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ORIGINAL CONTRIBUTIONS

Levels of Maternal Serum Alpha-fetoprotein (AFP) in Pregnant Women and Subsequent Breast Cancer Risk

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High maternal serum alpha-fetoprotein (AFP) levels during pregnancy may be instrumental in reducing the subsequent risk of breast cancer. This hypothesis was tested in a nested case-control study using stored frozen sera accrued between 1959 and 1966 by the University of California at Berkeley Child Health and Development Studies (CHDS) group from a cohort of pregnant women. Cases with histologically confirmed breast cancer were identified from California Cancer Registry files covering their date of enrollment in the CHDS until 1994. Controls were selected from the CHDS cohort by using randomized recruitment. Third-trimester maternal serum AFP levels were analyzed by using both a radioimmunoassay and an immunoenzymatic method. After controlling for multiple confounders in logistic regression models, the authors found an inverse association between high levels of maternal serum AFP (top quartile) during the index pregnancy and the risk of breast cancer. The protective effect of high levels of maternal serum AFP varied by age at first full-term pregnancy (age 20 years or less: odds ratio (OR) = 0.43, 95% confidence interval (CI) 0.28–0.65; age 21–23 years: OR = 0.62, 95% CI 0.41–0.92). After age 27 years, the estimated risk exceeded unity (OR = 1.67, 95% CI 1.14–2.45). These study findings suggest that some of the protection against breast cancer conferred by early first full-term pregnancy may result from high levels of maternal serum AFP. After age 27 years, a high maternal serum AFP level is not protective and may increase risk. *Am J Epidemiol* 1998;148:719–27.

alpha-fetoproteins; breast neoplasms; breast neoplasms/prevention & control; fetal proteins

One factor that many studies have shown to confer long-term protection against subsequent breast cancer is early age at first full-term pregnancy (1–3). Among other physiologic and biochemical changes taking place during pregnancy, an increase occurs in circulating maternal levels of alpha-fetoprotein (AFP), a three-domain glycoprotein structurally similar to serum albumin (4). AFP, which is synthesized during embryonic development primarily by the fetal liver and yolk sac, enters the amniotic fluid through fetal renal excretion, crosses the placenta (possibly by diffusion), and enters the maternal circulatory system (5). Reported AFP concentrations in maternal serum (average, 2.6 ng/ml) are different from those in nonpregnant women (6). By the eighth week of gestation, the mean level of pooled sera is 11.3 ng/ml (7) and

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Abbreviations: AFP, alpha-fetoprotein; CHDS, Child Health and Development Studies; CI, confidence interval; OR, odds ratio.

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rises to a peak (approximately 250 ng/ml) at about the 32nd week of pregnancy. It then declines slightly until term (8).

Based on the experimental findings of Mizejewski et al. (9) and Allen et al. (10), Jacobson and Janerich (11) have hypothesized that the high maternal serum levels of AFP are protective against subsequent breast cancer. They postulate that maternal serum AFP in contact with estradiol (E_2) is transformed into a substance (transformed AFP (tAFP)) that, depending on the type and concentration of bound ligands, inhibits growth in estrogen-sensitive tissue.

Indirect evidence from epidemiologic studies supports a protective role of elevated fetal protein levels during pregnancy. Using indicators of high maternal serum AFP, Jacobson et al. (12) and Thompson et al. (13) found a protective effect against subsequent breast cancer risk. However, the Jacobson et al. study investigators found that multiple births, which are known to produce high AFP levels, were protective against breast cancer while a multiple birth prior to a last birth was not.

To our knowledge, no previous studies have evaluated breast cancer risk in relation to measured maternal serum levels of AFP. To directly test the hypothesis that high maternal serum levels of AFP are protective against subsequent breast cancer risk, we conducted a nested case-control study using third-trimester maternal serum levels of AFP as the exposure.

