Published in final edited form as:

Obstet Gynecol. 2010 September ; 116(3): 673–678. doi:10.1097/AOG.0b013e3181ea2dd1.

Relationships between Cell-Free DNA and Serum Analytes in First and Second Trimesters of Pregnancy

Neeta L. Vora, M.D.^{1,2}, Kirby L. Johnson, Ph.D.², GERALYN Lambert-Messerlian, Ph.D.³, Hocine Tighiouart, M.S.⁴, Inga Peter, Ph.D.⁵, Adam C. Urato, M.D.¹, and Diana W. Bianchi, M.D.^{1,2}

¹ Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Tufts Medical Center, Boston, MA

² Division of Genetics, Department of Pediatrics, Floating Hospital for Children at Tufts Medical Center, Boston, MA

³ Department of Pathology and Laboratory Medicine, Women and Infants Hospital and the Alpert School of Medicine at Brown University, Providence, RI

⁴ Institute of Clinical Research and Health Policy Studies, Tufts Medical Center

⁵ Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY

Abstract

Objective—Circulating cell-free DNA and maternal serum analytes are indicators of fetal and placental condition. Little is known about the relationship of these noninvasive markers to each other, particularly in the first trimester. Our goal was to assess the relationship between first and second trimester cell-free DNA levels and maternal serum screening markers.

Methods—First and second trimester residual maternal serum samples from 50 women were obtained. First trimester (pregnancy-associated plasma protein A [PAPP-A] and β -hCG), and second trimester serum analytes (β -hCG, alpha-fetoprotein [AFP], unconjugated estriol and inhibin A) had been measured at the time of sample receipt. All fetuses were male, as confirmed by birth records. Cell-free DNA was extracted and measured by real-time quantitative polymerase chain reaction (PCR) amplification using glyceraldehyde phosphate dehydrogenase (*GAPDH*) and *DYS1* as markers of total DNA and fetal DNA, respectively. Determination of linear associations between first and second trimester serum markers and cell-free DNA levels using Pearson correlations was performed.

Results—Statistically significant correlations between first trimester PAPP-A multiples of the median (MoMs) and both total ($r=0.36$, $p=0.016$) and fetal ($r=0.41$, $p=0.006$) DNA in the first trimester were observed. There were no significant correlations between first trimester serum hCG or any second trimester serum marker with DNA levels.

Conclusions—Correlation between serum PAPP-A and first trimester circulating cell-free fetal and total DNA levels is a novel finding. PAPP-A is a glycoprotein of placental origin, and its correlation to cell-free fetal DNA in maternal serum suggests a common tissue origin, through apoptosis of placental cells. However, since PAPP-A and cell-free DNA were only marginally correlated and cell-free DNA can be reliably detected in the first trimester, the addition of cell-free DNA to serum screening strategies may be helpful in predicting adverse pregnancy outcome.

Introduction

Maternal serum is routinely analyzed in pregnant women to screen for chromosomal abnormalities such as Down and Edwards syndrome, and neural tube defects. In addition, there are many reports of an association between abnormal levels of individual analytes and poor

fetal or placental health. For example, while elevated alpha-fetoprotein (AFP) in the second trimester indicates a high risk for neural tube and ventral wall defects, it is also reported to be associated with other fetal abnormalities and adverse obstetrical outcomes such as intrauterine growth restriction.¹ Similarly, a low pregnancy-associated plasma protein A (PAPP-A) in the first trimester indicates a high risk for Down and Edwards syndrome, and is associated with poor pregnancy outcomes, such as preterm delivery, fetal growth restriction, and spontaneous loss.^{1,2} While informative in the first trimester, PAPP-A has not been shown to be useful in the second trimester, as its level does not differ between unaffected and Down syndrome fetuses during this time.³ The second trimester serum markers of placental origin, β -hCG and inhibin A, can also be elevated in pregnancies with adverse outcomes.¹

Circulating cell-free fetal DNA is released into the maternal circulation as a result of placental apoptosis.^{4,5} Increased levels of cell-free fetal DNA are associated with abnormal placental development.⁶ Cell-free fetal DNA is elevated in pregnancies with fetal Down syndrome,^{7–10} and has been suggested as an additional serum marker to improve detection.¹⁰ Cell-free fetal DNA levels are also increased in other complications of pregnancy that involve the placenta, such as preeclampsia, intrauterine growth restriction, and invasive placentation.^{11–17} Thus, both Down syndrome and abnormal placentation appear to cause a change in serum analytes and an increase in cell-free fetal DNA.

