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Placental characteristics as a proxy measure of serum hormone and protein levels during pregnancy with a male fetus

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Abstract

Objective—In utero exposure to steroid hormones may be related to risk of some cancers such as testicular germ cell tumors (TGCT). To determine whether placental characteristics are good surrogate measures of maternal biomarker levels, we evaluated the correlations in mothers of sons at higher (whites, n = 150) and lower (blacks, n = 150) risk of TGCT. Associations with birth weight were also examined.

Methods—All mothers, participants in the Collaborative Perinatal Project, were primigravidas who gave birth to male singletons. Associations between placental weight and placental thickness and third trimester biomarker levels were evaluated using linear regression. Partial correlation coefficients for placental characteristics and birth weight were also estimated.

Results—Placental weight was positively correlated with alpha fetoprotein (AFP), sex hormone-binding globulin (SHBG), testosterone, estradiol and estriol in whites, and AFP and estriol in blacks. Placental thickness was not associated with any biomarker. After adjustment for placental weight, birth weight was not correlated with any biomarker.

Conclusions—In these data, placental weight was modestly correlated with third trimester biomarker level; however, it appeared to be a better surrogate for third trimester biomarker level than birth weight. Placental thickness had limited utility as a surrogate measure for biomarker levels.

Keywords

cancer risk; placental weight; birth weight; maternal hormones

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INTRODUCTION

The intrauterine hormonal milieu may be an important determinant of the risk of some hormone-related tumors such as breast cancer and testicular germ cell tumors (TGCT) [1-5]. This hypothesis has proven difficult to test, as maternal hormone levels and pregnancy biomarkers are not routinely measured and cancer outcomes occur decades later. Many studies have attempted to overcome these problems by examining the relationship between maternal or perinatal factors such as birth weight and/or placental weight and cancer under the assumption that these characteristics are good proxy measures of in utero hormone or biomarker conditions.

A number of studies have examined relationships between birth weight, as well as other maternal factors, and maternal biomarker levels [6-18]; however, there are few studies evaluating the utility of placental characteristics as markers of exposure in utero and the existing studies have primarily focused on placental weight [7,11,13]. To assess the validity of placental size (weight and thickness) as a proxy for maternal hormone and protein biomarker levels during pregnancy we evaluated the correlation between placental weight, placental thickness and birth weight with maternal alpha fetoprotein (AFP), sex hormone-binding globulin (SHBG), testosterone, estradiol and estriol levels in third-trimester serum samples from mothers of populations at higher (white Americans) and lower (black Americans) risk of TGCT. Further we explored the relative utility of these biomarkers (AFP, SHBG and hormones) compared to one another.

MATERIALS AND METHODS

We used data from a study of 300 black and white mothers who participated in the Collaborative Perinatal Project (CPP) to evaluate our hypothesis. Briefly, the CPP was a cohort study originally designed to examine perinatal risk factors for neurologic disorders in offspring [19]. Between 1959 and 1964 the study enrolled 48,197 women upon presenting for prenatal care at 12 medical centers in the United States; centers were located in Baltimore MD, Boston MA, Buffalo NY, Memphis TN, Minneapolis MN, New Orleans LA, New York NY (two centers), Philadelphia PA, Portland OR, Providence RI and Richmond VA. The study was purposefully not intended to be representative of the United States as each clinical site utilized its own sampling approach and selection ratio (varying from 10-100% of eligible women either by enrolling a random or systematic sample or all women). There were 142,130 pregnancies among the 48,197 women, including 54,390 pregnancies that were prospectively (observed) captured by the CPP, and 87,777 prior histories retrospectively reported by mothers upon enrollment of the index pregnancy. Children born during the study period were followed until 7 years of age. Of the 54,390 prospectively observed pregnancies, 13,248 white males and 13,109 black males survived for at least one year. As part of data collection, the mothers were asked to donate non-fasting blood samples at approximate 8-week intervals throughout their pregnancies. Serum samples were stored in glass vials at -20 degrees Celsius with no recorded thaws.

Details of the study population of 300 black and white mothers have been previously described [20,21]. Inclusion criteria were based on characteristics of both the mother and the baby. Maternal inclusion criteria included: first pregnancy, length of gestation between 26 and 48 weeks, and availability of blood samples from both the first and third trimester. Inclusion criteria based on characteristics of the infants included: male sex, singleton birth, birth weight of at least 500 grams, baby lived for at least one year, and no diagnoses of undescended testes, late developing testes, retractile testes or other malformations possibly related to maternal hormone levels (e.g., CNS, genitourinary, inguinal hernia, hydrocele,

supernumerary nipples). In total, 162 black and 652 white mothers satisfied the study inclusion criteria. The primary limiting criterion was the availability of first trimester blood samples as the median entry time into the study for the entire CPP population was 20 weeks' gestation. Additionally, the nulliparity criterion restricted the pool of eligible participants to approximately one-third of the whole population. Each of the 162 black mothers was matched to a white mother on the closest blood draw dates. The matches were then reordered to select the 150 pairs best matched on draw dates. The length of gestation for the 300 selected mothers ranged from 30 to 43 weeks.