MATERIALS AND METHODS

Study population

Cases and controls were selected from among the pregnant women enrolled in the Child Health and Development Studies (CHDS), which were designed and initiated in 1959 at the University of California, Berkeley, by Dr. Jacob Yerushalmy in cooperation with the Kaiser Foundation Research Institute and the Permanente Medical Group. The main objectives were to investigate the relation of genetic, medical, and environmental factors before and during pregnancy to the development of the offspring (14). A gravida registry card containing basic medical and sociodemographic information was prepared at the time of enrollment of 15,528 women, and it was updated during the pregnancy. Included was information such as reproductive history, ethnic background, occupation, years of education, date of last menstrual period, and date of delivery. Of this initial group, 12,552 women took part in an in-depth interview in which information was collected on health-related matters, such as smoking habits and consumption of alcoholic beverages.

To investigate the relation between high AFP levels and breast cancer risk among women enrolled in the CHDS, the following eligibility criteria were used: each woman must have 1) completed an interview questionnaire; 2) been of legal age (21 years) or married; 3) delivered one or more liveborn or stillborn infant(s) from the index pregnancy; 4) had a blood sample taken and frozen as serum during the last trimester of the index pregnancy, which was the last pregnancy of cases and controls during the CHDS enrollment period of June 1959–September 1966; and 5) continued to be a California resident so that had she developed breast cancer, she would be included in the California Cancer Registry or have had her death recorded in California. Follow-up of cohort members was conducted by CHDS personnel using license records from the department of motor vehicles. Statewide mortality files were also used to ascertain possible deaths among the cohort.

Cases in this study were all women in the CHDS cohort who met these five eligibility criteria and who had histologically confirmed primary breast cancer (*International Classification of Diseases,* Ninth Revision, code 174) as identified in the files of the California Cancer Registry through 1994. The CHDS cohort was matched to tumor registry data by using the California Cancer Registry, which is part of the California Public Health Foundation, in conjunction with CHDS personnel. Identifying data for each woman were transferred to the Cancer Registry by the CHDS. The Cancer Registry then matched possible cases by using these identifiers. Controls were eligible members of the cohort who had not been diagnosed with breast cancer; they were "probability matched" to cases by distribution of the birth dates of cases using a randomized recruitment technique described by Weinberg and Sandler (15) and Weinberg and Wacholder (16). This method allowed individual random selection of controls on the basis of age within 5 years of the age of a case.

A total of 286 cases were identified by using the California Cancer Registry, and 225 of them met the eligibility criteria for this study. From the CHDS cohort, a total of 348 women who met the entry criteria were selected from a list of 851 eligible controls and alternates, giving a total of 573 women in the study. At the time of this study, 20 controls had died of competing causes.

Exposure assessment

From 1959 to 1966, serum samples from the CHDS population were stored at −20°C and then shipped to the National Institutes of Health in Bethesda, Maryland, where they were again held at −20°C. Later, the

samples were transferred to the National Cancer Institute–Frederick Cancer Research Facility in Frederick, Maryland, where they were maintained at −20°C. The concentration of AFP is relatively stable in human serum, even when subjected to repeated freezing (at −20°C) and thawing and to hours at room temperature between each freezing episode (17). It has also been shown that human AFP in serum is stable for more than a month at room temperature, although purified AFP is not (18). To assess the possibility of protein degradation in the CHDS samples, two assays of serum levels of AFP were performed at the Reproductive Hormone Laboratory, Duke University Medical Center, Durham, North Carolina. The first, a radioimmunoassay (Double Antibody AFP; Diagnostics Products Corporation, Los Angeles, California), enabled both intact and fragmented AFP molecules to be assessed; the second, an immunoenzymetric assay (TANDEM-E AFP; Hybritech Corporation, San Diego, California), used two epitope-specific monoclonal antibodies to detect intact protein only. Although data from the two assays were highly correlated (r = 0.95), there were outliers in each data set. Therefore, we analyzed each assay as well as the average of the two assays separately. In all three analyses, exposure was characterized as the number of nanograms of AFP per milliliter of maternal serum. All three analyses yielded similar results; therefore, the average of the two assays was used as the indicator of exposure (described in the analysis that follows).

