Elevated Second-Trimester Maternal Serum hCG: A Marker of Inadequate Angiogenesis

CATHERINE Y. SPONG, MD, ALESSANDRO GHIDINI, MD, GARY A. DILDY, MD, CAROL A. LOUCKS, RN, MICHAEL W. VARNER, MD, AND JOHN C. PEZZULLO, PhD

Objective: To measure angiogenin, a potent inducer of neovascularization and interleukin-6, as an indicator of acute inflammation, in second-trimester amniotic fluid of patients with elevated maternal serum hCG.

Methods: In this case-control study, 20 patients with elevated maternal serum hCG (at least 2.0 multiples of median) at triple screen were matched 2:1 with controls on the basis of year of amniocentesis, parity, and race. Inclusion criteria were 1) singleton gestation, 2) no evidence of anomalies, and 3) genetic amniocentesis. Amniotic fluid was immunoassayed for angiogenin and interleukin-6. The immunoassay sensitivity for angiogenin was 0.026 ng/mL, interassay coefficient of variation 4.6%, and intra-assay coefficient of variation 2.9%. For interleukin-6, the immunoassay sensitivity was 2.37 pg/mL, interassay coefficient of variation 2.7%, and intra-assay coefficient of variation 1.9%. Angiogenin and interleukin-6 values were normalized by using natural log transformation for statistical analysis. Statistical analysis included analysis of variance and stepwise regression, with $P < .05$ significant.

Results: After correcting (by multivariate regression) for gestational age at sampling and nulliparity, amniotic fluid angiogenin levels were significantly lower in the study subjects than in controls (26% ± 11% lower, $P = .004$), whereas the interleukin-6 levels did not change significantly (34% ± 40% lower, $P = .3$).

Conclusion: Amniotic fluid angiogenin levels are significantly lower in patients with elevated maternal serum hCG at triple screen, suggesting inadequate angiogenesis, but interleukin-6 values do not differ significantly. (Obstet Gynecol 1998;91:605–8. © 1998 by The American College of Obstetricians and Gynecologists.)

Maternal serum levels of hCG are measured commonly as part of a second-trimester genetic screening for chromosomal anomalies (triple screen). Pregnant women with unexplained elevations of hCG serum levels have an increased risk of subsequent preeclampsia, fetal growth restriction, and preterm delivery.[1–5] Multivariate analysis has shown that after controlling for preeclampsia, elevated second-trimester hCG levels are not predictive of fetal growth restriction or preterm delivery, but only of hypertensive complications.[2] The link between second-trimester hCG levels and hypertension is unknown presently.

Experimental evidence suggests that three factors can influence production of hCG by the trophoblast. First, number of trophoblastic cells (predominantly syncytiotrophoblast) in the placenta is correlated with maternal serum hCG levels.[6] Second, the degree of oxygenation of trophoblastic cells has been shown to influence hCG production. Studies[6–8] using cultured villi from term placentas found that prolonged low oxygen tension decreased synthesis and release of hCG compared with normoxic conditions; these findings have been attributed to a decrease in syncytium formation because trophoblast survival was documented by a continued ability to synthesize protein and by recovered ability to form syncytium and to release hCG once the hypoxic conditions were withdrawn. Third, inflammatory cytokines, such as interleukin-6, have been shown to stimulate hCG synthesis in vitro.[9]

To establish which process underlies the elevation in maternal serum hCG levels, we have measured amniotic fluid angiogenin and interleukin-6 in a consecutive series of women who underwent genetic amniocentesis because of abnormal triple screening and who had elevated serum levels of hCG. Interleukin-6 is an inflammatory cytokine, and elevations of interleukin-6 concentration in amniotic fluid have been associated with intrauterine infection.[10] Angiogenin is a potent

inducer of neovascularization and a known marker of ischemia in several tissues including placenta.[9]