Placental weight (g) and thickness (mm) were measured according to standard protocol [22,23]. Placental thickness at the center of the chorionic disc was recorded in units of 1 mm, by piercing the disc with a knitting needle on which millimeter marks were inscribed. We evaluated placental thickness as both a continuous variable and a dichotomous variable because of the bimodal distribution of the data. Gestational age was calculated based on the last menstrual period in rounded weeks. Maternal characteristics were recorded at enrollment and throughout pregnancy by study interviewers. Maternal age was coded as age at (enrollment) in years, and maternal height was measured in inches. Maternal weight prior to pregnancy was self-reported in pounds. Body mass index (BMI) was calculated from maternal height and weight.

Laboratory Assays

The selected biomarkers were assessed at the Reproductive Endocrine Research Laboratory of the University of Southern California Keck School of Medicine. Estriol, estradiol, and testosterone were determined by well-established and validated radioimmunoassay methods [24,25]. SHBG and AFP were quantified by direct chemiluminescent immunoassay using the Immulite analyzer. (Siemens Medical Solutions Diagnostics, Malvern, PA) A total of 68 samples from a single blood pool were assayed for quality control (QC) purposes. Each sample was assigned a unique, random identification number in order to blind subject identities. Samples were analyzed in 17 batches with each batch containing four QC samples. The intra-assay coefficient of variation ranged from 3.7% to 12.6% for all hormones, while the inter-assay coefficients of variation ranged from 4.8% to 13.3%. We did not estimate levels of free testosterone or free estradiol using SHBG and total testosterone or total estradiol because the estimation methods are not reliable during pregnancy [26].

Statistical analysis

The mean and standard deviation for the pregnancy characteristics were calculated stratified by maternal race (white or black). Multiple linear regression modeling was employed to estimate the mean change in placental weight for an increase of one standard deviation in first and third trimester pregnancy hormone level adjusting for gestational age at blood draw in weeks, study center, maternal age (years), pre-pregnancy BMI (kg/m^2), maternal smoking (yes/no) and birth weight (grams). Multiple logistic regression modeling was used to estimate the association between placental thickness (dichotomized at > 20 mm vs. ≤ 20 mm) and first and third trimester biomarker level adjusting for gestational age at blood draw, study center, maternal age, birth weight, pre-pregnancy BMI and maternal smoking. The distributions of the studied markers did not sharply deviate from normality and no transformation was deemed necessary. We evaluated non-linear associations using multi-knot splines and found no deviation from linearity. First and third trimester biomarker levels divided by the population standard deviation were used in the primary statistical analyses. We used one standard deviation increment for each biomarker measurement to facilitate comparability among the regression estimates.

We adjusted for gestational age at blood draw because the variable was a matching factor in the study design. Maternal age was included in the model because white mothers in our study population were older than black mothers. Although pre-pregnancy BMI and smoking status at registration did not differ between the two groups, adjustment for these factors resulted in changes of varying magnitude in the standardized beta estimates for the change in pregnancy characteristics; thus, they were also included in the final models. Finally, because birth weight and placental weight are correlated ($r = 0.65$), and because we wanted to examine the association of placental weight above and beyond any association with birth weight, regression models evaluating placental weight and placental thickness were adjusted for birth weight. The results did not differ substantially when birth weight was removed from the model.

We calculated partial correlation coefficients for the individual first and third trimester biomarker measurements with the pregnancy characteristics adjusting for gestational age at blood draw in weeks, study center, maternal age, pre-pregnant BMI and maternal smoking. We further evaluated the partial correlations for placental weight adjusting for birth weight, placental thickness (continuous in mm) adjusting for birth weight, and birth weight adjusting for placental weight. All tests of significance were two sided with $\alpha = 0.05$. Statistical analyses for the study were performed using SAS software, version 9.1 (SAS Institute, Inc. Cary, NC).

RESULTS

As noted above the white mothers tended to be older than the black mothers (Table 1). Pre-pregnancy BMI and pregnancy weight gain were similar between the two groups.