Data analysis

The AFP levels of each woman during the index pregnancy were characterized as the difference (residual) between the woman's assay value and the estimated mean AFP level determined for that day of gestation. Therefore, "high" was used to characterize women whose serum levels were elevated (top 25 percent) as compared with the levels of all other women (the other 75 percent). This allowed adjustment for differing dates of blood draw throughout the data set. The mean AFP curve was estimated by using local linear regression ("lowess"), a nonparametric smoothing technique that employs weighted regression by using varying subsets of the data to estimate the curve at each gestational age, that is, a weighted average over the number of days of gestation. For these data, a bandwidth of 70 percent of the data was used to estimate a given point on the curve (19). To contend with heteroscedasticity in the original scale of measurement, the lowess and the residual values were determined by using logs of the original values. Figure 1 shows the mean curve, on a log scale, displayed with the individual data points.

Multiple logistic regression in SAS Proc Genmod (20) was used to compute odds ratios. SAS Proc Genmod allows "offsets" to adjust for the randomized recruitment sampling probabilities used to select controls. Information on potential confounders and effect modifiers was gathered from the interview data compiled from the questionnaires administered to all eli-

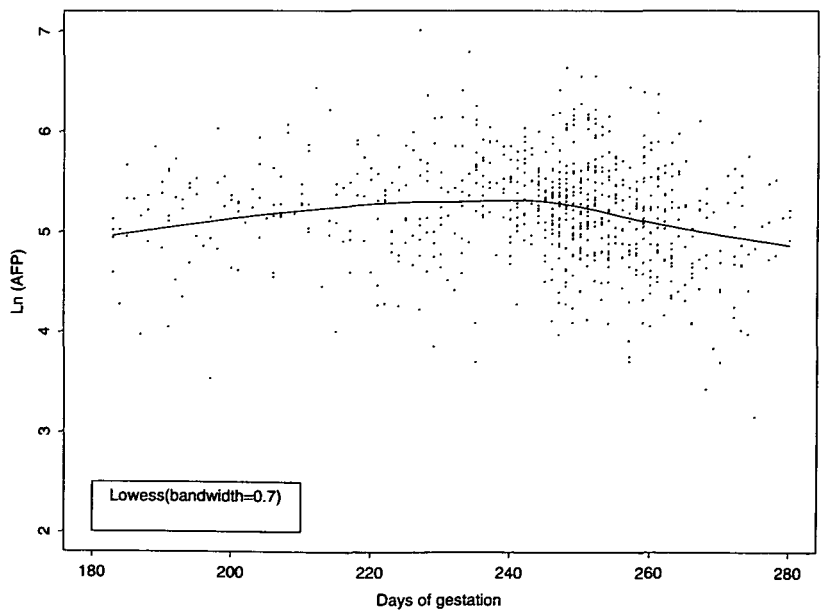

FIGURE 1. Ln (alpha-fetoprotein (AFP) ng/ml) for all subjects by the number of days of gestation from day 182 to day 280, Child Health and Development Studies, Berkeley, California, 1959–1966.

gible cohort members during pregnancy. Included were day of gestation at blood draw, race, maternal age at index delivery, age at first full-term pregnancy, number of previous pregnancies, number of abortions and miscarriages up to the time of the index pregnancy, alcohol consumption, prepregnancy weight, height, years of education, and age at menarche. Since the factors that could be potential confounders of an AFP-breast cancer association were unknown, the various breast cancer risk factors described above were evaluated to determine whether they met the criteria for confounding. The analysis was completed in two parts: 1) by examining the association between the potential confounders and breast cancer outcome among those without high levels of maternal serum AFP and 2) by determining which factors were associated with the exposure (top quartile of maternal serum AFP) among the controls.

The factors found to be associated with breast cancer among those with low levels of AFP included race, age, and age at first full-term pregnancy. The factors determined to predict levels of maternal serum AFP among the controls included race, age, and height. All potential confounders were evaluated during the modeling process. Of all potential confounders considered, only race and age at index pregnancy exhibited associations with both the outcome and the exposure of interest and substantially changed (more than 10 percent) the relative risk for the exposure variable. In addition to race and maternal age at delivery, we included age at first full-term pregnancy because the relative risk for breast cancer in women with high AFP levels varied as a function of age at first full-term pregnancy, as discussed below.