Because cell-free DNA and maternal serum markers are thought to be indicators of fetal and placental health, our goal was to assess the relationship between first and second trimester serum analytes and cell-free fetal and total DNA levels towards potential future improvements to serum screening.

Materials and Methods

This study was approved by the Institutional Review Boards at Women and Infants' Hospital and Tufts Medical Center. First and second trimester residual maternal serum samples from 50 women were randomly selected and obtained between February and May of 2007 from routine prenatal screening in the Department of Pathology and Laboratory Medicine at Women and Infants' Hospital without regard to results. First trimester was defined as between 10 weeks, 3 days and 13 weeks, 6 days of gestation; second trimester was between 15 and 20 completed weeks of pregnancy. Only samples collected from a pregnancy resulting in the birth of a singleton male were selected. Birth outcomes (e.g. karyotype) were not determined, as the objective of the study was to compare serum analytes to cell-free DNA levels towards potential future improvements to serum screening. Multiple gestations, women with insulin-dependent diabetes and pregnancies resulting from *in vitro* fertilization (IVF) were excluded. Serum analytes (PAPP-A, β -hCG, AFP, unconjugated estriol and inhibin A) were measured at the time of sample receipt using the AccessR Immunoassay System (Beckman Coulter, Inc., Brea, CA). Total β -hCG was measured on first trimester serum samples at the time of the present study, and an aliquot was prepared upon sample thaw for DNA testing.

Cell-free DNA was extracted from all samples and measured by real-time quantitative polymerase chain reaction (PCR) amplification as previously described.^{18–19} DNA was extracted from 400 μ L of serum using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA), according to the blood and body fluid protocol. DNA was eluted in 50 μ L of the elution buffer. Real time quantitative PCR amplification was performed to amplify the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) to show that DNA had been successfully extracted from the serum sample. PCR amplification of *DYS1*, a Y chromosome sequence, was used to detect male DNA. All samples were analyzed in triplicate. DNA analysis was performed in Boston on samples coded in Rhode Island. A female staff member processed and handled all samples to minimize the risk of contaminating samples with male DNA.

Additional variables included maternal age and weight, gestational age, parity, and smoking (Table 1). Determination of linear associations between first and second trimester cell-free DNA and serum markers within and across trimesters using Pearson correlations was performed.

Multiples of the median (MoMs) were log transformed and correlation coefficients were calculated. Both cell-free DNA and serum markers were adjusted for maternal weight using partial correlations. The minimum correlation that can be detected when $n=50$ at $\alpha=0.05$ and with a power of 0.80 is $r=0.39$ using Fisher's z transformation. Statistical significance was assigned when $p < 0.05$.

Results

Both *GAPDH* and *DYSI* were detected and amplified in all samples, therefore the sensitivity of detection of both markers was 100%. In the first trimester, statistically significant correlations were observed between PAPP-A MoMs and both total ($r=0.36$, $p=0.016$) and fetal ($r=0.41$, $p=0.006$) DNA levels (Table 2). Neither *DYSI* nor *GAPDH* levels correlated significantly with any of the other measured analytes (Table 3). First trimester *DYSI* and second trimester *DYSI* levels were significantly correlated ($r=0.37$; $p=0.015$). It is not known if this relationship holds across all maternal and gestational ages, as the sample size is too small to conduct subgroup analysis for these variables.

Discussion

Our objective was to examine the relationship between cell-free DNA levels and serum markers in both first and second trimesters of pregnancy. Our analysis demonstrates that there is a marginal, yet statistically significant, correlation between first trimester serum PAPP-A and first trimester cff and total DNA levels. Both are markers of placental function, as PAPP-A is a glycoprotein of placental origin, and cff DNA in maternal serum derives predominantly from apoptosis of placental cells.

