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Elevated Maternal Serum Alpha-Fetoprotein With Low Unconjugated Estriol and the Risk for Lethal Perinatal Outcome

Peter A. Benn,[1]* Alicia Craffey,[1] Donna Horne,[1] Lisa Ramsdell,[1] and John F. Rodis[2]

[1]*Division of Human Genetics, Department of Pediatrics, University of Connecticut Health Center, Farmington, Connecticut*

[2]*Division of Maternal Fetal Medicine, Department of Obstetrics, University of Connecticut Health Center, Farmington, Connecticut*

Objective: To determine whether a combination of elevated maternal serum alpha-fetoprotein (MSAFP) and low unconjugated estriol (E3) concentration identifies pregnancies at particularly high risk for fetal abnormality or poor outcome.

Methods: Pregnancy outcomes were reviewed for women with elevated MSAFP (≥2.0 MoM) from our database of 50,315 women who had received triple marker testing from 1993–1998. Outcomes for those with low E3 (≤0.7 MoM) were compared with those with normal E3 (>0.7 MoM). The incidences of fetal death, neural tube defects, chromosome abnormalities, congenital abnormalities, preterm birth, small-for-gestational age (SGA), twins, and inaccurate dates were compared in the two groups using Fisher's exact test with $P < 0.05$ considered significant.

Results: Of the 50,315 women screened, 1,435 (2.85%) had an elevated MSAFP. Pregnancy outcomes were obtained in 94% of those with elevated MSAFP and 70% of all patients screened. Neural tube defects were present in 57 fetuses/infants (21 anencephalic, 29 spina bifida, 7 encephalocele) of which 46 (81%) had an elevated MSAFP. Of the 1,435 women with an elevated MSAFP, 199 (14%) had a low E3. Compared to those women with elevated MSAFP but normal E3, women with elevated MSAFP and low E3 were at significantly increased risk for fetal death (20.6% vs. 2.8%, relative risk (RR) 8.9), anencephaly (9.0% vs. 0.1%, RR 122.8) and chromosome abnormality (2.5% vs. 0.6%, RR 4.0).

Conclusions: Pregnancies complicated by elevated second trimester MSAFP and low E3 are at a particularly high risk (32%) for lethal perinatal outcomes. Twins, while a common cause of elevated MSAFP, are rarely found when an elevated MSAFP is associated with low E3. *J. Matern.-Fetal Med. 2000;9:165–169.*

Key words: neural tube defects; prenatal screening; chromosome abnormalities; fetal death

INTRODUCTION

Second trimester maternal serum alpha-fetoprotein (MSAFP) concentrations are routinely measured to identify pregnancies at increased risk for an open neural tube defect in the fetus [1]. Elevation in MSAFP concentration is also considered an indicator of placental insufficiency, explaining the association between high MSAFP values and low birthweight, fetal and perinatal loss, and pregnancy complications [2].

At the same time in pregnancy that open neural tube defect screening is performed, unconjugated estriol (E3) and human chorionic gonadotropin (hCG) levels are also usually measured and combined with MSAFP results to help identify pregnancies at increased risk for fetal Down syndrome and trisomy 18 [3,4]. Maternal serum E3 levels are low when anencephaly is present [5,6], consistent with decreased production of precursors of E3 by the fetal adrenal cortex [8,9]. The combination of elevated MSAFP and low E3 is also expected to identify some cases where recent

*Correspondence to: Peter A. Benn, Division of Human Genetics, Department of Pediatrics, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-6140.
E-mail: BENN@NSO1.UCHC.EDU

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fetal death has occurred [10]. Low E3 may also be an indicator of placental dysfunction [11,12] and rare cases may also be attributable to disorders in steroid biosynthesis [13].

Since elevated MSAFP and low E3 have both been associated with impaired placental function, and both are expected to identify anencephaly, we wished to test the hypothesis that an evaluation of the E3 concentration would assist in the interpretation of an elevated MSAFP result. We therefore reviewed the outcomes of a series of pregnancies with elevated MSAFP and compared those cases with low E3 concentrations to the group with normal E3 levels.