In the preliminary analysis, two different reference groups were used to assess high levels of AFP and breast cancer risk. Top and bottom quartiles of AFP were evaluated by using the middle two quartiles of the residuals as the reference group, and a high AFP level was evaluated by comparing the top quartile of AFP with the lower three quartiles, controlling for race, age, and age at first full-term pregnancy. Low levels of AFP (lowest quartile of residuals) were not associated with outcome when the middle two quartiles of AFP were used as the reference category; therefore, the lower three quartiles of AFP level were used together as the reference level. All results reported below are based on the dichotomized AFP variable, using "high" (top quartile) versus "other" (bottom three quartiles).

Initially, a model was specified that allowed us to enter age at index pregnancy and age at first full-term pregnancy linearly. However, a stratified analysis was also done by using SAS Proc Freq (21) as an initial check of the validity of the linear model. Once it was determined that there was no obvious indication of nonlinearity, our candidate model was constructed by using all necessary confounders and the interaction term of age at first full-term pregnancy and AFP. Then, fractional powers of age were considered that might modify the effect of age at first full-term pregnancy at higher age levels. In addition, we used categorical variables for age at first full-term pregnancy greater or less than 35 years and other representations, and a range of spline models as well as piecewise linear regression, to assess a difference in slope by age at first full-term pregnancy (22). None of the proposed transformations produced a model that was an improvement over the linear form. Also considered were piecewise linear functions of age at first full-term pregnancy. Specifically, allowing changes in slope for an age at first full-term pregnancy of either 21 or 27 years was considered. Again, these complications did not yield a significant improvement over the linear form. The final model simultaneously adjusted for race, age at index delivery, and age at first full-term pregnancy and for an AFP level by age at first full-term pregnancy interaction.

RESULTS

Table 1 shows the final model with the risk of breast cancer, estimated by using logistic regression, among women with the highest quartile of maternal serum AFP. For women in this study, a high maternal serum level of AFP during the index pregnancy was associated with breast cancer risk. There was an increase in risk with each change in year of maternal age at index

TABLE 1. Adjusted risks* and 95% confidence intervals for breast cancer among study participants with the highest quartile of maternal serum alpha-fetoprotein and other risk factors: Child Health and Development Studies, Berkeley, California, 1959–1966

Variable	Odds ratio	95% CI†
High maternal serum levels of alpha-fetoprotein	0.05	0.01–0.50
Race		
White‡	1.00	
Black	1.83	1.21–2.77
Asian	1.10	0.46–2.62
Age (years) at index pregnancy	1.06	1.03–1.09
Age (years) at first full-term pregnancy	1.05	1.01–1.09
High alpha-fetoprotein × age at first full-term pregnancy	1.12	1.03–1.23

* The risk associated with each variable was estimated by using the odds ratio and was adjusted for all other variables listed.
† CI, confidence interval.
‡ Referent.

pregnancy (odds ratio (OR) = 1.06, 95 percent confidence interval (CI) 1.03–1.09). The ages of the women in the study ranged from 17 to 44 years. Cases were aged 18–43 years at termination of index pregnancy (mean age, 31.00 years), and controls were aged 17–44 years (mean age, 31.03 years). Therefore, the ages at index pregnancy among women in the study spanned 27 years, making this factor important, because women who were older when they participated had an inherently higher risk of developing breast cancer. Additionally, there was a difference in risk based on race. With white women as the reference category, the odds ratio for black women was 1.83 (95 percent CI 1.21–2.77); for Asian women, it was 1.10 (95 percent CI 0.46–2.62). Since only 26 Asian women were included in the study, the point estimate may reflect unstable data.

Table 2 shows selected demographic data and risk factors for cases and controls. After we controlled for week of blood draw, Asian women had consistently higher mean serum levels of AFP than did black or white women during the third trimester of the index pregnancy.

As illustrated in figure 2, controls showed the expected peak level of serum AFP at 32 weeks of gestation, while the AFP levels among cases are represented by a rather flat curve instead of a steep upward curve. In every quartile of age at first full-term pregnancy, controls had higher mean serum levels of AFP than did cases during weeks 32–33 of gestation.

