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Combination of Elevated Maternal Serum Alpha-Fetoprotein (MSAFP) and Low Estriol Is Highly Predictive of Anencephaly

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Increased levels of second trimester maternal serum alpha-fetoprotein (MSAFP) have long been established as a marker for neural tube defects (NTDs). In addition, decreased levels of maternal estriol in the third trimester have been reported in pregnancies with anencephalic fetuses. The purpose of this study was to evaluate whether early second trimester unconjugated serum estriol (uE3) is an independent predictor of NTDs. The study included 57,031 patients who underwent maternal serum screening with MSAFP at 14–22 weeks gestation. Of these, 23,415 also had uE3 measurements. There were 63 cases of NTD, an overall incidence of 1.1 per 1,000. Elevated MSAFP (⩾2.5 MOM) was detected in 1,346 patients, 48 of which had NTDs. Decreased uE3 (⩽0.5) was detected in 1,437 patients, 17 of which had NTDs. The incidence of NTDs was significantly higher in patients with low uE3, compared to patients with normal/high uE3 (1.15% vs. 0.09%, $P < 001$). Finally, 51 patients had both increased MSAFP and decreased uE3; 16 of these had NTDs, 14 of which were anencephalics. In conclusion, both elevated MSAFP and low maternal serum estriol are predictive of NTD but have a low sensitivity. The combination of abnormally elevated MSAFP and low estriol is highly predictive of NTD in particular anencephaly. Am. J. Med. Genet. 75:297–299, 1998.

KEY WORDS: estriol; neural tube defects; anencephaly; prenatal diagnosis

INTRODUCTION

Urinary and maternal serum estriol have been employed in the past to predict placental insufficiency, and thereby to evaluate fetal well-being in the second half of pregnancy. At that time it was noted that estriol production is extremely low in pregnancies with anencephalic fetuses [Frandsen et al., 1961; Shahwan et al., 1969; Dean et al., 1977]. With the advent of electronic fetal heart rate monitoring and ultrasonography, however, estriol measurements for determination of fetal well-being became obsolete and were abandoned. Increased second trimester maternal serum alpha-fetoprotein (MSAFP) has long been established as a marker for neural tube defects (NTDs). Conversely, decreased levels of MSAFP and low maternal serum unconjugated estriol (uE3) have been known to be predictive of Down syndrome (DS). Thus, MSAFP and uE3, in conjunction with the β subunit of human chorionic gonadotropin (β-hCG), are routinely being employed as part of the "triple screen," to assess DS risk. Now that uE3 is routinely measured again, we sought to evaluate whether it can be of any predictive value in screening for NTDs, and in particular anencephaly.

MATERIALS AND METHODS

This retrospective study included 57,031 patients with singleton pregnancies, who underwent second trimester maternal serum marker biochemical screening at 14–22 weeks gestation, over a 5-year period (March 1991–May 1996). MSAFP alone was measured in 33,616 patients, whereas 23,415 patients also had serum uE3 level determinations, a part of the "triple

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TABLE I. Value of Second Trimester Serum Markers for Prediction of Neural Tube Defects

	RR[a] (95% C.I.)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
High MSAFP	132.4 (72.2–246.3)	76.2	97.7	3.6	100
Low uE3	12.7 (6.4–24.2)	45.9	93.8	1.2	99.9
High MSAFP and Low uE3	349.0 (182.9–645.3)	43.2	99.9	31.4	99.9

[a]RR, relative risk.

screen,'' as ordered by the referring physician. Reagents were obtained from Sanofi Diagnostics Pasteur Inc. (Marnes-la-Coquette, France). A competitive radioimmunoassay technique was used for MSAFP and uE3 assays. Results were obtained from Quest Diagnostics Database (formerly Corning Clincial Laboratories, Teterboro, NJ and Auburn Hills, MI). Alpha-fetoprotein values were adjusted clinically for maternal weight, whereas estriol was not. Markers were reported in gestational age-corrected multiples of the medians (MOMs). Pregnancy complications and outcome were obtained and documented for all patients using a physician-completed questionnaire. Data were incorporated into the Quest Diagnostics Database, Teterboro, NJ.

The performance of MSAFP and estriol as predictors of NTDs, particularly anencephaly, were evaluated according to standard clinically applied cutoffs: 2.5 MOM for MSAFP, and 0.5 MOM for uE3. Statistical analysis consisted of X^2-test, and ANOVA, using StatView® for the Macintosh (Abacus Concepts, Inc. Berkeley, CA). A *P*-value of <0.05 was considered significant.