Concerning the biological plausibility for the correlation between PAPP-A and cell-free DNA, PAPP-A is a protease for insulin growth factor (IGF) binding protein 4. Lower levels of PAPP-A are associated with higher levels of IGF binding protein 4, and thus, less free IGF. Insulin growth factor regulates fetal growth by stimulating cell proliferation and differentiation and plays a role in trophoblast invasion of the decidua.^{20,21} Cell-free DNA is thought to be mainly released from the placenta and is a result of trophoblastic apoptosis.^{5,22} If there is abnormal trophoblast invasion in early pregnancy and decreased subsequent cell proliferation, this could result in poor placental development and an increase in placental apoptosis, which would then result in high cff DNA levels with correspondingly low PAPP-A levels. Further studies are needed to test this hypothesis.

This study shows an early marginal correlation in the first trimester between PAPP-A and cell-free fetal DNA. In the second trimester, total and fetal DNA are not correlated with any of the serum analytes, even the analytes specifically of placental origin. This is surprising given that the source of fetal DNA measured in the maternal circulation is hypothesized to predominantly originate from the placenta. These results raise the question of extra-placental sources of fetal DNA and suggest that such sources may differ between trimesters. This possibility warrants further investigation as to the source of cell-free DNA throughout pregnancy.

Prior reports in the literature demonstrate that a low PAPP-A value and a high cell-free fetal DNA measurement are both associated with adverse pregnancy outcomes. Results from the First and Second Trimester Evaluation of Risk (FASTER) trial showed that a PAPP-A measurement of less than the 5th percentile was associated with spontaneous pregnancy loss

at < 24 weeks, preeclampsia, low birth weight, gestational hypertension, preterm birth, stillbirth, preterm premature rupture of membranes, and placental abruption.²⁰ Low PAPP-A and high DNA levels both suggest placental abnormalities. Future work is needed to determine whether this correlation has clinical significance. While abnormal serum markers are indicators of poor placental health and are associated with adverse pregnancy outcomes, there is no currently accepted practice for managing pregnancies in women with abnormal serum markers. Since cell-free fetal DNA can be detected in the first trimester, it may have utility, in conjunction with other analytes, as an early marker of adverse obstetric outcomes.²³

Markers in serum screening have been chosen based on their ability to provide independent information. Low correlation between serum markers is required to improve Down syndrome detection rates.^{24,25} Addition of fetal DNA as a marker of Down syndrome may improve screening performance, especially in the second trimester where all correlations are low. On the other hand, recent strategies have emerged in screening to take advantage of high correlations of marker levels. High correlation of a marker across trimesters can be used to improve prenatal screening for Down syndrome by calculating ratios of the levels of the same serum markers measured in the first and the second trimester (cross trimester ratios).²⁶ Use of cross trimester ratios was also found to improve the performance of the integrated screening test and lower the false positive rate.²⁶ We found a statistically significant correlation between cell free fetal DNA in the first and second trimester ($p=0.015$). Future work studying cell free DNA levels across trimesters can be done to determine whether this could improve screening performance. However, the utility of cell-free fetal DNA can ultimately only be realized with a gender independent marker. In this study, a Y chromosome sequence was used a marker of fetal DNA, meaning that only half of pregnancies can be analyzed. Although these data show the feasibility of using cell-free DNA as a biomarker, a gender independent marker is essential for widespread clinical implementation.

