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Alpha-Fetoprotein Concentrations in Maternal Serum: Relation to Race and Body Weight

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We confirmed the relation between maternal weight and serum alpha-fetoprotein concentration and have shown a further relation—to ethnic origin. Of these two, maternal weight is the more closely related. Oriental, white, and Hispanic women showed no significant differences in serum AFP concentrations when corrections for maternal weight were applied. Black women showed consistently higher values, by an average of 10% at each week of gestation. These corrections only affected values falling just above or below the 95th centile cutoff. We conclude that corrections for maternal weight and race should be applied when values for alpha-fetoprotein in maternal serum are being interpreted.

Additional Keyphrases: *cutoff point · variation, source of · neural tube defects*

Screening of maternal serum for alpha-fetoprotein (AFP) for the detection of neural tube defects has been initiated in several European countries, and a few centers in the United States now provide this test on a voluntary basis. In screening programs a median value is usually first established for normal singleton pregnancies and then a "cutoff" point is selected, above which assay of a second serum sample or sonography is recommended. Biological factors and assay conditions are two variables that obviously affect the median serum AFP value; maternal weight is a third, less-obvious one (*1, 2*): maternal weight and AFP concentration in serum apparently are inversely related. Race has been suggested as a fourth variable. Shapiro et al. (*3*) reported a significant difference between values for AFP in maternal serum from Asian and Caucasian women in the United Kingdom. However, Macri et al. (*4*) found no significant difference between values for blacks and whites in New York. In both cases, these conclusions were based on data from small sample populations.

We collected data, which included race and maternal weight, during a pilot program for screening maternal serum in California, conducted between 1978 and 1980. Here we compare the relation between these two variables and AFP concentration in serum.

Methods

AFP in maternal serum was determined by radioimmunoassay, with use of a double-antibody technique (*5*). We established our reference intervals by measuring AFP in at least 100 serum samples for each week of gestation between 14 and 24 weeks. These samples were collected from normal singleton pregnancies.

Gestation was calculated as the number of weeks since the last menstrual period. In cases where the serum samples were collected between two weeks, those for the last completed week plus four days or more were included in the next gestational week. A median and 95th centile (about twice the median) were established. Within-assay variation (CV) did not exceed 5% and usually was <3%.

Enrollment in the program was entirely voluntary and, after an explanation, women wishing to participate were asked to complete a form, which included spaces for entering height, weight, and age, and the ethnic origin of both the mother and the father, as well as reproductive history and family occurrence of neural tube defects. We usually collected one 6- to 8-mL sample of venous blood between 16 and 20 weeks of gestation. We extracted information concerning the outcomes of pregnancies from medical charts and obstetrical records and included the birth date and weight, as well as the result of the newborn examination. All this information was coded and stored in a computer.

The covariance analysis used to evaluate the data was computed on the logarithms of serum AFP by the Biomedical Computer Program BMDP2V (*6*). The geometric means listed are the antilogs of the arithmetic means.

Results

We measured AFP concentrations in serum from 10 715 second-trimester pregnancies. Of these, 9054 women indicated their race, which was categorized as black, Hispanic, Oriental, white, or "other." We evaluated the distribution of values for AFP in maternal serum between 14 and 22 weeks of gestation in 8297. We excluded 757 women because they listed race as "other." We also excluded instances of twin pregnancies, gestation of uncertain duration, and data from blood samples taken before or after the dates selected.

Table 1 shows the AFP distribution for each ethnic group. It includes 439 black and 619 Oriental women. There appeared to be no difference between Hispanics and whites, but blacks had the highest mean values at each week, and values for Orientals fell between these two groups. Of the black women, 8.1% had above-normal values for AFP (≥95th centile), as compared with 6.6% for Orientals, 5.6% for Hispanics, and 4.5% for whites. This percentage in Oriental women was eliminated when a correction was applied for maternal weight, but the percentage for blacks was unaffected by this correction (Table 2). Oriental women, on the average, weighed less than each of the other ethnic groups.

The ethnic origin of both parents was recorded for 7594 couples. Although the higher values in black women were slightly accentuated when their mates were also black, this difference was insignificant.