SUBJECTS AND METHODS

Between April 1993 and December 1998, a total of 50,315 women underwent triple marker (MSAFP, hCG, and E3) testing as part of a prenatal Down syndrome and open neural tube defect detection program. MSAFP and the beta subunit of hCG concentrations were measured using Hybritech (San Diego, CA) Tandem E enzyme immunoassays or Bayer (Norwood, MA) immunoassays and E3 was measured using either the Amersham (Arlington Hts., IL) Amerlex (Kodak) or Diagnostic Systems Laboratories radioimmunoassays. The low-level sensitivity for both E3 assays was established as 0.15 ng/mL. An MSAFP value of at least 2.0 multiples of the median (MoM) was used to define the group of women with pregnancies screen-positive for an open neural tube defect. Details of the screening policies used have been described previously [14,15]. Detection rates refer to the proportion of affected pregnancies identified without adjustment for late second or third trimester fetal losses.

Follow-up information on pregnancy outcome was collected by contacting the offices of referring physicians and requesting completion of a questionnaire for each pregnancy. Data were also collected from regional maternal-fetal medicine referral centers and the cytogenetics laboratory. For the time period covered by this study, follow-up was available for 94% of all screening tests with elevated MSAFP and approximately 70% of all patients screened. To determine gestational age at term, ultrasound or last menstrual period (LMP) data provided at the time of screening was combined with birth date and those births prior to 37 weeks were considered preterm. Small for gestational age (SGA) was defined as a birthweight less than the 10^{th} centile [16].

Following an initial observation that E3 levels were frequently low in pregnancies where fetal death, anencephaly, or chromosome abnormality was present, outcomes for patients with elevated MSAFP were reviewed to determine whether a low E3 level was useful in distinguishing different outcomes. The E3 level was considered low if the value was less than or equal to 0.7 MoM. This cut-off was selected on the basis that the combination of MSAFP at least 2.0 MoM and E3 less than or equal to 0.7 MoM should identify most cases of anencephaly [5–7] and have a theoretical false-positive rate of approximately 0.6%.

Statistical analyses were carried out using the SPSS Statistical Package (Chicago, IL). Comparison of the proportions of women with various pregnancy outcomes was carried out using Fisher's exact test with $P < 0.05$ considered statistically significant.

RESULTS

Of the 50,315 women screened, 1,435 (2.85%) had an MSAFP value ≥2.0 MoM. A total of 57 neural tube defects were present (21 anencephaly, 29 spina bifida, and seven encephalocele) in women for whom there was no established evidence for a neural tube defect prior to screening. Eleven of the neural tube defects were identified in those patients with normal MSAFP levels (two anencephaly, seven spina bifida, and two encephalocele). Two neural tube defects were present in fetuses with chromosome abnormalities (one case of trisomy 13 and one case of triploidy) and two of the fetuses with spina bifida also had ventral wall defects. The detection rates, false-positive rates, and positive predictive values (percentage of patients with positive test results that had a fetus with a neural tube defect) for testing based on MSAFP ≥2.0 MoM are summarized in Table 1. Use of a 2.5 MoM cut-off for MSAFP would have resulted in a 1.12% screen-positive rate with the detection of one less case of anencephaly and four fewer other neural tube defects (detection rate 72%).

For the total population screened, a total of 20 isolated fetal ventral wall defects were known to be present (i.e., omphalocele or gastroschisis not in association with a chromosome abnormality or neural tube defect). Of these 20 cases, MSAFP was >2.0 MoM in 19 pregnancies (detection rate 95%). For these 20 affected pregnancies, median MSAFP, hCG, and E3 values were 6.13 MoM, 1.12 MoM, and 1.05 MoM, respectively.

Pregnancy outcome was reviewed for all patients with an MSAFP value equal or greater than 2.0 MoM (Table 2). The elevation in MSAFP was explained by fetal death in 5.3% of the cases (median MSAFP 2.78 MoM, hCG 1.27 MoM, E3 0.84 MoM). Fetal anomalies, other than neural tube defects, ventral wall defects, or chromosome abnormalities were noted in 2.9% of the cases (median MSAFP 2.29 MoM, hCG 1.45 MoM, E3 1.05 MoM). A high proportion (14.4%) of the elevated MSAFP cases were associated with preterm delivery (median MSAFP 2.35 MoM, hCG 1.45 MoM, E3 1.08 MoM) and SGA births were noted in 6.3% of the cases (median MSAFP 2.31 MoM, hCG 1.52 MoM, E3 0.90 MoM).