The correlation of second trimester serum markers and fetal DNA has been examined in a prior study.¹⁰ Our results are in agreement that the correlation of markers is low. The correlation (r) of AFP, unconjugated estriol or β -hCG with fetal DNA ranged from 0.1 to 0.2 in both studies. The results for inhibin were more variable; 0.3 in the prior study, but -0.09 presently. Nevertheless, either of these correlations is low enough to suggest a benefit from addition of fetal DNA levels to second trimester screening. Indeed, Farina and colleagues calculated that fetal DNA measurement could add 5% detection to second trimester Down syndrome screening over routine quad markers alone.¹⁰

A limitation of the study is that pregnancy outcomes were not determined, other than to confirm that there was a birth of a live born singleton male. However, the objective of the study was to compare serum analytes to cell-free DNA levels towards potential future improvements to serum screening and not to assess for pregnancy outcome. In addition, due to the small sample, it is possible that marginal correlations that were reported (i.e. correlation of cell-free fetal DNA and PAPP-A) or those that were undiscovered would be further delineated from a larger sample size. Nevertheless, there are a number of strengths in our study. We used the same patients for the first and second trimester data, 98% of patients were non-smokers, and IVF patients were excluded. Prior data has shown that smoking in pregnancy increases levels of total cell free DNA by three fold.²⁷ In addition, although one study has shown that IVF does not affect levels of cell-free fetal DNA,²⁸ these pregnancies were excluded as some studies suggest that serum analytes in pregnancies conceived with assisted reproductive technologies may be abnormal.^{29,30}

Our observation that PAPP-A and cell-free DNA are correlated in the first trimester reinforces the idea of cell-free DNA as a marker of placental development. Further studies should be done to determine whether pregnancies with abnormal placentas show an increase in trafficking of

total and fetal DNA, and whether this increase correlates with other placental secretory products. If patients with abnormally low PAPP-A and high cell-free DNA levels have poor pregnancy outcomes, these combined markers could potentially identify pregnancies in the first trimester that warrant increased surveillance.

Acknowledgments

Supported by NIH T32 HD049341 and HD42053-07

Dr. Bianchi is a member of the Clinical Advisory Board and she holds equity in Artemis Health, Inc. Artemis Health had no role in the design or conduct of this study.

References

1. Gagnon A, Wilson RD, Audibert F, Allen VM, Blight C, Brock JA, et al. Obstetrical complications associated with abnormal maternal serum markers analytes. *J Obstet Gynaecol Can* 2008;30:918–49. [PubMed: 19038077]
2. Montanari L, Alfei A, Albonico G, Moratti R, Arossa A, Beneventi F, et al. The impact of first-trimester serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein A on the diagnosis of fetal growth restriction and small for gestational age infant. *Fetal Diagn Ther* 2009;25:130–35. [PubMed: 19279389]
3. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *J Med Screen* 2003;10:56–104. [PubMed: 14746340]
4. Bischoff FZ, Lewis DE, Simpson JL. Cell-free fetal DNA in maternal blood: kinetics, source and structure. *Hum Reprod Update* 2005;11:59–67. [PubMed: 15569699]
5. Tjoa ML, Cindrova-Davies T, Spasic-Boskovic O, Bianchi DW, Burton GJ. Trophoblastic oxidative stress and the release of cell-free feto-placental DNA. *Am J Pathol* 2006;169:400–4. [PubMed: 16877342]
6. Maron JL, Bianchi DW. Prenatal diagnosis using cell-free nucleic acids in maternal body fluids: a decade of progress. *Am J Med Genet C Semin Med Genet* 2007;145C:5–17. [PubMed: 17299735]
7. Lo YM, Lau TK, Zhang J, Leung TN, Chang AM, Hjelm NM, et al. Increased fetal DNA concentrations in the plasma of pregnant women carrying fetuses with trisomy 21. *Clin Chem* 1999;45:1747–51. [PubMed: 10508120]
8. Zhong XY, Burk MR, Troeger C, Jackson LR, Holzgreve W, Hahn S. Fetal DNA in maternal plasma is elevated in pregnancies with aneuploid fetuses. *Prenat Diagn* 2000;20:795–8. [PubMed: 11038456]
9. Lee T, LeShane ES, Messerlian GM, Canick JA, Farina A, Heber WW, et al. Down syndrome and cell-free fetal DNA in archived maternal serum. *Am J Obstet Gynecol* 2002;187:1217–21. [PubMed: 12439507]
10. Farina A, LeShane ES, Lambert-Messerlian G, Canick J, Lee T, Neveux L, et al. Evaluation of cell-free fetal DNA as a second trimester maternal serum marker of Down syndrome pregnancy. *Clin Chem* 2003;49:239–42. [PubMed: 12560345]
11. Alberry MS, Maddocks DG, Hadi MA, Metawi H, Hunt LP, Abdel-Fattah SA, et al. Quantification of cell-free fetal DNA in maternal plasma in normal pregnancies and pregnancies with placental dysfunction. *Am J Obstet Gynecol* 2009;200:98.e1–e6. [PubMed: 19121662]
12. Leung TN, Zhang J, Lau TK, Chan LYS, Lo D. Increased maternal plasma fetal DNA concentrations in women who eventually develop preeclampsia. *Clin Chem* 2001;47:137–9. [PubMed: 11148193]
13. Wright CF, Burton H. The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis. *Hum Reprod Update* 2009;15:139–51. [PubMed: 18945714]
14. Hahn S, Huppertz B, Holzgreve W. Fetal cells and cell free fetal nucleic acids in maternal blood: new tools to study abnormal placentation? *Placenta* 2005;945:141–4.
15. Nakib A, Desbriere R, Bonello N, Bretelle F, Boubli L, Gabert J, et al. Total and fetal cell-free DNA analysis in maternal blood as markers of placental insufficiency in intrauterine growth restriction. *Fetal Diagn Ther* 2009;26:24–8. [PubMed: 19816026]

