

Estrogen and α -Fetoprotein Levels in Maternal and Umbilical Cord Blood Samples in Relation to Birth Weight

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Abstract

Birth weight has been associated with a subsequent risk of breast cancer. The present study examined associations between birth weight and pregnancy estrogens and α -fetoprotein (AFP). The concentrations of estradiol, estriol, and AFP were measured in maternal and umbilical cord blood samples from 194 women during pregnancy and at birth. Birth weight was significantly positively correlated with maternal serum estradiol and estriol levels in the 29th week (estradiol: $r = 0.16$, $P = 0.03$; estriol: $r = 0.29$, $P = 0.001$) and at delivery (estradiol: $r = 0.20$, $P = 0.01$; estriol: $r = 0.41$, $P < 0.0001$) after controlling for covariates. The umbilical

cord estriol level was moderately but significantly correlated with birth weight ($r = 0.15$, $P = 0.049$). There was no significant association between umbilical cord serum estradiol and birth weight. There was no significant association between birth weight and maternal serum AFP in any gestational week. Umbilical cord AFP was significantly inversely correlated with birth weight ($r = -0.16$, $P = 0.04$). Umbilical cord AFP was unrelated to cord levels of estradiol and estriol. The data suggested a greater exposure to estriol and a lower exposure to AFP among high birth weight babies. (Cancer Epidemiol Biomarkers Prev 2006;15(8):1469–72)

Introduction

It has been proposed that breast cancer may have a prenatal origin (1). Estrogen has central role in the development of breast cancer. A variable level of estrogen exposure *in utero* has been suggested to explain the hypothesized relationships between perinatal characteristics and breast cancer risk. Among these perinatal factors, birth weight has been most consistently associated with a subsequent risk of breast cancer among offspring (2–7). Several studies examined the association between pregnancy estrogens and birth weight (8–15), but data are still limited. Most of these studies primarily focus on maternal estrogen levels.

α -Fetoprotein (AFP), an α_1 -glycoprotein that is formed in the yolk sack and in the fetal liver, plays an important role in the regulation of fetal growth (16). AFP has antiestrogenic properties and, therefore, may have an implication in the estrogen environment of the fetus. A high level of AFP in maternal serum was associated with the reduced risk of maternal breast cancer (17). Lambe et al. (18) observed that AFP levels during pregnancy varied by ethnicity, suggesting that AFP may reflect the ethnic differences in breast cancer risk in the mother and in the offspring.

The present study examined associations between birth weight and the concentrations of estrogens and AFP in maternal and umbilical cord blood samples during pregnancy and at birth.

Materials and Methods

The present study was based on participants in a longitudinal study investigating the relationships between maternal life-style, pregnancy hormones, and gestational and neonatal

factors. Women who visited a maternal clinic in Gifu, Japan and were identified as pregnant were recruited between May 2000 and October 2001. A total of 600 pregnant women agreed to participate in the study. Informed consent was obtained from each woman. This study was approved by the institutional review board.

Of the 600 women, 8 had a spontaneous or induced abortion, 34 moved or changed clinics, and 23 chose to end their participation. We did not obtain the precise number of women who were recruited on their first visit. However, we obtained information showing that, among women who gave birth in the clinic during the study period, 52.2% participated in the study. The actual participation rate should be higher because women who may have visited the clinic for the first time during middle or late pregnancy were included in the denominator. Each woman responded to a health questionnaire designed to obtain demographic characteristics, smoking status, and past medical and reproductive histories at the time of enrollment or first blood drawing.

Women visited the clinic to attend a health checkup at approximately the 10th and 29th weeks of gestation. Clinical and auxological data for the mother and fetus or newborns were recorded at these visits and at delivery. Maternal and cord blood samples were obtained at each visit and at delivery. Umbilical cord artery blood was immediately drawn after birth. The blood samples were centrifuged, and the sera were stored at -80°C until assayed. Urine sample was also stored at -80°C until assayed. The relationships of blood hormone levels to urinary biomarkers, such as isoflavones metabolites, will be described elsewhere.

We restricted the subjects for the present study to those who had had parity no more than once previously and had a singleton birth of a newborn girl. Out of the total of 250 women, women who had taken hormonal medications during the index pregnancy ($n = 10$), had been diagnosed with hypertension ($n = 1$), diabetes mellitus ($n = 1$), or thyroid disease ($n = 3$) before or during the index pregnancy, or had a fetus with a major anomaly ($n = 0$) were excluded. Twelve women who had not responded to the health questionnaire were also excluded. Another 30 women were excluded because no umbilical blood samples had been obtained. Thus, 194 pregnant women and their newborn girls were part of the present study.