The ratio of maternal serum AFP values in black and white women varied slightly at each week of gestation but averaged 1.1 to 1, a significant difference ($p \leq 0.001$) but not as great as that resulting from variations in maternal weight. Although the latter increased with each week of gestation, at (e.g.) 17 weeks there was a decrease of 4.8 μg/L for each 9.1-kg (20-lbs.) increase in weight over 63.6 kg (140

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Table 1. AFP Distribution by Race: Median Serum AFP, in μg/L (and n)

Gestational age, weeks	Race			
	Hispanic	White	Oriental	Black
14	46 (7)	43 (103)	43 (15)	51.5 (6)
15	41.5 (40)	46 (389)	51 (45)	47 (35)
16	47 (186)	50 (1981)	52 (196)	53.5 (110)
17	51 (192)	53 (1843)	53 (139)	59 (108)
18	56 (118)	57 (1092)	62 (94)	67 (71)
19	65 (84)	62.5 (590)	66 (53)	66 (51)
20	65 (41)	66 (313)	70 (39)	75 (30)
21	67.5 (20)	67 (163)	69 (24)	64 (17)
22	86 (7)	79.5 (70)	128.5 (14)	89 (11)
Total	(695)	(6544)	(619)	(439)

Table 2. Serum AFP in Ethnic Groups Adjusted for Maternal Weight and Gestational Age[a]

	Mean body weight, kg	AFP, μg/L[b]	Adjusted AFP, μg/L
Hispanic	61.8 (383)	53.5	52.6
White	61.8 (4822)	53.3	53.6
Oriental	52.5 (483)	57.5	54.4
Black	65.5 (288)	59.3	59.2

[a] Adjusted to a mean maternal weight of 61.4 kg (135 lbs.) at 17.1 weeks in 5976 women weighing between 36.4 and 127 kg and delivering only live infants weighing more than 2500 g. [b] Geometric mean.

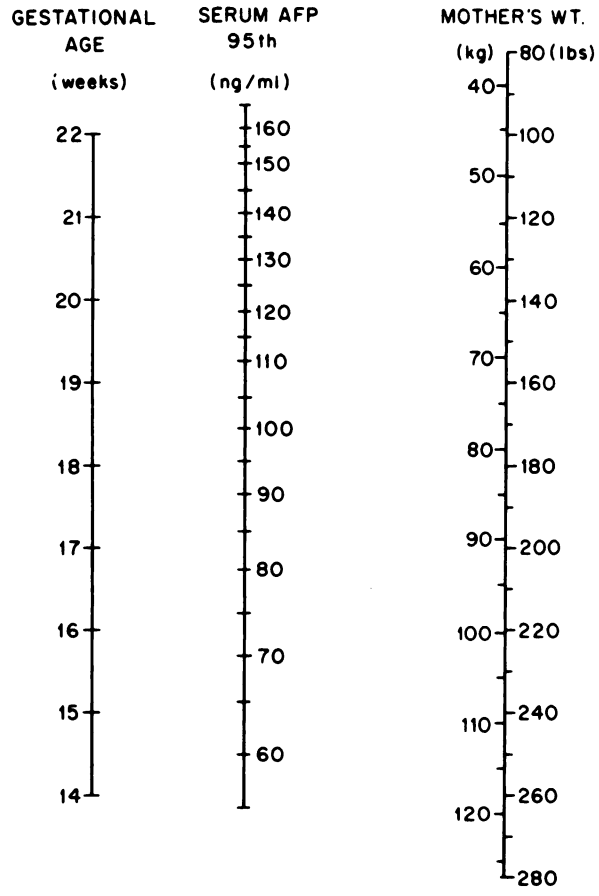

Fig. 1. Nomogram constructed to identify 95th centile serum AFP at each week of gestation for maternal weights between 36.4 kg (80 lbs.) and 127 kg (280 lbs.)

lbs.) and a 5.5 μg/L increase in the AFP value (Table 3) for each 9.1-kg (20-lbs.) decrease in weight.

Table 3. 95th Centile Serum AFP (μg/L) at Maternal Weights of 36.4, 63.6, and 90.9 kg

Gestational age, weeks	Maternal wt.		
	36.4 kg (80 lbs.)	63.6 kg (140 lbs.)	90.9 kg (200 lbs.)
14	87	75	65
15	94	81	70
16	102	88	76
17	110	96	83
18	120	104	90
19	130	113	97
20	141	122	106
21	153	132	115
22	166	144	124

Discussion

We have confirmed a relation between maternal weight and values for serum AFP and have demonstrated a second variable to be taken into account: ethnicity. Of these two, maternal weight has the greater effect. We have constructed a nomogram for quick correction of the 95th centile between 14 and 22 weeks for women weighing between 36.4 kg (80 lbs.) and 127 kg (280 lbs.) (Figure 1). Values for black and white women are compared in Figure 2. These differences varied slightly at different gestational ages, averaging 10%. Most serum AFP screening centers have constructed their median and 95th centiles from non-black populations so that multiplication of these values by 1.1 should provide the corresponding expected values for black women.