Initially, logistic regression models with no interaction terms showed no main effect of an elevated level of maternal serum AFP. After we examined all interactions of AFP and the possible confounders, it became clear that there actually was a very strong main effect of maternal serum AFP level, but it varied dramatically by age at first full-term pregnancy. In fact, there was no way to determine a weighted summary figure for the main effect of AFP across strata, because the odds ratios for a high AFP level at different ages at first full-term pregnancy varied from protective to an apparent increased risk after approximately age 27 years. Table 3 shows the odds ratios of breast cancer estimated by using logistic regression for

TABLE 2. Distribution of mean maternal serum levels of alpha-fetoprotein (AFP) (ng/ml) among cases and controls, by selected sociodemographic characteristics and breast cancer risk factors: Child Health and Development Studies, Berkeley, California, 1959–1966

	Cases (n = 225)			Controls (n = 348)		
	No. observed*	Mean AFP level	Standard deviation	No. observed*	Mean AFP level	Standard deviation
Race						
White	147	215.3	112.4	255	208.1	125.3
Black	66	165.0	69.4	78	185.8	98.9
Asian	11	359.7	186.4	15	320.3	170.3
Age (years) at index pregnancy						
≤20	13	202.9	88.5	17	264.5	194.1
>20–≤23	23	172.7	72.4	40	226.5	186.9
>23–≤27	29	201.4	96.1	48	212.3	99.8
>27–≤30	40	206.1	107.8	50	237.2	116.1
>30–≤35	62	217.4	115.4	98	189.7	97.7
>35	58	217.1	140.0	95	191.3	112.3
Age (years) at first full-term pregnancy						
≤20	48	173.6	78.0	93	203.4	140.3
>20–≤23	45	197.1	101.2	102	208.6	132.3
>23–≤27	70	212.8	124.9	77	210.8	104.6
>27	61	235.9	127.2	76	205.4	107.9
No. of previous full-term pregnancies						
0	48	203.4	80.5	66	241.2	176.7
1	60	221.4	130.9	80	208.1	100.5
2	57	219.0	120.8	77	212.7	123.7
3	25	224.1	144.1	53	190.6	98.7
≥4	35	161.0	67.8	72	185.0	104.2

* Total in each category could differ because of missing data.

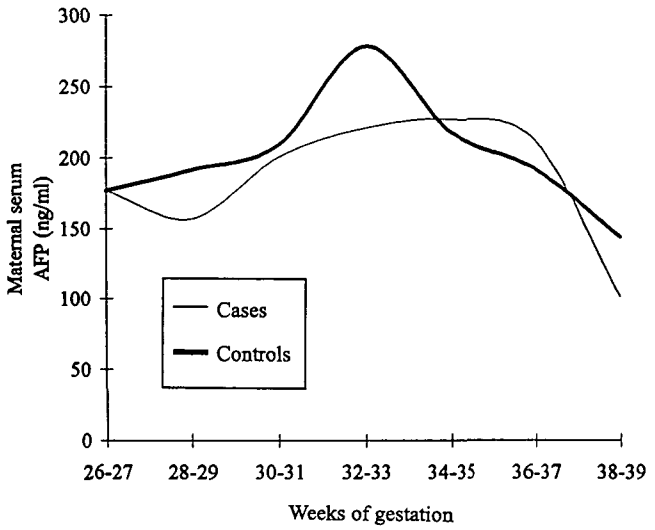

FIGURE 2. Mean maternal serum levels of alpha-fetoprotein (AFP ng/ml) by the number of weeks of gestation for cases and controls, Child Health and Development Studies, Berkeley, California, 1959–1966.

TABLE 3. Estimated odds ratios for breast cancer among study participants, from logistic regression analysis, for a high* level of maternal serum alpha-fetoprotein and by age at first full-term pregnancy: Child Health and Development Studies, Berkeley, California, 1959–1966

Age (years) at first full-term pregnancy	Odds ratio†	95% confidence interval
16	0.33	0.14–0.79
20	0.52	0.29–0.93
24	0.82	0.54–1.25
26	1.03	0.67–1.59
30	1.63	0.89–3.09
34	2.59	1.01–6.65
38	4.10	1.14–14.74

* Highest quartile versus lowest three quartiles.
† Adjusted for race and for age at index pregnancy.