Received 2/28/06; revised 4/12/06; accepted 5/25/06.

Grant support: Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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doi:10.1158/1055-9965.EPI-06-0158

Serum estradiol was measured by RIA using kits purchased from the Roche Diagnostic Japan (Tokyo, Japan). Serum estriol was measured by RIA using kits purchased from Abbott Japan Co. Ltd. (Tokyo, Japan). Serum AFP was measured by chemiluminescent immunoassay using kits purchased from Abbott Japan. The interassay coefficients of variation were 2.6% for estradiol, 10.9% for estriol, and 7.1% for AFP.

Many studies have reported that birth weight is positively associated with umbilical cord insulin-like growth factor-I (IGF-I) levels (19-22). We included measurement of umbilical cord IGF-I levels. Serum IGF-I was measured by immunoradiometric assay using kits purchased from TFB, Inc. (Tokyo, Japan). The interassay coefficient of variation was 5.6%.

Blood data were missing for 3 women at the 10th week and for 11 women at delivery because no blood samples had been obtained. Serum estriol was not measured in three umbilical cord samples because blood volume was insufficient. Serum IGF-I was not measured in 11 umbilical cord samples. Serum estriol was undetectable (<6 ng/mL) for most of the women ($n = 157$, 80.9%) in the 10th week of gestation; the value of assay sensitivity minus one unit (=5 ng/mL) was assigned for them.

For statistical analyses, Spearman rank correlation was used to assess the association between birth weight and estradiol, estriol, and AFP because the concentrations of these estrogens and AFP were not normally distributed. Several factors covering the spectrum of the likely measured confounders were used for adjustment. Several variables related to demographic factors, reproductive history, lifestyle factors, and clinical data during pregnancy were examined as potential confounders. These variables included age, parity, length of gestation, smoking status, years of education, pre-pregnancy height and weight, weight gain, and weeks of gestation at the time of measurement. By regressing birth weight and each hormone or AFP separately on the confounders, we accomplished adjustment for these potential confounders. The correlations (Spearman) between the residuals were then calculated. We also calculated the mean birth weight according to quartiles of serum hormone and AFP levels using the analysis of covariance method. All statistical analyses were done using Statistical Analysis System (SAS Institute, Cary, NC).

Results

The women ranged in age at entry from 17 to 40 years (mean, 28.7). The mean (SD) length of gestation was 39.6 (1.0) weeks. The means (SD) of height, pre-pregnancy weight, and weight gain were 158.0 (5.0) cm, 51.7 (7.6) kg, and 9.5 (3.0) kg, respectively. About half of the women (56.9%) had given birth previously. The percentages of current and ex-smokers were 4.7% and 21.9%, respectively.

The serum levels of estrogen and AFP and their relations to birth weight are shown in Table 1. Maternal serum estradiol levels in the 29th week and at delivery were significantly positively correlated with birth weight after controlling for covariates. There was no significant association between umbilical cord serum estradiol and birth weight. Maternal serum estriol levels in the 29th week and at delivery were significantly positively associated with birth weight. Cord estriol level was moderately but significantly correlated with birth weight. There was no significant association between birth weight and maternal serum AFP at any gestational week. Umbilical cord AFP was significantly inversely correlated with birth weight. In the cord blood, AFP level was unrelated to estradiol and estriol levels; the correlation coefficients were 0.05 and -0.01, respectively. The mean birth weight according to quartile of hormone and AFP values was also calculated. The mean birth weight was 13.3% higher in the highest quartile than the mean birth weight in the lowest quartile of maternal

estriol level at delivery. The mean birth weight was 6.1% lower in the highest quartile than the mean birth weight in the lowest quartile of umbilical cord AFP level. The variation of birth weight according to umbilical cord estriol was small.

Scattergrams were presented to show statistically significant associations of birth weight with maternal serum estradiol and estriol levels and umbilical cord AFP level (Fig. 1).

There was a significant association between umbilical cord IGF-I and birth weight after controlling for the covariates ($r = 0.57$, $P < 0.0001$). The means of birth weight were 2,790.6 and 3,253.9 g for the lowest and the highest quartiles of umbilical cord IGF-I level, respectively.

