Circulating levels of cytokines during pregnancy: thrombopoietin is elevated in miscarriage

Brian W. Whitcomb, Ph.D.,[a,b] *Enrique F. Schisterman, Ph.D.,*[a] *Mark A. Klebanoff, M.D.,*[a] *Mona Baumgarten, Ph.D.,*[b] *Xiaoping Luo, M.D.,*[c] *and Nasser Chegini, Ph.D.*[c]

[a] Epidemiology Branch, Division of Epidemiology, Statistics and Prevention Research, National Institute of Child Health and Human Development, National Insttutes of Health, Bethesda, Maryland; [b] Department of Epidemiology and Preventive Medicine, School of Medicine, University of Maryland, Baltimore, Maryland; and [c] Department of Obstetrics and Gynecology, University of Florida, Gainsville, Florida

Objective: To evaluate the hypothesis that cytokine levels are associated with miscarriage risk using serum samples collected before report of miscarriage.
Design: A nested case-control study.
Setting: Biospecimens from the multisite Collaborative Perinatal Project, University of Florida, laboratory assessment of interleukin (IL)-1 receptor antagonist, IL-1β, IL-4, IL-6, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, thrombopoietin (TPO), and granulocyte colony-stimulating factor (G-CSF).
Patient(s): Cases of miscarriage (n = 439) were matched to controls (n = 373) by gestational age at sample collection.
Intervention(s): None.
Main Outcome Measure(s): Miscarriage.
Result(s): Increased risk of miscarriage was associated with elevated TPO (adjusted odds ratio [OR] 1.16, 95% confidence interval [CI] 1.00–1.36) and decreased G-CSF (adjusted OR 0.78, 95% CI 0.64–0.95). When analysis was restricted to samples collected more than 35 days before miscarriage, the effect of G-CSF was not observed (adjusted OR 0.96, 95% CI 0.72–1.28), whereas increased risk related to higher TPO remained.
Conclusion(s): Circulating levels of TPO may be associated with increased risk of miscarriage. (Fertil Steril® 2008;89:1795–802. ©2008 by American Society for Reproductive Medicine.)

Key Words: Cytokines, epidemiology, hematopoiesis, miscarriage, placentation

Human reproduction is a complex and highly regulated process. In humans this process is prone to failures. Fecundability has been estimated to be less than 30% (1). Estimates of the proportion of recognized pregnancies that end in miscarriage range from 15% to 31% (1, 2). Although some causes of miscarriage have been identified, the etiology is poorly understood (1, 2).

Immune-related cytokines are among the molecules recognized to play key roles in pregnancy (3–5). These cytokines are primarily produced by cells of the immune system, but are also expressed at the maternal–fetal interface by decidua and trophoblast cells. Among their major regulatory functions, cytokines participate in differentiation of naïve T-helper cells into T-helper type (Th)-1 cells, or Th2 cells. Murine and human studies have suggested a shift toward Th2 in successful pregnancy, although human studies have been less conclusive (5–15). Local production of cytokines by uterine and placental cells is considered to influence embryo implantation, decidualization, and placentation (3, 16).

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Reprint requests: Enrique F. Schisterman, Ph.D., Epidemiology Branch, DESPR, National Institute of Child Health and Human Development, National Institutes of Health, 6100 Executive Blvd., Rm. 7B03, Rockville, MD 20852 (FAX: 301-402-2084; E-mail: SchisteE@mail.nih.gov).

In addition, growth factors like vascular endothelial growth factor, thrombopoietin (TPO), granulocyte colony-stimulating factor (G-CSF), and granulocyte macrophage colony-stimulating factor (GM-CSF) have been observed in various stages of pregnancy (4, 17). The vascular endothelial growth factor and TPO are involved in angiogenesis and thrombopoiesis. These factors have been investigated in the context of preeclampsia, and both are critical for successful pregnancy (4, 17).

Previous studies suggesting altered cytokine production in miscarriage have included small study populations, evaluated few factors, or used samples collected at diagnosis of miscarriage, subsequent to fetal demise (5, 11, 18–23). Given the ambiguous findings regarding the association among Th1 and Th2 cytokines and miscarriage, the complex biological interplay between these factors, and the lack of information regarding the relation between TPO and miscarriage, we evaluated the hypothesis that cytokine levels are altered in miscarriage using a large number of serum samples collected before report of miscarriage for simultaneous assessment of multiple cytokines. In addition to a nested case-control approach, we used serum samples from women who experienced pregnancies that ended in miscarriage and normal pregnancies for a case-crossover analysis. These within-woman comparisons address factors that might differ between women who experience miscarriage and those who do not.