16. Lo YM, Leung TN, Tein MS, Sargent IL, Zhang J, Lau TK, et al. Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. *Clin Chem* 1999;45:184–8. [PubMed: 9931039]
17. Caramelli E, Rizzo N, Concu M, Simonazzi G, Carinci P, Bondavalli C, et al. Cell-free fetal DNA concentration in plasma of patients with abnormal uterine artery Doppler waveform and intrauterine growth restriction - a pilot study. *Prenat Diagn* 2003;23:367–71. [PubMed: 12749031]
18. Johnson KL, Dukes KA, Vidaver J, LeShane ES, Ramirez I, Weber WD, et al. Interlaboratory comparison of fetal male DNA detection from common maternal plasma samples by real-time. PCR *Clin Chem* 2004;50:516–21.
19. Wataganara T, Bianchi DW. Fetal cell-free nucleic acids in the maternal circulation: new clinical applications. *Ann N Y Acad Sci* 2004;1022:90–9. [PubMed: 15251945]
20. Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH, et al. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). *Am J Obstet Gynecol* 2004;191:1446–51. [PubMed: 15507981]
21. Byun D, Mohan S, Yoo M, Sexton C, Baylink DJ, Qin X. Pregnancy-associated plasma protein-A accounts for the insulin like growth factor (IGF)-binding protein-4 (IGFBP-4) proteolytic activity in human pregnancy serum and enhances the mitogenic activity of IGF by degrading IGFBP-4 *in Vitro*. *J Clin Endocrin Metab* 2001;86:847–54.
22. Alberry M, Maddocks D, Jones M, Abdel Hadi M, Abdel-Fattah S, Avent N, et al. Free fetal DNA in maternal plasma in anembryonic pregnancies; confirmation that the origin is the trophoblast. *Prenat Diagn* 2007;27:415–8. [PubMed: 17286310]
23. Dugoff L. First and second trimester maternal serum markers for aneuploidy and adverse obstetric outcomes. *Obstetrics and Gynecology* 2010;115:1052–61. [PubMed: 20410782]
24. Wright DE, Bradbury I. Repeated measures screening for Down's syndrome. *BJOG* 2005;112:80–3. [PubMed: 15663402]
25. Wald NJ, Bestwick JP, Morris JK. Cross-trimester marker ratios in prenatal screening for Down syndrome. *Prenat Diagn* 2006;26:514–23. [PubMed: 16739232]
26. Urato AC, Peter I, Canick J, Lambert-Messerlian G, Pulkkinen A, Knight G, et al. Smoking in pregnancy is associated with increased total maternal serum cell-free DNA levels. *Prenat Diagn* 2008;28:186–90. [PubMed: 18260159]
27. Pan PD, Peter I, Lambert-Messerlian GM, Canick JA, Bianchi DW, Johnson KL. Cell-free fetal DNA levels in pregnancies conceived by IVF. *Hum Reprod* 2005;20:3152–6. [PubMed: 16006472]
28. Henionen S, Ryynanen M, Kierkinen P, Hippelainen M, Saarikoski S. Effect of in vitro fertilization on human chorionic gonadotropin serum concentrations and Down's syndrome screening. *Fertil Steril* 1996;66:398–403. [PubMed: 8751737]
29. Hui PW, Lam YH, Tang MH, Ng EH, Yeung WS, Ho PC. Maternal serum pregnancy-associated plasma protein-A and free beta-human chorionic gonadotrophin in pregnancies conceived with fresh and frozen-thawed embryos from in vitro fertilization and intracytoplasmic sperm injection. *Prenat Diagn* 2005;25:390–3. [PubMed: 15906430]