Discussion

To our knowledge, eight studies have assessed the relationships between birth weight and estrogen concentration in maternal or umbilical cord blood (8-15). A positive association between maternal estriol and birth weight was consistently reported by Knopp et al. ($r = 0.13$ in the 27th week; ref. 9), Kaijser et al. (11), Peck et al. ($r = 0.32$ at third trimester; ref. 14), and Mucci et al. (increase of 8.4 g of weight per one SD of estriol in the 27th week; ref. 15). A significant positive association of maternal estradiol with birth weight was reported by Peck et al. ($r = 0.17$; ref. 14), which were similar to our result but not in others (9, 14, 15). A lack of association between cord estradiol and birth weight has been reported (8, 12). Only one study reported by Troisi et al. (13) included the measurements of both maternal and umbilical cord estrogen concentrations. In their study of 86 mothers and their newborns, birth weight was significantly correlated with maternal estriol ($r = 0.36$) but not with cord estriol ($r = -0.08$). They suggested that the lack of association of birth weight with hormones measured in the fetal circulation is not consistent with the hypothesis that greater estrogen concentrations in high birth weight babies mediate the positive association with breast cancer risk. There is no other report addressing the relationship between cord estriol and birth weight. In our study, both maternal estrone and cord estriol concentrations had a significant positive association with birth weight, suggesting a greater exposure to estriol among high birth weight babies. However, we should mention that the association between cord estriol and birth weight was modest. Estriol originates almost exclusively from fetal dehydroepiandrosterone sulfate (23). The observed stronger correlation of birth weight with maternal estriol than with cord estriol may indicate that estriol metabolism in mothers is relevant to the fetal growth. The reason for this is not clear. To measure the levels of estrogen to which a fetus is exposed, estrogen levels in maternal blood samples may not be adequate. However, as cord blood sampling is done at a single point in time (the moment of delivery), it is also possible that cord blood hormone levels may not reflect exposure levels. Both cord and maternal hormone data could be surrogate markers of the hormone environment of the fetus.

It is also possible that certain correlates of estrogen levels other than estrogen may be the determinant of birth weight. For example, IGF-I is a major growth promoter in the fetus. We included measurement of umbilical cord IGF-I levels. The associations of birth weight with maternal and cord estriol levels were attenuated after additional adjustment for IGF-I ($r = 0.29$, $P = 0.0003$ and $r = 0.04$, $P = 0.57$, respectively). However, considering that regulation of fetal growth by steroid hormones and growth factors is biologically complex and multifactorial, the use of statistical adjustment may be not appropriate.

To our knowledge, only one study has assessed the association between AFP and birth weight and reported that the umbilical cord AFP level was nonsignificantly inversely

Table 1. Maternal and cord estrogen and AFP levels and their associations with birth weight

	Mean	Median	Range	Crude <i>r</i> (<i>P</i>)	Adjusted* <i>r</i> (<i>P</i>)	Mean birth weight (g)*			
						Quartile 1	Quartile 2	Quartile 3	Quartile 4
Estradiol (pg/mL)									
10th week	2,687	2,305	632-9,090	0.08 (0.26)	0.08 (0.30)	3,038	2,842	2,987	3,088
29th week	21,754	20,600	7,390-38,000	0.11 (0.11)	0.16 (0.03)	2,922	2,950	3,015	3,095
Delivery	31,419	31,900	3,960-67,300	0.12 (0.10)	0.20 (0.001)	2,920	2,952	3,003	3,095
Cord	5,907	4,325	554-41,300	-0.15 (0.04)	-0.06 (0.44)	2,992	3,008	3,040	2,944
Estriol (ng/mL)									
10th week	5.7	5.0	5.0-18.5	0.13 (0.07)	0.14 (0.07)	—	—	—	—
29th week	91.0	85.8	31.4-301	0.21 (0.004)	0.29 (0.0001)	2,839	2,987	3,019	3,155
Delivery	239.4	218	35.4-707	0.25 (0.001)	0.41 (0.0001)	2,781	2,980	3,057	3,151
Cord	2,126	2,050	725-3,860	0.16 (0.03)	0.15 (0.049)	2,881	3,042	3,073	2,978
AFP (ng/mL)									
10th week	12.1	8.7	2.8-380	0.001 (0.98)	-0.04 (0.58)	2,955	3,039	3,000	2,980
29th week	283.9	261.0	87-723	-0.003 (0.96)	0.07 (0.34)	2,987	2,996	2,914	3,075
Delivery	203.0	185.0	47.8-954	-0.03 (0.70)	0.07 (0.38)	2,976	2,963	2,979	3,055
Cord	96,503	91,050	22,300-327,000	-0.24 (0.001)	-0.16 (0.04)	3,098	2,985	2,992	2,909

*Adjusted for age, parity, length of gestation, smoking status, years of education, pre-pregnancy height and weight, weight gain, and weeks of gestation at the time of measurement.