Of the 1,435 women with elevated MSAFP, 199 (13.9%) also had a low E3 level. These cases constituted 0.40% of the entire screened population. The data show that the most common reason for elevated MSAFP in association with low E3 was fetal death, with 41 (20.6%) of cases

TABLE 1. Summary of the Statistical Data for Neural Tube Defect Cases

	Anencephaly	Other neural tube defects	All neural tube defects
Median MoM (range)			
MSAFP	7.36 (0.31–12.99)	3.00 (0.86–19.90)	
hCG	0.52 (0.11–2.74)	0.98 (0.16–10.59)	
E3	0.37 (0.13–0.85)	0.93 (0.28–1.72)	
MSAFP ≥2.0 MoM			
Detection rate	19/21 (90%)	27/36 (75%)	46/57 (81%)
False-positive rate	2.82%	2.80%	2.76%
Positive predictive value	1.32%	1.88%	3.21%
MSAFP ≥2.0 MoM and E3 ≤0.7 MoM			
Detection rate	18/21 (86%)	5/36 (14%)	23/57 (40%)
False-positive rate	0.36%	0.39%	0.35%
Positive predictive value	9.0%	2.5%	11.6%

TABLE 2. Pregnancy Outcomes for Women With Elevated MSAFP and Either Low or Normal E3 Levels

Outcome	All cases no. (%)	E3 ≤0.7 MoM no. (%)	E3 >0.7 MoM no. (%)	Relative risk	95% CI	
Fetal death[1]	76 (5.3)	41 (20.6)	35 (2.8)	8.9	5.5–14.4	$P < 0.001$
Anencephaly	19 (1.3)	18 (9.0)	1 (0.1)	122.8	16.3–925.6	$P < 0.001$
Other neural tube defects	27 (1.9)	5 (2.5)	22 (1.8)[2]	1.4	0.5–3.8	NS
Chromosome abnormality	13 (0.9)	5 (2.5)	8 (0.6)[2]	4.0	1.3–12.2	$P < 0.05$
Ventral wall defect (isolated)	19 (1.3)	5 (2.5)	14 (1.1)	2.3	0.8–6.3	NS
Other abnormality	41 (2.9)	8 (4.0)[3]	33 (2.7)[4]	1.5	0.7–3.4	NS
Preterm[4]	206 (14.4)	32 (16.1)[5]	174 (14.1)[6]	1.17	0.8–1.8	NS
SGA[5]	90 (6.3)	28 (14.1)[5]	62 (5.0)[6]	3.1	1.9–5.0	$P < 0.001$
Normal	672 (46.8)	62 (31.2)	610 (49.4)	0.5	0.3–0.6	$P < 0.001$
Twins	211 (14.7)	0 (0.0)	211 (17.1)			$P < 0.001$
Outcome unknown	86 (6.0)	7 (3.5)	79 (6.4)			
Total	1,435 (100.0)	191 (100.0)	1,236 (100.0)			

[1]Spontaneous abortion, stillbirth, or in utero demise.
[2]Two cases with chromosome abnormalities and spina bifida.
[3]Four cardiac; two urogenital; two miscellaneous anomalies.
[4]Six urogenital; six cardiac; two gastrointestinal; and 21 miscellaneous anomalies.
[5]Twelve cases both preterm and SGA.
[6]Eleven cases both preterm and SGA.

attributable to demise. This compared with 35 (2.8%) cases of fetal death identified in women with elevated MSAFP but normal E3 levels. Risk for fetal death for the women with elevated MSAFP and low E3 was 8.9 times higher than that seen for women with elevated MSAFP and normal E3 levels. In general, our outcome database did not allow us to establish the precise time in gestation when demise occurred. However, we observed that a low E3 level did not appear to assist in the identification of those deaths that did not get recognized until the third trimester. Of the 41 fetal deaths in women with elevated MSAFP and low E3, six (14.6%) were not recognized until the third trimester, while in women with elevated MSAFP and higher E3 concentrations, 9 of 35 (25.7%) were not reported until the third trimester ($P > 0.05$).