Materials and Methods

In this case-control study, 20 women with elevated maternal serum hCG (at least 2.0 multiples of the median) who underwent genetic amniocentesis were compared with 40 controls chosen to be as comparable as possible, in the aggregate, in year of amniocentesis, race, and parity. Patients underwent amniocentesis at Georgetown University Medical Center, University of Utah School of Medicine, or Utah Valley Regional Medical Center between May 1993 and August 1996. Inclusion criteria were singleton gestation, gestational age of 15–24 weeks at amniocentesis, no evidence of fetal structural or chromosomal anomalies, and genetic amniocentesis. Gestational age was confirmed or established by ultrasonographic fetal biometry at time of amniocentesis (before 25 weeks' gestation). Small for gestational age was defined as birth weight less than the 10th percentile for gestational age.[11,12] Demographic data including gestational age at amniocentesis and delivery, pregnancy complications including prelabor rupture of membranes, chorioamnionitis, preterm labor, and neonatal birth weight were collected by chart review.

Angiogenin and interleukin-6 levels were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) validated for amniotic fluid. Samples were assayed in duplicate in a single batch. The angiogenin ELISA limit of detection for amniotic fluid was 0.026 ng/mL, and the inter- and intra-assay coefficients of variation were 4.6% and 2.9%, respectively. The angiogenin ELISA used is specific for angiogenin and does not cross-react or interact with human interleukin-1α, -1β, -2, -3, -4, -6, -7, or -8 or tumor necrosis factor α or β. The ELISA sensitivity for interleukin-6 was 2.37 pg/mL, interassay coefficient of variation 2.7%, and intra-assay coefficient of variation 1.9%. The ELISA used for interleukin-6 does not cross-react or interact with recombinant human interleukin-1α, -1β, -3, -4, -7, or -8 or tumor necrosis factor-α or -β.

Statistical analysis included χ^2 and Fisher exact test for categoric variables, one-way analysis of variance for continuous variables, Mann Whitney U test for data not distributed normally, and stepwise regression analysis with $P < .05$ considered significant (Statview 4.5; Abacus Concepts Inc., Berkeley, CA). Angiogenin and interleukin-6 levels were normalized with natural log transformation for statistical analysis. Stepwise regression was used as part of the model-building process to eliminate variables that either had no effect or that influenced the outcome only indirectly, by virtue of

Table 1. Obstetric Characteristics

	Elevated hCG	Control	P
Patient population	$n = 20$	$n = 40$	
Maternal age (y)	29.3 ± 7.9	37.0 ± 3.6	<.001
Race			.6
White	18 (90%)	38 (94%)	
Black	1 (5%)	1 (3%)	
Other	1 (5%)	1 (3%)	
Nulliparous	11 (55%)	10 (25%)	.04
GA at sampling (wk)	19.4 ± 3.5	17.2 ± 1.9	.004

GA = gestational age.
Data are presented as mean ± standard deviation or n (%).

their correlation with other variables that were related more strongly to outcome. This study was approved by the Institutional Review Board committees at Georgetown University Medical Center, University of Utah School of Medicine, and Utah Valley Regional Medical Center.

Results

Twenty gravidas with elevated hCG values were matched with 40 controls. Gestational age at sampling and maternal age were significantly different between the two groups, reflecting that patients with abnormal screening undergo amniocentesis at a later gestational age than those with advanced maternal age. Similarly, because patients with normal screening tests do not undergo amniocentesis, controls are represented primarily by patients undergoing amniocentesis for advanced maternal age. As a consequence, the rate of nulliparity also was significantly different between the two groups. Maternal race was not different between the groups (Table 1).

Neonatal birth weight, delivery type, gender of the infant, and Apgar scores less than 7 were not different between the two groups. Three study patients delivered before 37 weeks' gestation, compared with two in the control group ($P = .3$). In addition, three patients with elevated hCG levels delivered small for gestational infants, compared with three controls ($P = .9$). The rate of preeclampsia was not different between the groups (Table 2).