†A 5.0 ng/mL was assigned for 157 (80.9%) women with undetectable levels. The mean birth weight according to quartile of estriol level was not calculated.

associated with birth weight (12). There has been no study addressing the relationship of maternal AFP level to birth weight. We observed a modest but significant inverse correlation between umbilical cord AFP and birth weight. The association of maternal AFP with birth weight was almost null. Although human AFP peptide has shown to bind the estrogen receptor and estrogens, there was no

suggestion that the AFP level affects estrogen levels in either maternal or cord bloods in the present study. The AFP level was also unrelated to the IGF-I level ($r = -0.04$). AFP peptides inhibit both estrogen-dependent and estrogen-independent growth events (16). The implication of AFP in the pregnancy estrogen hypothesis as well as the etiology of breast cancer should be studied further.

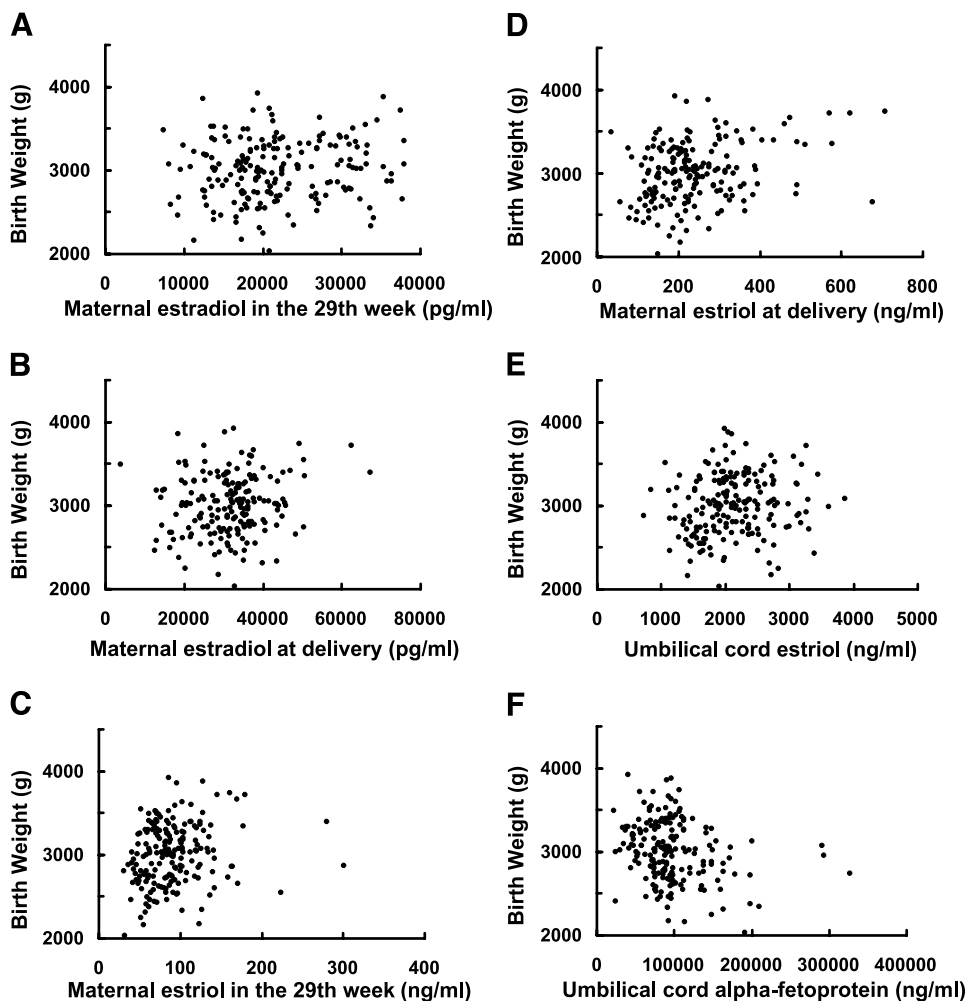


Figure 1. Birth weight versus maternal and umbilical cord estrogen and AFP levels.

One of the advantages of the present study is repeated measurement of estrogens and AFP during pregnancy. We did not observe any significant association of birth weight with these measures in the 10th week of gestation. Estrogen and AFP levels early in pregnancy may not be relevant to birth weight and later breast cancer risk. However, there was a limitation for the measurement of low estriol levels in early pregnancy. In addition, we should keep in mind that maternal values may only serve as proxy values for the fetal circulation.

Our study showed independent links between birth weight and pregnancy estriol and between birth weight and AFP levels. We inferred that pregnancy estriol and AFP may be associated with a breast cancer risk in offspring. However, there is an uncertainty about the predictive performance of these factors as indicators of risk. Implication of these factors in the future risk of breast cancer among offspring is worth further investigation.

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