TABLE 4. Odds ratios for breast cancer among study participants, by quartile of age at first full-term pregnancy: Child Health and Development Studies, Berkeley, California, 1959–1966

Quartile of age (years) at first full-term pregnancy	No. of cases	No. of controls	Odds ratio*	95% CI†
Quartile 1: ≤20‡	49	93	1.00	
Quartile 2: >20–≤23	45	102	0.86§	0.52–1.43
Quartile 3: >23–≤27	70	77	1.69	1.02–2.79
Quartile 4: ≥27	61	76	1.58¶	0.92–2.72

* The risk associated with each variable was estimated by logistic regression analysis and was adjusted for race and for age at index pregnancy.
† CI, confidence interval.
‡ Referent.
§ Breslow-Day (23) chi-square test (1 df) of no difference from quartile 1: $\chi = 0.25$ ($p = 0.62$).
¶ Breslow-Day (23) chi-square test (1 df) of no difference from quartile 3: $\chi = 0.03$ ($p = 0.85$).

women with a high maternal serum level of AFP at selected ages at first full-term pregnancy, controlling for age at index pregnancy and for race. This analysis showed a linear trend of an increasing risk of breast cancer with increasing age at first full-term pregnancy for women with a high AFP level.

To investigate the relation of AFP and age at first full-term pregnancy in more detail, we divided the data into quartiles by age at first full-term pregnancy and conducted a stratified analysis. Table 4 shows the odds ratios of breast cancer estimated by using logistic regression, with indicator variables representing the four quartiles of age at first full-term pregnancy and controlling for maternal age at index pregnancy and for race. Including a term for AFP did not materially change the results. There was no difference in breast cancer risk between the group whose age at first full-term pregnancy was 21–23 years and the reference group, whose age at first full-term pregnancy was 20 years or less (Breslow-Day (23) chi-square test (1 df): $\chi = 0.25$ ($p = 0.62$)). Both of the next two groups of age at first full-term pregnancy showed elevated point estimates as compared with an age at first full-term pregnancy of 20 years or less but were not different from each other (Breslow-Day chi-square test (1 df): $\chi = 0.03$ ($p = 0.85$)). However, when women whose age at first full-term pregnancy was less than 23 years were compared with women whose first birth occurred at greater than age 23 years, the two groups were significantly different (Breslow-Day chi-square test (1 df): $\chi = 3.90$ ($p = 0.05$)). Therefore, among the women in this study, age at first full-term pregnancy greater than 23 years was associated with an increased risk, regardless of the maternal serum level of AFP.

The results of a detailed analysis of the interaction of age at first full-term pregnancy and AFP are shown in table 5. By using logistic regression with indicator variables, we compared the odds ratios for breast cancer by quartile of age at first full-term pregnancy and high and low AFP levels with the reference level (age at first full-term pregnancy quartile 1 (age less than or equal to 20 years) and low AFP level). The pattern of breast cancer risk by quartile of age at first full-term pregnancy differed between women with low AFP levels and women with high AFP levels. The women with high levels had a decreased risk at younger ages at first full-term pregnancy and an increased risk at older ages at first full-term pregnancy relative to women in the reference category. At each quartile of age at first full-term pregnancy, these data also showed a linear increase in breast cancer risk among women with high AFP levels as age at first full-term pregnancy increased. Among those women with high

TABLE 5. Odds ratios for breast cancer risk among all study participants, by quartile of age at first full-term pregnancy (FFTPA) and high* or low† levels of maternal serum alpha-fetoprotein (AFP)‡: Child Health and Development Studies, Berkeley, California, 1959–1966

Quartile of age (years) at FFTPA	Low AFP		High AFP	
	OR§,¶	95% CI§	OR¶	95% CI
Quartile 1: ≤20	1.00#		0.46	0.18–1.17
Quartile 2: >20–≤23	0.82	0.47–1.45	0.59	0.25–1.39
Quartile 3: >23–≤27	1.60	0.91–2.82	1.57	0.73–3.38
Quartile 4: ≥27	1.36	0.75–2.46	2.26	0.99–5.17
p for trend	NS§		0.01	