Placental weight and placental thickness were not associated with any of the first trimester biomarker levels for either white or black participants (results not shown).

Among white mothers, a 16.5 gram increase in placental weight was positively associated with a one standard deviation increase in third trimester AFP [p-value = 0.03] (Table 2). Placental weight was also positively associated with third trimester SHBG [standardized beta (gram change in placental weight per standard deviation increase in biomarker) = 15.7, p-value = 0.02], testosterone [standardized beta = 42.1, p-value = 0.01], estradiol [standardized beta = 12.6, p-value = 0.05], and estriol [standardized beta = 20.0, $p < 0.01$] among white mothers. For black mothers, placental weight was positively associated with third trimester AFP [standardized beta = 20.5, $p < 0.01$] and third trimester estriol [standardized beta = 12.7, p-value = 0.04].

Placental thickness was not associated with any of the third trimester biomarker levels for either white or black participants (results not shown). With the exception of the association between testosterone and placental weight the associations between the biomarkers and placental weight, placental thickness or birth weight were not significantly different comparing black and white participants.

As expected, the third trimester biomarkers that were significantly associated with placental weight in the regression analysis were also positively correlated with placental weight when evaluating the partial correlation coefficients (Table 2). The magnitude of these associations, however, was stronger when birth weight was not included in the model. The continuous measure of placental thickness was not correlated with any of the third trimester biomarker levels (results not shown). Finally, with the exception of SHBG in white [partial corr = 0.22, p-value = 0.01] and black participants [partial corr = 0.17, p-value = 0.04] and estriol in black participants [partial corr = 0.29, p-value < 0.01] birth weight was not positively

correlated with third trimester biomarker levels; after adjusting for placental weight none of the third trimester biomarkers were correlated with birth weight (Table 2).

In a previously published manuscript [20], we evaluated associations between neonatal characteristics (birth weight, birth length, head circumference and gestational age) and maternal hormone levels, namely testosterone, estradiol and estrone. In that paper we reported a positive association between birth weight and third trimester estriol in blacks but not whites and no association between birth weight and testosterone or estradiol [20]. At the time of publication we did not have laboratory results for SHBG or AFP. Birth weight was not associated with third trimester AFP in either whites or blacks [standardized beta for birth weight and AFP in whites = 8.5, p-value = 0.85; standardized beta blacks = -6.6, p-value = 0.88] while birth weight was positively associated with SHBG among both white and black mothers [standardized beta for birth weight and SHBG in whites = 97.5, p-value = 0.01; standardized beta blacks = 63.7, p-value = 0.04]. (Table 2)

DISCUSSION

In the present study, a prospective evaluation of AFP, SHBG, hormone levels during pregnancy and placental characteristics, we found that placental weight was positively associated with third trimester AFP, SHBG, testosterone, estradiol and estriol among whites, and with third trimester AFP and estriol among blacks. However, these associations were modest based on the magnitude of the partial correlation coefficients. Results were not supportive of an association between any of the placental characteristics and first trimester maternal AFP, SHBG, and hormone levels. They were also not supportive of an association between third trimester maternal AFP, SHBG, and hormone levels and placental thickness. We followed up on results published in a previous manuscript [20] and reported that birth weight was positively associated with third trimester SHBG among both whites and blacks and third trimester estriol in blacks; however, in all instances the magnitude of the correlation between these biomarkers and placental weight was greater.

To our knowledge, only two studies have evaluated an association between placental weight and maternal estrogen levels during pregnancy [11,13]. The first, a study of multiparous women in three Scandinavian cities who gave birth to either a male or female infant, reported a positive association between estriol levels during pregnancy (at gestational weeks 17, 25, 33 and 37) and placental weight [11]. The authors also reported that birth weight was a stronger predictor of estriol levels than placental weight [11]; however, they used the geometric mean corrected area under the curve to estimate the total estriol load during pregnancy rather than an estimate of third trimester estriol levels as used in the current study. The second, a study of 230 white pregnant women who gave birth to either a male or female infant at Beth Israel Hospital in Boston Massachusetts, reported that estriol and SHBG were positively associated with placental weight at the 27th gestational week [13]. The findings of the present study, positive associations between third trimester estriol level and placental weight in white and black sons and third trimester SHBG level and placental weight in white sons, are consistent with these results. Further, the magnitude of the associations between placental weight and hormone level in whites is similar between the study in Massachusetts (mean change in placental weight per standard deviation increase in: estradiol 10.1, estriol 42.6 and SHBG 33.1) and our study (mean change in placental weight per standard deviation increase in: estradiol 16.8, estriol 30.2, and SHBG 29.6), suggesting that our results might be more broadly generalized to pregnancies with female fetuses and that placental weight, rather than birth weight, may be a surrogate for in utero hormone level in breast cancer studies.

