Inverse Correlation Between Maternal Weight and Second Trimester Circulating Cell-Free Fetal DNA Levels

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OBJECTIVE: Clinical applications of the analysis of cell-free fetal DNA in maternal plasma and serum are expanding. However, use of fetal DNA during prenatal screening requires knowledge of variables that might affect its levels in the maternal circulation. We conducted this study to estimate the effect of selected demographic factors on fetal DNA levels in the first and second trimesters of pregnancy.

METHODS: We developed a database that included fetal DNA levels and clinical information, such as maternal age, ethnicity, weight, and smoking history. We measured fetal DNA levels in maternal plasma and serum using real-time quantitative polymerase chain reaction amplification of a Y chromosome specific sequence. The fetal DNA data from fresh first trimester plasma and previously frozen second trimester serum samples were analyzed separately. Fetal DNA levels were adjusted according to gestational age and storage time and then analyzed in association with the demographic factors.

RESULTS: In the first trimester group, no significant association between maternal age, weight, ethnic background, or smoking and plasma fetal DNA levels was observed. In the second trimester group, a significant inverse correlation between maternal weight and serum fetal DNA level was demonstrated ($r = -0.26$, $P = .007$). This was especially prominent when the mothers weighed more than 170 lb ($P = .001$). Maternal age, ethnicity, and smoking were not significantly associated with the second trimester serum fetal DNA levels.

CONCLUSION: Fetal DNA levels are affected by maternal weight in the second trimester. A correction for this effect may be needed in larger-scale studies or for future clinical applications that measure cell-free fetal nucleic acids in maternal circulation. (Obstet Gynecol 2004;104:545–50. © 2004 by The American College of Obstetricians and Gynecologists.)

LEVEL OF EVIDENCE: II-3

The current standard of antenatal care in many parts of the world is to offer screening for certain fetal aneuploidies or anomalies through the analysis of multiple biochemical markers in maternal serum. These fetal-placental glycoproteins and hormones, namely, alpha fetoprotein, human chorionic gonadotropin, unconjugated estriol, and inhibin A, are released from the feto-maternal unit into the maternal circulation. Deviation of the levels of these analytes from the expected median value at each gestational age could reflect fetal or placental pathology.[1] An additional fetal genetic marker, cell-free fetal DNA, has been hypothesized to originate from either placental apoptosis or the trafficking of intact fetal cells through the fetomaternal interface.[2] The analysis of circulating fetal DNA levels, therefore, may also be useful to identify certain abnormalities of the fetus or the placenta.

In a previous study, we showed that fetal DNA levels were independent of the other serum analytes, and could be combined with the quadruple serum screening markers to modestly improve the detection performance of Down syndrome screening.[3] In addition, fetal DNA measurement may be useful to detect women at risk to develop preeclampsia later in gestation.[4] The possibility of broader clinical implementation, however, cannot be accomplished without knowledge of the potential factors that might influence fetal DNA levels.

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Dr. Wataganara's maternal-fetal medicine fellowship was supported in part by the Anandamahidol Foundation, Thailand. Dr. Peter was supported by the Agency for Healthcare Research and Quality grant T32 HS00060. Funding was also received from grants NO1-HD43204 and RO1-HD42053 (Dr. Bianchi) from the National Institutes of Health.

The authors thank Jacob Canick, PhD, Angela Chen, MD, Erik LeShane, BS, and Thomas Lee, MD, for sample recruitment and analysis of fetal DNA levels.

0029-7844/04/$30.00
doi:10.1097/01.AOG.0000137352.93110.15

Several demographic variables have been reported to affect the level of maternal serum proteins in the first and second trimester of pregnancy. Factors that affect serum markers include ethnic background, maternal weight, and smoking status.[5] We hypothesized that fetal DNA levels might be affected by factors that influence the placentally derived serum proteins. In this retrospective study, we investigated the association between fetal DNA levels in maternal plasma or serum during the first and second trimesters of pregnancy and these demographic factors. The effect of maternal age was also explored. Knowledge of the effects of these surrounding variables upon fetal DNA levels may enable us to interpret the results of this novel genetic analysis more accurately.