Table 1

Descriptive demographics of study patients

Variable	Category	Mean (SD)	n (%)
Maternal age (years) ¹		30.9 (6.3)	-
Weight (lbs) ²		150.1 (31.3)	-
Gestational age (weeks)	First trimester	12.2 (0.94)	-
	Second trimester	16.7 (1.19)	-
Maternal Race	Non-black	-	44 (88)
	Black	-	5 (10)
	Unknown	-	1 (2)
Smoking status	Non-smoker	-	48 (96)
	Smoker	-	1 (2)
	Unknown	-	1 (2)
Parity	Primiparous	-	17 (34)
	Multiparous	-	32 (64)
	Unknown	-	1 (2)

¹ Maternal age calculated at estimated day of delivery² Maternal weight determined in the first trimester

Table 2

Correlation analysis of first trimester analytes and DNA levels

		Maternal age	Gestational age	β-hCG*	PAPP-A (MoM)	DYS	GAPDH (log)
Maternal age	r	1	-0.29	0.07	0.10	-0.26	-0.07
	p value	-	0.061	0.67	0.51	0.086	0.66
Gestational age	r	1	1	-0.23	0.09	0.10	0.12
	p value	-	-	0.14	0.56	0.54	0.44
β-hCG	r	1	1	1	-0.0008	-0.02	-0.14
	p value	-	-	-	0.99	0.89	0.37
PAPP-A (MoM)	r	1	1	1	1	0.41	0.36
	p value	-	-	-	-	0.006	0.016
DYS	r	1	1	1	1	1	0.18
	p value	-	-	-	-	-	0.26
GAPDH (log)	r	1	1	1	1	1	1
	p value	-	-	-	-	-	-

PAPP-A: pregnancy-associated plasma protein A, MoM: multiples of the median

GAPDH : glyceraldehyde phosphate dehydrogenase

Bold indicates probability values ≤ 0.05

* hCG MoMs were unavailable

Table 3

Correlation analysis of second trimester analytes and DNA levels

Gestational age		β -hCG (MoM)	AFP (MoM)	Unconjugated estriol (MoM)	Inhibin A (MoM)	DYS	GAPDH (log)
Gestational age	r	1	0.29	0.17	-0.037	0.20	0.09
	p value	-	0.063	0.28	0.017	0.21	0.58
β -hCG (MoM)	r	1	1	0.27	0.50	0.13	-0.13
	p value	-	-	0.087	0.001	0.41	0.41
AFP (MoM)	r		1	0.01	0.01	0.22	-0.10
	p value		-	0.94	0.94	0.13	0.53
Unconjugated estriol (MoM)	r			1	-0.26	0.10	-0.16
	p value			-	0.094	0.54	0.30
Inhibin A (MoM)	r				1	-0.09	-0.08
	p value				-	0.55	0.63
DYS	r					1	0.10
	p value						0.54
GAPDH (log)	r						1
	p value						-

AFP: alpha-fetoprotein, PAPP-A: pregnancy-associated plasma protein A, MoM: multiples of the median

GAPDH : glyceraldehyde phosphate dehydrogenase

Bold indicates probability values ≤ 0.05