The relationship between maternal biomarker levels and placental weight is of interest because birth weight and other perinatal characteristics have been associated with TGCT in a number of studies [4,27-33]. However, only one study to date has included placental weight in their evaluation of perinatal characteristics and risk of TGCT [31]. Akre and colleagues reported a significantly increased risk of seminoma with increasing placental weight in a nested case-control study of men born in Sweden between 1920 and 1978 [31]. The authors of the study emphasized the role of higher levels of pregnancy estrogens, represented by perinatal characteristic proxies including higher placental weight, as the potential mechanism for increased seminoma TGCT risk [31]. However, in the context of our results it is difficult to know whether the increased risk of TGCT is due to in utero estrogen level, as the associations between placental weight and the estrogens were not different between whites and blacks, and TGCT occurs more frequently among whites than blacks. Based on our results it could be hypothesized that the increased risk of TGCT in the Swedish study could be due to increased exposure to testosterone in utero, given that increased placental weight was positively associated with third trimester testosterone level in whites but not in blacks.

Our study demonstrates that placental weight was a better surrogate for in utero third trimester biomarker levels than birth weight; suggesting that placental weight could also be, if available, a surrogate measure of in utero AFP, SHBG, and hormone levels in cancer studies where birth weight was used previously. Unlike birth weight, placental weight is not captured on the birth certificate and may not be available to all studies. However, placental weight is often captured in the medical record, thus obtainable, and placental weights are readily available for entire populations, such as in Norway, Sweden and Finland.[34-36]

The present study has several strengths; the major advantages were that the study was conducted prospectively, included mothers of more than one ethnic group, included only mothers pregnant with male fetuses, and included only mothers giving birth to their first child. The evidence suggesting that maternal biomarker levels may be affected by fetal sex and maternal parity make the last two advantages critical to the extrapolation of results to TGCT studies [14,37,38]. In addition, the sample size permitted sufficient statistical power to study the associations separately in populations at lower (blacks) and higher (whites) risk of TGCT [20,21].

Although our results are consistent with those of others [11,13], there are limitations to our study. The biomarkers levels were measured on samples stored for approximately 40 years; however, prior studies of freeze-thaw cycles of serum levels in stored samples have shown that steroid hormones are fairly robust [39]. In addition, the biomarker levels in the present study are equivalent to those reported in other studies examining newly drawn samples [40]. Finally, because a large number of comparisons were analyzed caution should be exercised in interpreting the results.

Presumably, placental characteristics and birth weight represent the integrated effect of growth over the entire pregnancy, and if AFP, SHBG, and hormone levels during pregnancy play a role in health related outcomes later in life, it is most likely a result of these concentrations over some period of pregnancy. We evaluated biomarker levels during the first trimester, but did not observe substantial associations with placental characteristics or birth weight. Follow-up of this research question with multiple measures of biomarkers over the third trimester would better elucidate the correlation of pregnancy biomarkers and placental size or birth weight. Follow-up of these results in non-first born infants and females is also warranted.

In conclusion, our results suggest that the utility of placental weight as a surrogate measure for third trimester AFP, SHBG, and hormone levels of first born male infants was modest overall. The other characteristics evaluated, placental thickness and birth weight, had limited utility as surrogate measures for third trimester levels.

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Table 1

Distributions of maternal and neonatal characteristics in white and black participants

Characteristics	White (n=150)		Black (n=150)	
	mean	std dev	mean	std dev
Placental weight (g)	438.2	79.6	430.0	89.7
Birth weight (g)	3323.9	394.2	3323.9	394.2
Placental thickness (mm)	23.5	3.0	20.6	4.7
Maternal age (years)	22.3	3.7	19.6	4.3
Maternal height (cm)	63.9	2.6	63.5	2.7
Pre-pregnant weight (kg)	56.0	8.3	56.9	9.5
Pre-pregnant BMI (kg/m ²)	21.3	3.1	21.9	3.3
Pregnancy weight gain (kg)	9.7	4.0	10.7	5.1
Gestational age (wk)	39.3	2.0	38.6	2.4
<i>Third trimester biomarker levels</i>				
AFP (IU/mL)	101.1	190.1	155.2	207.2
SHBG (nmol/L)	159.1	504.0	206.3	543.8
Testosterone (ng/mL)	0.6	1.2	2.0	2.0
Estradiol (ng/mL)	8.7	19.2	9.7	18.4
Estriol (ng/mL)	5.0	11.3	7.0	11.3
Preeclampsia	<u>n</u>	<u>(%)</u>	<u>n</u>	<u>(%)</u>
Yes	2	(1.3%)	7	(4.7%)
No	148	(98.7%)	143	(95.3%)
Anemia during pregnancy				
Yes	11	(7.3%)	52	(34.7%)
No	139	(92.7%)	96	(64.0%)
Unknown	0	(0.0%)	2	(1.3%)
Gestational diabetes				
Yes	2	(1.3%)	3	(2.0%)
No	148	(98.7%)	145	(96.7%)
Unknown	0	(0.0%)	2	(1.3%)
Maternal smoking during pregnancy				
Yes	53	(35.3%)	43	(28.7%)
No	97	(64.7%)	107	(71.3%)