Angiogenin and interleukin-6 were detected in all samples of amniotic fluid. After correcting (via multivariate regression) for gestational age at sampling and nulliparity, amniotic fluid angiogenin levels were significantly lower in the study patients compared with the controls (26% ± 11% lower, $P = .004$), whereas the interleukin-6 levels did not change significantly (34% ± 40% lower, $P = .3$). All covariates in the multiregression

Table 2. Pregnancy Outcome

	Elevated hCG (n = 20)	Control (n = 40)	P
GA at delivery (wk)	37.9 ± 5.7	39.3 ± 1.6	.005
Vaginal delivery	16 (80%)	30 (75%)	.4
Birth weight (g)	3037 ± 645	3330 ± 577	.08
SGA < 5th percentile	3 (15%)	3 (8%)	.9
Female gender	10 (50%)	18 (45%)	.8
5-min Apgar < 7	3 (15%)	1 (3%)	.1
Preeclampsia-eclampsia	2 (10%)	4 (10%)	.9
Preeclampsia causing PTD	2 (10%)	0	.1
PTD (< 37 wk)	3 (15%)	2 (5%)	.3

GA = gestational age; SGA = small for gestational age; PTD = preterm delivery.

Data are presented as mean ± standard deviation or n (%).

analysis for angiogenin were significant, and no variables were forced into the analysis.

Discussion

Our intent in undertaking this study was to determine which process underlies the elevation of maternal serum hCG in the second trimester. We used two amniotic fluid cytokines—angiogenin and interleukin-6—as markers of the two pathophysiologic processes that could be involved in hCG elevation: placental ischemia and inflammation, respectively. We have found that amniotic fluid angiogenin levels are significantly lower in patients with elevated serum levels of hCG at triple screen, after controlling for maternal age, gestational age at sampling, nulliparity, and year of amniocentesis. We used stepwise regression analysis to account for the confounding variables that were present inevitably between the study and control groups, given the design of the study, and that could not be prevented by improved matching criteria. Indeed, because abnormal serum screening and advanced maternal age are the most common indications for early second-trimester amniocentesis, it is difficult to match patients for maternal age and parity.

We did not find evidence of an association between maternal serum hCG elevation and amniotic fluid inflammatory cytokine levels, such as interleukin-6. This lack of association cannot be ascribed to a lack of effect of interleukin-6 on cytotrophoblast, because studies have shown that inflammatory cytokines, including interleukin-6 or -1 and TNF-α, are involved in hCG release by normal human trophoblast. Interleukin-6 receptors are present on trophoblastic cells and the presence of a cytokine-mediated regulatory network of hCG release by trophoblast has been documented.[9,13] At a significance level of .05, the power of detecting an effect of the size we observed is 18%. To obtain a

significant result with this size effect would require 75 patients with elevated hCG levels and 150 control patients.

Our observation of low levels of amniotic fluid angiogenin in cases with elevated maternal serum hCG levels sheds some light on the intrauterine processes that occur during the early second trimester. Events critical to appropriate placental vascular development are known to occur beginning around 15 weeks' gestation. A second wave of trophoblast invasion into the spiral arteries is associated with subsequent conversion of the spiral arteries into uteroplacental arteries. Direct observations of this crucial period are lacking because of the lack of access to placental tissue from ongoing pregnancies. We postulate that the low amniotic fluid angiogenin levels we have found signal inadequate placental angiogenesis and neovascularization, two processes that are required for normal placental development. As a result, placental ischemia (ie, decreased perfusion) ensues, to which the trophoblast responds typically by proliferation. The hyperproliferative ischemic trophoblast would manifest by increased hCG secretion.

Supporting this hypothesis is the observation that preeclampsia, the pregnancy complication associated most commonly with second-trimester hCG elevation, is characterized histologically by exaggerated trophoblast proliferation at the implantation site. Because this trophoblastic proliferation also is associated with failure of the trophoblast to invade the myometrial spiral arteries progressively and to convert them into large capacitance vessels, a more generalized maturation defect in the trophoblast may be taking place during the early second trimester in pregnancies destined to develop preeclampsia.[14] Indeed, complete or partial absence of physiologic conversion of the spiral arteries is the most outstanding of the uteroplacental findings associated with preeclampsia. Our MEDLINE search (1966–1996) using as search terms hCG, ischemia, hypoxia, and placenta identified no publications on histopathologic placental findings in women with elevated second-trimester serum hCG levels. Because not all patients with elevated second-trimester hCG levels proceed to develop preeclampsia, other factors or variables must play a role. The low rate of preeclampsia-eclampsia in our study and control groups, probably due to our small sample size, prevents us from drawing any meaningful conclusion to this end. A sample about twice the size of ours would be required to detect a significant difference in the incidence of preeclampsia-eclampsia between the two groups. Moreover, the lack of histopathologic placental documentation in our study does not allow correlations between second-trimester serum and amniotic fluid findings and under-