MATERIALS AND METHODS

We developed a database that included previously measured maternal fetal DNA levels and newly acquired clinical information regarding the study subjects, such as maternal age, ethnicity, weight, and smoking status. This clinical information was obtained from the electronic database systems from Women and Infants' Hospital, Providence and Boston Medical Center, Boston, Massachusetts. The original databases were created as part of routine patient care in the maternal serum screening and family planning programs, respectively. Fetal DNA levels were measured and analyzed in previously published studies.[6–8] All of the studies were approved by the institutional review boards at Tufts-New England Medical Center and Boston Medical Center, Boston, Massachusetts and Women and Infants' Hospital, Providence, Rhode Island. We pooled all of the primary studies into a new database that included both the relevant clinical information and fetal DNA measurements from 181 and 226 pregnant women in their first and second trimesters, respectively.

Blood samples from the first trimester pregnant women were collected between August 2002 and April 2003 at Boston Medical Center. Archived serum samples obtained from second trimester pregnant women were collected between March 1999 and June 2001 and were frozen at −20°C until analysis. Only one sample per subject was included for statistical analyses. All fetal DNA quantitation assays were performed in the Division of Genetics, Tufts-New England Medical Center. Extraction of DNA and real-time quantitative polymerase chain reaction for the Y chromosome specific *DYS1* sequence were performed as previously described.[7] Levels of fetal DNA were expressed in genome equivalents per milliliter (GE/mL). Fetal gender was confirmed by genetic analysis of the products of conception or at delivery.

After the exclusion of known female and aneuploid fetuses, 91 first trimester samples and 126 second trimester samples were eligible for further analyses. Due to the difference in the nature of samples included in the database, first trimester and second trimester samples were analyzed separately. The first group comprised fetal DNA data from freshly processed first trimester plasma samples from women who opted for elective termination of pregnancy for social indications (ie, no known fetal pathology). The second group included fetal DNA data from archived second trimester serum samples that were the residuals from routine clinical second trimester maternal serum screening. Therefore, the prevalence of fetal pathology in these 2 groups should be similar to that which occurs in the general population.

Fetal DNA levels in each group were analyzed in connection with maternal age, weight, ethnicity, and smoking status. If an association was observed from linear regression analysis of the continuous variables, further categorization of this trait was implemented to find the demarcation point that might be useful clinically. Box plots representing each category were then created for clearer graphic presentation. The patients' ethnicity was identified as Caucasian, African American, Hispanic, Asian, or unspecified (other ethnic groups or unknown).

In the first trimester group, the association between gestational age, maternal age, and maternal weight on the logarithmically transformed plasma fetal DNA levels was estimated using linear regression analysis after adjustment for gestational age. The Kruskal-Wallis nonparametric analysis of variance (ANOVA) test was used to evaluate the difference in median levels of plasma fetal DNA among the identified ethnic groups, as well as between the smokers and nonsmokers.

In the second trimester group, fetal DNA levels in archived serum samples were corrected for gestational age and freezer storage time, based on the previously reported degradation rate of −0.66 GE/mL of fetal DNA per month at −20°C.[6] The relationships of gestational age, maternal age, and maternal weight on the logarithmic transformed serum fetal DNA levels were estimated using linear regression analysis. Maternal weights were categorized, and the nonparametric ANOVA test was applied to detect the differences in median serum fetal DNA levels among each weight group. The nonparametric ANOVA test was also used to evaluate the difference in median levels of serum fetal DNA levels among the identified ethnic groups and by smoking status. Due to the known physiologic increase in circulating fetal DNA levels with advancing gestational age, adjustment of fetal DNA levels according to the gestational week was performed before further analysis in both first and second