Fetal anencephaly was present in 18 (9.0%) of pregnancies with an elevated MSAFP and low E3, compared to only one case with elevated MSAFP and normal E3 concentrations (relative risk (RR) 122.8). Five (2.5%) fetal chromosome abnormalities were identified in women with low E3 levels (one case each of 47,XX,+18 with omphalocele; trisomy 13; 45,X; 45,X/46,XY; and dup(4p) with abnormal ultrasound findings present in the trisomy 18 and 45,X cases). Eight (0.6%) chromosome abnormalities were found in the fetuses of women with normal E3 concentrations (two cases of triploidy, two cases of trisomy 16 mosaicism, and one case each of trisomy 13, trisomy 16; an unbalanced translocation, and del(11q) with associated ultrasound detectable anomalies for the trisomy 13; trisomy 16, mosaic trisomy 16, unbalanced translocation and triploid karyotypes). The incidence of chromosome abnormality in the women with low E3 levels was significantly higher than seen for patients with higher levels of E3 (RR 4.0). A combination of high MSAFP and low E3 did not appear to preferentially identify pregnancies with preterm delivery, but SGA births did appear to be significantly more common

in this group relative to that seen for women with elevated MSAFP and normal levels of E3 (RR 3.1).

DISCUSSION

Our data shows that the most common reason for elevated MSAFP and low E3 was fetal death, which accounted for 21% of such cases (Table 2). A further 9% were attributable to anencephaly, an observation that is consistent with that of Yaron et al. [7]. In addition, 2.5% were associated with chromosome abnormalities. Combining the categories of fetal death, anencephaly, and those chromosome abnormalities incompatible with long-term survival, approximately 32% of the cases were nonviable. This can be compared with a combined risk of approximately 3.6% for these same categories of abnormality in pregnancies of women with elevated MSAFP but normal E3 levels.

The association between elevated MSAFP and fetal death is well-established [17,18]. In many instances, it is likely that a clinically unrecognized demise had occurred prior to screening, but there is also evidence that an elevated MSAFP level has some predictive value in identifying those pregnancies at increased risk for later loss [19,20]. Our data does not allow us to draw any conclusions regarding the relative risk for loss when viability has been definitively established at the time of screening. The presence or absence of a low E3 level did not appear to provide any additional help in identifying those pregnancies in which loss was not reported until the third trimester. Autopsies, karyotyping, and ultrasound examinations were not consistently carried out and the causes of death were likely to be diverse.

Although both elevated MSAFP and low E3 can individually be expected to be associated with pregnancies with impaired placental function [2,11,12], the combination did not appear to preferentially identify prematurity beyond that expected with elevated MSAFP alone. However, the incidence of SGA babies appeared to be increased relative to that seen in cases with elevated MSAFP but normal E3 levels. This association may be artifactual; unrecognized inaccuracy in gestational age assessment is more likely in patients with elevated MSAFP alone and this type of error can lead to an incorrect interpretation of the pregnancy duration (and expected weight) at term. The data in Table 2 also shows that an elevated MSAFP and low E3 is unlikely to be attributable to twins. Low E3 is associated with placental sulfatase deficiency [21], congenital adrenal hypoplasia [21], Smith-Lemli-Opitz syndrome [22], and other disorders associated with enzyme defects in the pathway to estriol [13], but each of these are not expected to be associated with elevated MSAFP.

The primary significance of an elevated MSAFP and low E3, therefore, appears to be in the identification of a subgroup of pregnancies where risk for second trimester fetal death or severe abnormality (anencephaly and aneuploidy) is particularly high. Patients with elevated MSAFP are routinely referred for targeted ultrasound examination irrespective of the values of other serum analytes. Fetal death, anencephaly, and at least those chromosome abnormalities that are associated with gross malformations will be readily identified during the sonogram. A formal change in the screening protocol to incorporate the E3 value in the interpretation of the MSAFP result is therefore probably unnecessary. However, using E3 concentration to help identify pregnancies at substantially higher risk for fetal death, anencephaly, and chromosome abnormality is of value to the extent that it can alert the obstetrician, prior to the scan, to the much greater probability of distressing findings. In turn, patients with elevated MSAFP and low E3 can be more prepared for the possibility of a severe problem in their pregnancies.

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