lying pathologic processes, as documented by placental findings. Larger studies are necessary to correlate laboratory findings (hCG and amniotic fluid angiogenin levels) with both clinical (preeclampsia) and histopathologic (placental) findings.

References

1. Wenstrom KD, Owen J, Boots LR, DuBard MB. Elevated second trimester human chorionic gonadotropin levels in association with poor pregnancy outcome. Am J Obstet Gynecol 1994;171:1038–41.
2. Gonen R, Perez R, David M, Dar H, Merksamer R, Sharf M. The association between unexplained second-trimester maternal serum hCG elevation and pregnancy complications. Obstet Gynecol 1992; 80:83–5.
3. Lieppman RE, Williams MA, Cheng EY, Resta R, Zingheim R, Hickok DE, et al. An association between elevated levels of human chorionic gonadotropin in the midtrimester and adverse pregnancy outcome. Am J Obstet Gynecol 1993;168:1852–7.
4. Tanaka M, Natori M, Kochno H, Ishimoto H, Kobayashi T, Nozawa S. Fetal growth in patients with elevated maternal serum hCG levels. Obstet Gynecol 1993;81:341–3.
5. Kuller JA, Sellati LE, Chescheir NC, Helwick JJ, Lincoln-Boyen B, Boor-Smith C, et al. Outcome of pregnancies with elevation of both maternal serum alpha-fetoprotein and human chorionic gonadotropin. Am J Perinatol 1995;12:93–7.
6. Esterman A, Finlay TH, Dancis J. The effect of hypoxia on term trophoblast: Hormone synthesis and release. Placenta 1996;17:217–22.
7. Fox H, Path MC. Effect of hypoxia on trophoblast in organ culture. Am J Obstet Gynecol 1970;107:1058–64.
8. Alsat E, Wyplosz P, Malassine A, Guibourdenche J, Porquet D, Nessmann C, et al. Hypoxia impairs cell fusion and differentiation process in human cytotrophoblast, in vitro. J Cell Physiol 1996;168: 346–53.
9. Li Y, Matsuzaki N, Masuhiro K, Kameda T, Taniguchi T, Saji F, et al. Trophoblast-derived tumor necrosis factor induces release of human chorionic gonadotropin using interleukin-6 (IL-6) and IL-6

10. Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor. Association with infection. J Clin Invest 1990;85:1392–400.
11. Williams RL, Creasy RK, Cunningham GC, Hawes WE, Norris FD, Tashiro M. Fetal growth and perinatal viability in California. Obstet Gynecol 1982;59:624–32.
12. Lubchenco LO, Hansman C, Dressler M, Boyd E. Intrauterine growth as estimated from liveborn birth-weight data at 24 to 42 weeks of gestation. Pediatrics 1963;32:793–800.
13. Taniguchi T, Matsuzaki N, Jo T, Saji F, Taga T, Hirano T, et al. Interleukin-1 (IL-1) induced IL-6 and IL-6-receptor mediated release of human chorionic gonadotropin by choriocarcinoma cell lines (Jar and HCCM-5) activates adenosine 3'5' monophosphate independent signal transduction pathway. J Clin Endocrinol Metab 1992;74:1389–95.
14. Redline RW, Patterson P. Pre-eclampsia is associated with an excess of proliferative immature intermediate trophoblast. Hum Pathol 1995;26:594–600.

receptor-dependent system in the normal human trophoblasts. J Clin Endocrinol Metab 1992;74:184–91.

Address reprint requests to:
Catherine Y. Spong, MD
Laboratory of Developmental Neurobiology
NICHD, NIH
Building 49, Room 5A-38
9000 Rockville Pike
Bethesda, MD 20892