)	2.6 (1 - 6.4)	0.039
Preterm delivery	18 (21.4%)	9 (4.5%)	5.8 (2.5 - 13.4)	<0.0001
PPROM	6 (7.1%)	1 (0.5%)	15.2 (1.8-128.6)	0.001
IUGR	7 (8.3%)	3 (2%)	5.9 (1.5 - 23.6)	0.004
Placental abruption	6 (7.1%)	2 (1%)	7.6 (1.5 - 38.4)	0.004
IUFD	2 (2.4%)	-	n.c.	0.029

n.c. = not calculable

the further course and the outcome of pregnancy in the remaining 84 positive cases and in the 199 negative controls. We found (Table 3) that in the group of positive patients there were 18 PD versus 9 in the group of negative controls (21.4% vs 4.5%; p <0.0001; OR 5.8; 95% CI 2.5-13.4); 6 PPROM cases versus only one in controls (7.1% vs 0.5%; p 0.001; OR 15.2; 95% CI 1.8-128.6); 7 IUGR cases versus 3 in controls (8.3% vs 2%; p 0.004; OR 5.9; 95% CI 1.5-23.6); 10 PE/HELLP syndrome cases in both groups (11.9% vs 5%; p 0.039; OR 2.6; 95% CI 1-6.4); and 6 cases of placental abruption versus 2 in controls (7.1% vs 1%; p 0.004; OR 7.6; 95% CI 1.5-38.4). Finally, among the women who tested positive for NTD screening, 2 cases of third-trimester IUFD versus none among negative controls were found (2.4% vs 0%; p 0.029; OR not calculable [n.c.]).

Regarding neonatal outcome (Table 4), compared with the group of 199 controls with normal MSAF levels (< 2.5 MoM), the group with MSAFP levels ≥2.5

Table 4. Neonatal outcome (live births) and MSAFP levels

No. of women	MSAFP ≥2.5 MoM 82	MSAFP <2.5 MoM 199	OR (CI 5% - 95%)	p
Birth weight (M ± SD)	2833 ± 886	3354 ± 529	n.c.	<0.0001
SGA	16 (20.7%)	7 (4%)	6.6 (2.6-16.9)	<0.0001
Apgar score at 1' between 1 and 3	7 (8.5%)	6 (3%)	3.0 (1 - 9.2)	0.045
Apgar score at 5' between 1 and 3	3 (3.7%)	1 (0.5%)	7.5 (0.8 - 73.4)	0.007
NICU transfer	12 (14.6%)	3 (1.5%)	11.2 (3.1- 40.9)	<0.0001

SGA = Small for Gestational Age; M ± SD = Mean ± Standard deviation
NICU = Neonatal Intensive Care Unit; n.c. = not calculable

MoM, which consisted in 82 patients after excluding 2 patients with IUFD, had a lower birth weight on average (2833 vs 3354; p <0.0001; OR n.c.); a significantly higher SGA rate (20.7% vs 4%; p <0.0001; OR 6.6; 95% CI 2.6-16.9); a higher rate of very low Apgar score (between 1 and 3) at 1 minute (8.5% vs 3%; p 0.045; OR 3.0; 95% CI 1-9.2) and at 5 minutes (3.7% vs 0.5%; p 0.007; OR 7.5; 95% CI 0.8-73.4), and a significantly higher number of NICU transfers (14.6% vs 1.5%; p <0.0001; OR 11.2; 95% CI 3.1-40.9).

Discussion

In our study we reviewed a case series of singleton pregnancies with "unexplained" elevated MSAFP levels (≥2.5 MoM) in the second trimester. In order to evaluate the course and outcome of these pregnancies, after excluding any possible causes for MSAFP elevation, we compared these selected cases with a group of women who had normal MSAFP levels (<2.5 MoM) over the same period.

The results of our study are in agreement with most reports published so far, thus confirming that, in the absence of any specific causes, MSAFP elevation in the second trimester is an independent risk factor for the course and outcome of pregnancy (2-10).

Based on these considerations, it may be useful to implement an intensive management program that, in addition to routine clinical and instrumental tests, would also include increased maternal/fetal monitoring: particularly, monitoring of blood pressure and any related clinical changes; fetal growth monitoring; uterine artery Doppler flow study (which could reveal abnormalities associated with a greater risk of developing maternal/fetal complications) (11); cervical length measurement, possibly accompanied by a fetal fibronectin test, as a method of preterm labor screening; and sonographic monitoring of placental structure to detect any hyperechogenicity or any structural abnormalities of the placenta that may be associated with an adverse pregnancy outcome (12, 13).

Simpson (2) investigated the course of 650 singleton pregnancies without NTDs and with elevated MSAFP levels in the second and in the third trimester. He came to the conclusion that the second-trimester "unexplained" MSAFP elevation is associated with an increased risk of PD, PPROM, IUGR, and LBW; no correlation was found between MSAFP elevation in the third trimester and poor maternal/fetal outcome. Cardonick (10) reported the association of high MSAFP levels in late second trimester and early third trimester with poor maternal/fetal outcome. He concluded his study underlining the need to optimize the timing of screening. His observations are sustained by the evidence of our own study; in contrast, unlike our findings, Simpson (2) did not point to any correlation with preeclampsia.

Moreover, in our opinion there is another very significant finding in the literature that might explain the increase in MAFP levels: the existence of a modification in the placental organ. Ramus (12) studied three groups of women with and without hyperechogenicity of the placenta associated with MSAFP elevation. He came to the conclusion that women with elevated MSAFP levels associated with a hyperechogenic placenta were at greater risk of maternal/fetal complications. Yaron (14) investigated the correlation between maternal serum levels of the three markers

(AFP, βhCG and unE_3) that are routinely measured in the second trimester (16-20 wks) and poor maternal/fetal outcome. He found that high levels of AFP and βhCG (>2.5 MoM) and low levels of unE_3 (<0.5 MoM) are associated with a poor maternal/fetal outcome. Moreover, he claimed that changes in the serum levels of these markers might be explained by underlying modifications in the function and structure of the placenta (15-17). Yaron concluded that these patients should be closely monitored by blood pressure measurement and ultrasound scanning that would have to include not only fetal growth assessment, but also Doppler flow velocimetry.

Unfortunately, in our study we could not obtain an accurate evaluation of the placenta. However, based on our findings, a structural modification of the placenta might be suspected, across which AFP might diffuse from the fetoplacental to the maternal circulation, thus explaining its increased levels in maternal serum.

The association between elevated MSAFP levels in the second trimester and an adverse perinatal outcome was also confirmed by our study.

Recently some authors (18) investigated a number of patients with elevated MSAFP levels and recommended that these patients be managed as usual, since adverse events can usually be anticipated by routine management and, when not, they can be closely monitored. However, the results of our study, together with those of previous reports in the literature, underline the need of intensifying clinical and instrumental tests in all those cases presenting with "unexplained" MSAFP elevation in the second trimester, in order to improve maternal/fetal outcome and consequently perinatal morbidity and mortality. Further prospective studies are therefore needed to determine if and when intensive management may improve the outcome of these pregnancies.

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Correspondence: Salvatore Anfuso
Department of Gynecology, Obstetrics and Neonatology, University of Parma, Italy
V.le A. Gramsci
43100 Parma
Tel. 3494719258 - Fax 0521/290508
E-mail sa.anfuso@tiscali.it; www.actabiomedica.it