Table 1. Demographic Description of the Population Enrolled in the Study

	First trimester (n = 91)	Second trimester* (n = 126)
Fetal DNA levels (GE/mL) by ethnicity		
Caucasian	12.3 (6.1, 13.5) n = 15	43.1 (30.1, 60.5) n = 82
African American	11.8 (5.2, 22.1) n = 43	43.3 (29.7, 62.1) n = 7
Hispanic	11.1 (4.9, 19.4) n = 17	48.0 (38.1, 64.3) n = 25
Asian	11.0 (3.1, 17.2) n = 3	46.6 (38.7, 90.6) n = 4
Total	11.2 (5.4, 19.8)	43.6 (31.9, 61.4)
Gestational age (wk)	8 (7, 9)	16 (16, 18)
Maternal age (y)	24 (21, 28)	27 (21, 32)
Maternal weight (lb)	144.5 (125, 175)	144 (129, 171)
Fetal DNA levels (GE/mL) by maternal weight (lb)		
≤ 130	–	47.3 (34.2, 62.1) n = 33
131–150	–	47.2 (38.1, 68.0) n = 33
151–170	–	42.6 (30.1, 73.6) n = 19
171–190	–	36.1 (27.0, 47.9) n = 15
≥ 191	–	34.6 (28.9, 51.3) n = 16
Smokers (%)	36.2	8.3

GE/mL, genome equivalents per milliliter.

Values are median (25th, 75th percentile) except where otherwise specified. The total number in each category depended upon the availability of the data.

* Fetal DNA levels after correction for storage time.

trimester groups.[9] Statistical significance was assigned when the *P* value was less than .05. All statistical analyses were performed using SAS/STAT software (SAS Institute, Inc., Cary, NC).

RESULTS

Descriptive statistics, including medians and 25th and 75th percentile ranges, were generated for all studied variables (Table 1). In the first trimester group, the median (25th, 75th percentile) gestational age was 8 weeks (7, 9), and plasma fetal DNA level was 11.2 GE/mL (5.4, 19.8). Linear regression analysis of plasma fetal DNA levels indicated a highly significant association with gestational age ($P < .001$). The calculated incremental rate of plasma fetal DNA levels was 0.2 per week of gestation on the logarithmic scale, which corresponds to an average of 21% increase in plasma fetal DNA per gestational week in the first trimester. The median (25th, 75th percentile) maternal age in this group was 24 years (21, 28). After adjustment for gestational age, no significant correlation between fetal DNA levels and maternal age was found ($r = 0.09$, $P = .40$).

Median plasma fetal DNA levels in Caucasian, African-American, Hispanic, and Asian populations (Table 1) did not statistically differ ($P = .85$). There was no correlation between plasma fetal DNA levels and maternal weight after adjustment for gestational age ($r = -0.006$, $P = .95$). With smoking history available for all individuals, 32 of 91 women in the first trimester group reported a smoking history. A nonparametric unpaired *t* test indicated no significant difference in fetal DNA levels between smokers and nonsmokers ($P = .58$). Information regarding the number of cigarettes smoked was, however, not available.

In the second trimester group, the median (25th, 75th percentile) gestational age of the patients was 16 weeks (16, 18), and the serum fetal DNA level corrected for freezer storage time was 43.6 GE/mL (31.9, 61.4) (Table 1). Linear regression analysis with logarithmic transformation of serum fetal DNA levels indicated no significant association with gestational age in the second trimester ($P = .11$). Despite this *P* value, probably due to a lack of statistical power, adjustment for gestational age was performed for the most accurate estimation. A lower rate of increase of fetal DNA levels in the second trimester compared with the first trimester was previously shown.[4] The median (25th, 75th percentile) maternal age in this group was 27 years (21,32). No significant association was found between fetal DNA levels and maternal age and ethnicity ($P = .36$ and 0.45, respectively).