* Highest quartile of maternal serum AFP.
† Lowest three quartiles of maternal serum AFP.
‡ As compared with women in the lowest quartile of FFTPA and low maternal serum AFP.
§ OR, odds ratio; CI, confidence interval; NS, not significant.
¶ Estimated by logistic regression analysis and adjusted for maternal age at index pregnancy and for race.
# Referent.

versus low AFP levels, the increase in breast cancer risk was greater.

In table 6, the odds ratios from a series of stratified analyses are compared with the odds ratios computed by using logistic regression for high AFP levels in each quartile of age at first full-term pregnancy. Results in the "Continuous variable" columns were generated by entering the data separately from each quartile of age at first full-term pregnancy into the final model, that is, the age of the women during their first full-term pregnancy was analyzed as a continuous variable within each stratum. For the "Indicator variable" odds ratios, one equation was used to compute the results, with quartile 3 as the reference level. The information in this table shows that the linear increase in the risk of breast cancer among women with high levels of AFP was present and similar across all quartiles of age at first full-term pregnancy, whether the method of analysis was stratified analysis using frequency counts or the more complicated logistic regression analysis in Proc Genmod (20), either adjusted or unadjusted for confounding.

This study was based on data from women in their first pregnancy and those in their second or later pregnancy. We found that the association of high AFP levels with reduced breast cancer risk was strongest among those women whose first pregnancy was also the index pregnancy. We also found that in this group, the breast cancer risk estimated from logistic regression was 0.09 (95 percent CI 0.01–0.81) for those who had high maternal serum levels of AFP and were aged 18 years at the index pregnancy and first pregnancy (data not shown). For women in a second or later pregnancy at index pregnancy, the odds ratio for breast cancer risk was 0.49 (95 percent CI 0.22–1.11) for a high versus a low AFP level at age 18 years (data not shown). Although the increase in risk was less in the first pregnancy group, the pattern of risk was the same for both groups, increasing as age at first full-term pregnancy increased.

The analysis indicated that a high level of AFP had a protective effect only when a woman had an early first full-term pregnancy. If her first full-term pregnancy occurred after age 27 years, high serum levels of AFP did not decrease the risk of breast cancer and may have increased the risk.

DISCUSSION

The results of this study suggest that AFP can have a differential effect on breast tissue, depending on factors associated with early age at first full-term pregnancy. This finding was unexpected but is biologically plausible. Many studies have found that early age at first full-term pregnancy protects against later breast cancer risk (24–26). Research using animal models suggests that the protective effect of early age at first full-term pregnancy could be produced by a

TABLE 6. Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk among women with high* maternal serum levels of alpha-fetoprotein (AFP) as compared with women with low† maternal serum levels of AFP, by quartile of age at first full-term pregnancy, from unadjusted and adjusted analyses: Child Health and Development Studies, Berkeley, California, 1959–1966

Quartile of age at first full-term pregnancy	Unadjusted data						Adjusted data‡			
	Stratified analysis		Indicator variable		Continuous variable		Indicator variable		Continuous variable	
	OR§	95% CI	OR¶	95% CI	OR¶	95% CI	OR¶	95% CI	OR¶	95% CI
1	0.48	0.19–1.20	0.43	0.28–0.65	0.45	0.23–0.91	0.47	0.14–1.54	0.44	0.22–0.87
2	0.66	0.28–1.55	0.62	0.41–0.92	0.66	0.41–1.08	0.72	0.23–2.26	0.64	0.39–1.04
3	1.22	0.59–2.55	1.00#		0.98	0.65–1.47	1.00#		0.95	0.62–1.45
4	1.35	0.63–2.90	1.67	1.14–2.45	1.92	0.96–3.85	1.69	0.58–4.98	1.89	0.91–3.92