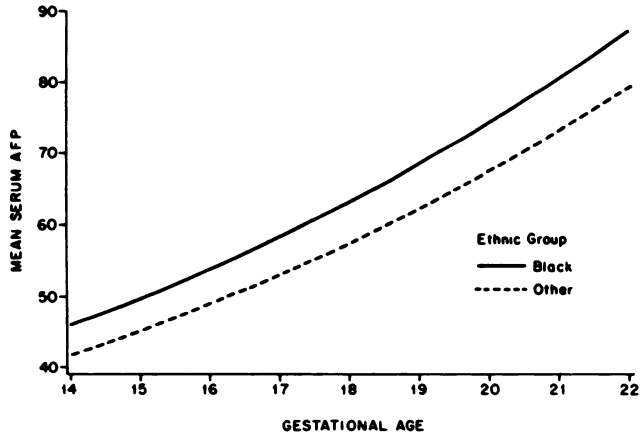

Fig. 2. Mean (geometric) serum AFP, in μg/L, of black and white women at each week of gestation

The geometric mean is practically the same as the median. Values average 10% more for black women

When we corrected for both maternal weight and ethnic origin, we found that 4.7% of about 500 women with apparently above-normal values were now normal and 0.4% of those who had had "normal" values were now above normal. However, these corrections only affected those just above or below the 95th centile cutoff, and would not have changed the sensitivity of maternal serum AFP screening in detecting neural tube defects.

The nomogram and the correction for black women together provide a simple method for adjusting the 95th centile. We estimate that this would decrease by about 20 the expected number of above-normal values for AFP in a center screening 10 000 women per year.

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Development and Clinical Evaluation of a Microcentrifugal Analyzer Method for Determining Creatine Kinase MB Isoenzyme

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We have adapted to a microcentrifugal analyzer an immunoinhibition assay for measuring the activity of creatine kinase MB by using an inhibitory antibody for the M monomer. The method actually measures half the MB activity, but results are not multiplied by two because atypical isoenzymes of creatine kinase, including BB, IgG–BB, and the isoenzyme derived from mitochondria, are also detected, if they are present. Results correlated well with an electrophoresis method for 36 serum samples. Myocardial infarction was assessed in 175 patients admitted to our coronary-care unit, with respect to sensitivity (100%) and specificity (98%) when a decision point of 100 U/L (30 °C) was chosen for total creatine kinase activity (dithiothreitol-activated) and 6 U/L (30 °C) for the isoenzyme (by immunoinhibition). Atypical isoenzymes are easily recognized and confirmed by electrophoresis when the MB activity (by immunoinhibition) exceeds 6 U/L and 20% of the total creatine kinase activity.

Measurement of creatine kinase (EC 2.7.3.2) isoenzyme MB (CK-MB)[3] in patient's serum is an accepted procedure for assessing myocardial damage, particularly in cases of suspected infarction (*1, 2*). In separation of the CK isoenzymes by electrophoresis, CK-MB migrates between the other two common types of CK, CK-MM and CK-BB. Measurements of CK-MB are now possible by electrophoresis (*3, 4*), column chromatography (*5*), immunoinhibition (IIA) with inhibiting antibody specific for the M monomer (*6–10*), and immunoseparation with double antibodies (*11, 12*).

The centrifugal analyzer is widely used for various biochemical procedures. However, sequential additions of reagents to the same rotor require a rotor with which a second reagent can be added after a preliminary assay has been done. The Multistat III microcentrifugal analyzer (MCA; Instrumentation Laboratory, Lexington, MA 02173) has this capacity for two reagent additions, and we evaluated its suitability for determining CK-MB activity by IIA. We correlated results for CK-MB by IIA and by electrophoresis, and performed a clinical study of 175 patients consecutively admitted to a coronary-care unit. We also report the calculated predictive values for myocardial infarction as related to CK-MB determined by the IIA method and identification of atypical CK results by this method.

Materials and Methods

For electrophoresis analysis of the CK isoenzymes we used the Helena method (Helena Laboratories, Beaumont, TX 77704) with Sclavo staining reagent (Sclavo, Inc., Wayne, NJ 07470) (*13*), with *N*-acetylcysteine as the activating agent. Fluoride was added to a final concentration of 60 mmol/L, to inhibit completely any adenylate kinase (EC

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[3] Nonstandard abbreviations: CK-MB, CK-BB, CK-MM, isoenzymes of creatine kinase; IIA, immunoinhibition assay; MCA, microcentrifugal analyzer.

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