Table 2

Standardized regression coefficients, partial correlation coefficients and 95% confidence intervals for placental weight, birth weight and third trimester maternal serum biomarker concentrations among white and black sons.

	White				Black			
	Estimate ^a	Corr ^b	(95 % CI)	p-value	Estimate ^d	Corr ^b	(95 % CI)	p-value
Placental weight (grams)								
AFP	17.3	0.16	(-0.02, 0.33)	0.07	22.4	0.31	(0.14, 0.47)	<0.01
SHBG	29.6	0.31	(0.14, 0.46)	<0.01	16.6	0.23	(0.05, 0.39)	0.01
Testosterone	41.7	0.19	(0.01, 0.35)	0.04	5.9	0.06	(-0.12, 0.24)	0.51
Estradiol	16.8	0.19	(0.01, 0.35)	0.04	17.2	0.22	(0.04, 0.39)	0.02
Estriol	30.2	0.31	(0.14, 0.46)	<0.01	26.2	0.33	(0.16, 0.48)	<0.01
Placental weight (grams) adjusted for birth weight								
AFP	15.7	0.20	(0.02, 0.36)	0.03	20.5	0.35	(0.18, 0.50)	<0.01
SHBG	16.5	0.22	(0.04, 0.38)	0.02	9.2	0.15	(-0.03, 0.32)	0.09
Testosterone	42.1	0.25	(0.07, 0.41)	0.01	10.2	0.13	(-0.05, 0.31)	0.15
Estradiol	12.6	0.18	(0.00, 0.35)	0.05	8.6	0.13	(-0.05, 0.31)	0.14
Estriol	20.0	0.26	(0.09, 0.42)	<0.01	12.7	0.19	(0.01, 0.36)	0.04
Birth weight (grams)								
AFP	8.5	0.02	(-0.16, 0.19)	0.85	-6.6	-0.01	(-0.18, 0.15)	0.88
SHBG	97.5	0.22	(0.04, 0.38)	0.01	63.7	0.17	(0.01, 0.33)	0.04
Testosterone	27.5	0.03	(-0.15, 0.20)	0.77	0.8	0.00	(-0.16, 0.17)	0.98
Estradiol	42.0	0.10	(-0.08, 0.27)	0.28	64.8	0.16	(-0.01, 0.32)	0.06
Estriol	76.1	0.17	(-0.01, 0.33)	0.07	117.7	0.29	(0.13, 0.43)	<0.01
Birth weight (grams) adjusted for placental weight								
AFP	-41.4	-0.11	(-0.28, 0.07)	0.23	-58.3	-0.18	(-0.35, 0.00)	0.05
SHBG	12.5	0.04	(-0.15, 0.21)	0.70	13.8	0.06	(-0.12, 0.24)	0.54
Testosterone	-131.4	-0.17	(-0.34, 0.01)	0.07	-54.0	-0.14	(-0.31, 0.05)	0.14
Estradiol	-20.5	-0.06	(-0.24, 0.12)	0.49	22.6	0.08	(-0.11, 0.26)	0.40
Estriol	-15.0	-0.04	(-0.22, 0.14)	0.65	48.0	0.14	(-0.04, 0.31)	0.13

Each biomarker was rescaled by dividing by its standard deviation from the entire population

^aEstimate is the change in placental weight or birth weight per standard deviation increase in biomarker adjusted for study center, gestational age (wks), maternal age (yrs), pre-pregnancy BMI (kg/m²), maternal smoking (yes/no) and birth weight (g) or placental weight (g) as listed

^b Partial correlation coefficient for individual biomarker adjusted for study center, gestational age (wks), maternal age (yrs), pre-pregnancy BMI (kg/m²), maternal smoking (yes/no) and birth weight (g) or placental weight (g) as listed

^c P-value for the interaction of race and the biomarker