RESULTS

Of the 57,031 patients in the study, 33,616 had measurements of MSAFP alone, and 23,415 also had serum uE3 measurement. There were 63 cases of NTDs confirmed either postnatally or after termination of pregnancy, an overall incidence of 1.1 per 1,000. Elevated MSAFP was detected in 1,346 patients, 48 of which had NTDs (3.6%). Of the 23,415 patients who had both MSAFP and uE3 measurements, there were 34 cases of NTD, an incidence of 1.4 per 1,000. Decreased uE3 was detected in 1,437 patients, 17 of which had NTDs (1.2%). Both increased MSAFP and decreased uE3 were found in 51 patients, 16 of which had NTDs, an incidence of 31.2%. Among patients who had both MSAFP and uE3, 14 cases of anencephaly were observed. All these patients had increased MSAFP and low estriol. The relative risk (RR), sensitivity, specificity, positive and negative predictive values for MSAFP, uE3, and their combination for the prediction of NTD, are presented in Table I. All but one patient with a low uE3 and NTD also had increased MSAFP. The one patient with NTD, normal MSAFP, and low uE3, had an MSAFP level of 1.9 MOM. Further stratification of the NTDs into anencephaly and other NTDs revealed that the greatest contribution to the decreased estriol is by anencephalic fetuses. Although estriol was lower in other NTDs, this was not statistically significant (Table II).

DISCUSSION

The association between elevated alpha-fetoprotein (AFP) and neural tube defects was first recognized in 1972 when Brock and Sutcliffe found that all cases with anencephaly and most cases with spina bifida had elevated amniotic fluid level of AFP [Brock et al., 1972]. It was subsequently found that MSAFP is also elevated in such cases, forming the basis of population-based screening for NTDs [Wald et al., 1974]. The first connection between low estriol and anencephaly was made by Frandsen and Stakemann more than 10 years earlier [Frandsen et al., 1961]. They reported that mothers of anencephalic fetuses have one-tenth the amount of estrogens as mothers of normal infants. Since then, there have been numerous reports concerning low estriol and anencephaly [Allen et al., 1974; MacDonald and Siitreri, 1965; Tulchinsky et al., 1977; Dean et al., 1977]. Most of these studies date back to the 1960s and 1970s, when estriol was used for evaluation of placental dysfunction and fetal well-being.

It is apparent that all the previously cited publications concern estriol levels were determined in the second half of pregnancy. With the emerging use of estriol as a part of the "triple screen," little has been reported regarding low estriol in the early second trimester and

TABLE II. Mean Maternal Serum Levels of MSAFP and uE3 in Patients With Anencephaly, Other NTDs, and Normal Controls

		MSAFP (MOM)	*P*-value[a]	uE3 (MOM)	*P*-value[a]
Anencephaly	14	8.29 ± 3.24	< 0.0001	0.28 ± 0.08	< 0.0001
Other NTDs	20	4.30 ± 2.50	< 0.0001	0.88 ± 0.03	0.16[b]
Normals	23,381	1.14 ± 0.54		1.02 ± 0.45	

[a]*P*-values compared to normals, ANOVA.
[b]Not significant.

anencephaly. Kostiuk et al. [1992] found that patients with NTDs had significantly elevated levels of MSAFP (median MOM = 5.95), and significantly lower levels of uE3 (median MOM = 0.2), compared to normal controls. They stated that "... the biological basis of altered levels of uE3 in pregnancies with fetal NTDs is unclear."

Low maternal serum levels of estriol found with anencephalic fetuses can be attributed to adrenal hypoplasia resulting from lack of pituitary ACTH production [MacDonald and Siitreri, 1965]. Normally, the fetal adrenal glands produce dehydroepiandrosterone sulfate (DHAS) which, after hydroxylation in the fetal liver, is converted to estriol in the placenta. In anencephalic fetuses, there is lack of ACTH production, with resulting decrease in DHAS levels, and thus low estriol.

Obviously, the effects of the nonfunctional pituitary in anencephalic fetuses are apparent in the second half of pregnancy. The question is, however, when does the pituitary ACTH become critical for estriol production? Sagen et al. [1979] measured the maternal venous plasma concentrations of total estriol and MSAFP in a woman with an anencephalic fetus, beginning at the seventh week of pregnancy until term. They found that in the first trimester, estriol values were in the lower normal range. However, beginning in the second trimester and throughout gestation, estriol concentration was constantly subnormal. This would suggest that in anencephalic fetuses, estriol levels would already be below normal, at the time of maternal serum screening.

We thus sought to evaluate whether these data could be of any additional value in the diagnosis of anencephaly and other NTDs. The results of our study show that all cases of anencephaly had a low uE3 and high MSAFP. In addition, the uE3 level in other nonanencephalic NTDs was lower than in normal controls (although not statistically significant). Thus, as an independent predictor of NTDs, decreased uE3 had a lower sensitivity than elevated MSAFP. However, the combination of low uE3 and high MSAFP was highly predictive of NTD, and in particular anencephaly.

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