* Highest quartile of maternal serum AFP.
† Lowest three quartiles of maternal serum AFP.
‡ Adjusted in logistic regression model for age at index pregnancy and for race.
§ Mantel-Haenszel odds ratio estimated by stratified analysis.
¶ From logistic regression analysis.
# Referent.

decrease in the number of stem cells and a change in the sensitivity to growth stimulation from hormonal stimuli in the remaining cells (27). AFP may be part of the process of desensitization to hormonally mediated growth stimulation. AFP has been shown to enhance or down-regulate growth in cell cultures, in animal models, and in two neoplastic cell lines (MCF-7 human and MTW9A rat breast cancer cell lines) (28–31). The active portion of human AFP, which can elicit this biphasic response from cells, has recently been synthesized and characterized by Mizejewski et al. (32) and has been found in the ligand-binding region of the molecule. In fact, it is hypothesized that the mechanism by which AFP affects cell growth is modulation of growth-factor(s) binding and action at the membrane level (33).

The ability to inhibit cell growth is dependent on the conformation of the AFP molecule, which makes the binding region accessible. The conformation of the molecule is altered in response to surrounding tissue levels of estrone, estriol, estradiol, progesterone, and other ligands (6, 34–37). In one study, women younger than age 20 years were shown to have slightly lower concentrations of pregnancy estrogens than older women did, and estrogen concentrations were slightly higher during the first versus subsequent pregnancies (38). Although the estrogen concentrations in the sera of women in this study were not known, the mean serum levels of AFP differed according to age at delivery, number of previous pregnancies, and race. Therefore, even if estrogen levels were constant across age at first full-term pregnancy, the ratio of AFP to estrogen would not have been constant. It then follows that early age at first full-term pregnancy and later age at first full-term pregnancy, during which levels of AFP and steroid hormones differ, could each have different effects on breast tissue.

The results of this study agree with the protective effect reported by Jacobson et al. (12) and Thompson et al. (13) using surrogate indicators (multiple births and hypertension) for a high level of AFP during the index pregnancy. However, we found this effect among only those women with a first full-term birth at less than age 27 years. In this study, unlike the two previous studies, we found that high AFP serum levels at first birth lower the breast cancer risk more than do high AFP levels in second or later births.

It seems unlikely that either misclassification or selection bias can account for the findings in this study. Misclassification of controls as not diseased, that is, using women who should have been classified as cases as controls instead, might have been a problem in a few instances, but cases as reported to the California Cancer Registry were documented as of 1994. Follow-up and lost to follow-up status was determined by personal contact (telephone and written), by accessing and scanning the death files, and by matching California Department of Motor Vehicles files to names and birth dates of cohort members. There is still a possibility that during the study, a very small portion of eligible breast cancer cases might have been misclassified as noncases because they resided in areas of California in which tumor registry data collection began only recently. However, cancer reporting to the California Cancer Registry for the five counties in the San Francisco Bay area of California in which the majority of the CHDS members live has been complete since 1969 and became complete for the whole state in 1988.

Failure to study cases and controls who were no longer California residents might have led to selection bias if levels of serum AFP had been linked to characteristics of those who had moved. The CHDS group found that the levels of education were higher among those who had moved, but no pattern of AFP levels by educational level was present among the controls (a randomly selected sample of the CHDS population).

As previously explained, AFP levels increase rapidly during the third trimester of pregnancy, reaching a peak at approximately 32 weeks of gestation (8). If time of blood draw during the third trimester was different by age at first full-term pregnancy, a selection bias might have resulted, creating a false association between this variable and high AFP levels. Analyzing serum levels in cases and controls relative to the mean AFP level at the time of blood draw, and not in terms of an absolute level during the trimester, made this bias unlikely. Nevertheless, a stratified analysis of quartiles of age at first full-term pregnancy by blood draw date for cases and controls was performed. The results of this analysis gave no indication that the association of high serum AFP level with age at first full-term pregnancy was an artifact of differential timing of blood draw by age at first full-term pregnancy and case status. In summary, results of this study indicate that high maternal serum levels of AFP in women with an early first full-term pregnancy are associated with a decreased subsequent breast cancer risk as compared with those with low serum levels of AFP.

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