In the second trimester group, a significant inverse correlation was found between serum fetal DNA levels and maternal weight after adjustment for gestational age ($r = -0.26$, $P = .007$), as demonstrated in Figure 1. Maternal weights were then categorized. The categorization was designed to be as detailed as possible, while the sample size in each category was still adequate for the analysis, as shown in Table 1. Serum fetal DNA levels were significantly different among the weight categories (overall $P = .003$). The closest categories that did not statistically differ among themselves (the first 3 and the

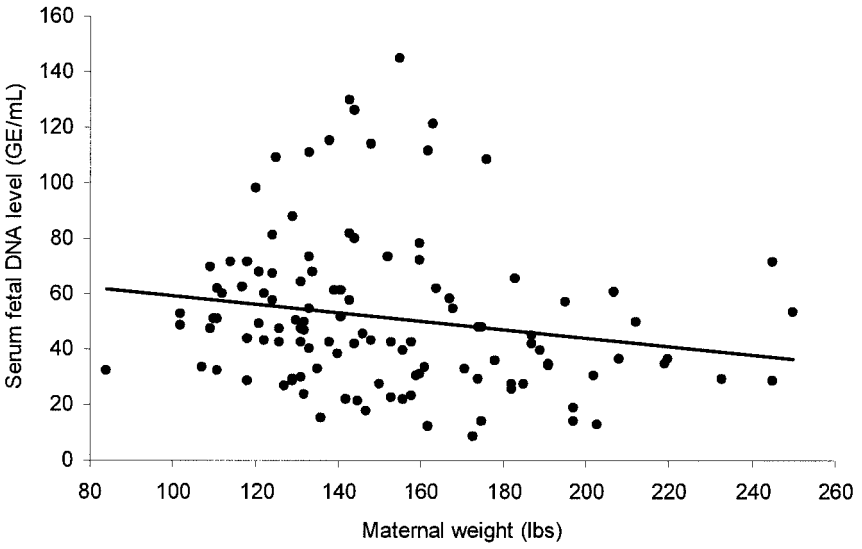

Fig. 1. Scatter plot demonstrating the correlation between serum fetal DNA levels and maternal weights in the second trimester. A significant negative association was found between fetal DNA levels and maternal weight after adjustment for gestational age ($P = .003$).

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last 2 categories) were then collapsed together forming 2 larger subgroups; 170 lb or less (n = 85) and more than 170 lb (n = 31). This also allowed us to better detect trends. The comparison between these 2 subgroups showed that median (25th, 75th percentile) serum fetal DNA levels were 26.5% (17.4%, 28.5%) lower in the heavier women ($P = .001$), as demonstrated in Figure 2.

One-hundred eight subjects in the second trimester group had a smoking history available. Nine of 108 women (8.3%) in the second trimester group reported a smoking history, and 99 of 108 were reported to be nonsmokers. No information regarding the amount of smoking per day was available. The median levels of serum fetal DNA were not different between smokers and nonsmokers ($P = .69$).

DISCUSSION

During the past few years, substantial knowledge has accumulated regarding the biology and potential clinical applications of cell-free nucleic acids of fetal origin in maternal circulation, as we have recently reviewed.[2] To translate the measurement of fetal cell-free nucleic acids into prenatal screening, certain variables that might affect the level of circulating fetal DNA need to be better understood. In the present study, we have demonstrated

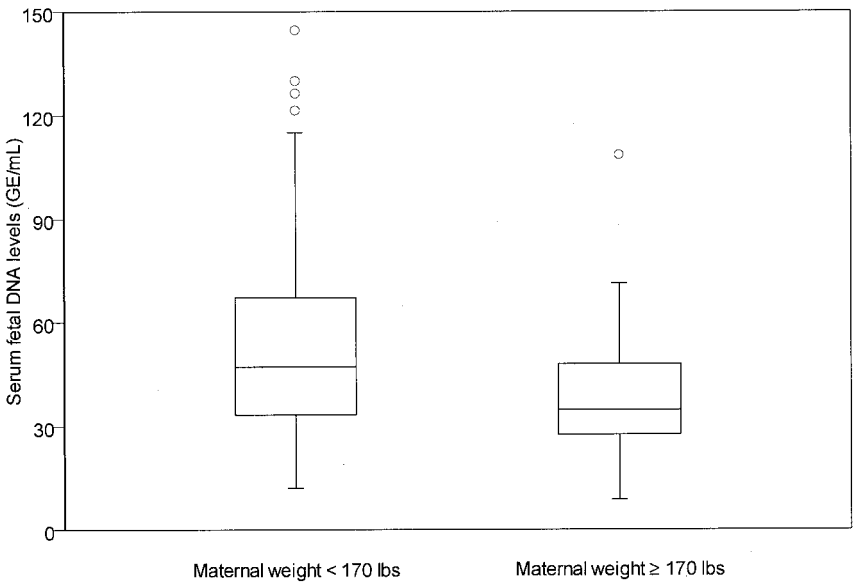

Fig. 2. Box plot of fetal DNA levels in the serum of pregnant women who weighed 170 lb or less, and more than 170 lb. The box represents the lower and upper quartiles; the line within the box is the median. The whiskers represent the range of the data, with the maximal observation shown separately by the circles. The levels of fetal DNA are significantly lower in the serum of pregnant women who weighed more than 170 lb ($P = .001$). GE/mL, genome equivalents per milliliter.

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an important inverse effect of maternal weight on the levels of circulating fetal DNA in the second trimester of pregnancy. Maternal age, ethnicity, and smoking were not significantly associated with fetal DNA levels.

It is standard practice for prenatal screening programs to adjust the analyte values for maternal weight.[10] Such adjustment is based on the fact that the placental-derived or fetal-derived markers tend to be more diluted in heavier women because they have a larger blood volume and, conversely, more concentrated in lighter women because of a smaller blood volume.[11] The inverse association between serum fetal DNA levels and maternal weights is in agreement with this concept and implies that the greater amount of body fluid found in heavier women may be responsible for this finding. However, this discordance was not observed in the first trimester pregnant women. The physiologic increase in plasma volume, which is proportionate to the maternal weight, does not begin until 8 weeks of gestation, and may contribute to the more pronounced effect of maternal weight on fetal DNA levels in the second trimester.[12] According to our findings, a correction factor of 26.5% may be required when fetal DNA analysis is performed in second trimester women, especially when they are heavier than 170 lb, where the effect of weight is particularly prominent.

We hypothesized that the aging process could affect the macromolecular and micromolecular compositions in the body fluid compartments by way of the alteration of structure and function of different organs, which could, theoretically, influence the concentration of various circulating analytes, including fetal DNA.[13] The independence of circulating fetal DNA levels and maternal age in both first and second trimester samples apparently indicates that fetal DNA levels do not need to be adjusted for maternal age when larger-scale studies are performed. However, it should be noted that this sample set included very few samples from women at very advanced maternal age (older than 40 years).

The effect of a patient's race on serum biochemical markers has been previously demonstrated, and adjustment for these factors is usually required.[14] Smoking has also been reported to alter the serum marker levels.[15] Morphologic changes of the villous barrier and the trophoblasts in the placenta of women who smoke may cause dysfunctional permeability, resulting in abnormal transfer of fetal-placental substrates.[16] Despite this, fetal DNA levels in our present study were not significantly affected by these demographic factors, although the number of Asian women and smokers in the database was small.

In conclusion, maternal weight inversely affects the levels of circulating cell-free fetal DNA in the second trimester of pregnancy. A correction for this effect may be needed in larger-scale studies or for future clinical applications that measure cell-free fetal nucleic acids in maternal circulation. The fact that fetal DNA is independent of maternal age makes it theoretically possible to improve the detection performance of fetal Down syndrome by adding fetal DNA analysis to the standard prenatal genetic screening algorithm. Future studies should address other potential variables that might affect circulating fetal DNA levels, including maternal diabetes, pregnancy after assisted reproductive technologies, gravidity, and parity.[5]

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Received March 8, 2004. Received in revised form May 18, 2004. Accepted June 3, 2004.