MATERIALS AND METHODS

Study Design and Population

Subjects were selected from the Collaborative Perinatal Project (CPP) cohort. The CPP was a multisite prospective study conducted from 1959 to 1974 that enrolled participants at presentation for prenatal care, and is described in detail elsewhere (24). Serum samples were collected at entry to the CPP and at subsequent bimonthly visits and stored at −20°C. Gestation was estimated using self-reported date of last menstrual period. Miscarriage was defined as involuntary loss of a clinically recognized intrauterine pregnancy (IUP) at less than 140 days of gestation. Subjects with serum samples collected less than 10 days before miscarriage (n = 355) or with unavailable serum samples (n = 36) were excluded. After exclusions, 439 cases of miscarriage were selected for this study.

Control selection followed a modified nested case-control design. Control serum samples were matched to cases by gestational age at sample collection to address underlying biologic variability of cytokine levels across gestation. Among women with eligible case samples, some also experienced normal pregnancies in the CPP; serum samples from these "crossover" women were preferentially sampled, allowing for the addition of a case-crossover analysis to address time-invariant confounding (25). The case-control analysis was limited to independent pregnancies—excluding normal pregnancies from 86 women—and therefore included serum samples for 439 cases and 373 controls. The case-crossover analysis included 186 serum samples from 86 case pregnancies and 100 serum samples from the control pregnancies of the same 86 women.

Exposure Assessment

Serum cytokine levels were measured using the multiplex Fluorokine MAP Human Cytokine detection system (R&D Systems, Inc., Minneapolis, MN) as previously described (26). Briefly, the assays use 96-well plates with 50 μL of sera in duplicates in a sandwich ELISA-based approach. The solid phase consists of fluorescent beads covalently linked with cytokine-specific monoclonal antibodies allowing capture of each cytokine and corresponding biotinylated antibody. After addition of streptavidin–phycoerythrin, intensity is measured using the Luminex 100 IS system (Luminex Corp., Austin, TX).

Preliminarily, we evaluated use of CPP serum samples by comparing cytokine levels with serum samples freshly collected at first trimester from healthy term pregnancies, as well as to levels of interleukin (IL)-6 measured by standard ELISA. The levels of most measured cytokines were consistent across these groups. The feasibility assessment is discussed in more detail in the Appendix. Multiplex assays evaluated concentrations of IL-1 receptor antagonist, IL-1β, IL-4, IL-6, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), G-CSF, and TPO.

Samples were randomly ordered by case status and batches organized by gestational age at sample collection. Case samples and matched controls were analyzed in the same batch. Because specimens had been collected previously and identifying information removed, the Office of Human Subjects Research at the National Institutes of Health and Institutional Review Board (IRB) at the University of Florida determined this study as exempt from further IRB review.

Risk Factor Assessment

Maternal age, race, and smoking status were considered as possible confounding factors based on having a suspected association with miscarriage, and a possible relation with cytokine levels (2, 27–29). Information on risk factors from the CPP examinations and interviews were recorded by study staff at entry into the study and at subsequent visits to prenatal care providers. Smoking was self-reported as cigarettes per day. Information on parity, gravidity, and previous pregnancy outcomes was abstracted from medical records. Information regarding factors measured at multiple time points was taken from the visit concurrent with sample collection, usually corresponding to the initial visit.

Statistical Analysis

Demographics, risk factors, and outcomes were evaluated among the 812 women comprising the study sample. For continuous variables, mean and standard error were calculated; for discrete variables, proportions were calculated. The study sample was grouped by case status into 439 case samples and 373 control samples, and bivariate relations between potential confounders and miscarriage were evaluated. For continuous variables Student's t tests were used and χ^2 tests were used to compare categorical variables. Values for the biomarkers were divided by their SD within the controls to yield standardized measures.

Conditional logistic regression models were used to estimate risk of miscarriage matched on gestational age in weeks at time of sample provision. These models were used to obtain crude and adjusted estimates of the odds ratio (OR). Specification of the final multivariable model was determined from bivariate analyses or biologic relevance (30), with confounding determined by a change in estimate of at least 10% (31). Estimated adjusted ORs correspond to the effect of one SD change in biomarker on odds of miscarriage. Conditional logistic regression models were also used for analysis of the self-matched case-crossover data, with each individual woman serving as the matching factor.

To evaluate the importance of the time between sample collection and miscarriage on estimates, observations were classified in overlapping categories—no restriction (i.e., not beyond the exclusion criteria), at least 14 days, at least 21 days, at least 28 days, and the most restrictive subset, at least 35 days from sample collection to miscarriage.

RESULTS

Characteristics of the 812 women in the study are shown in Table 1. Mean maternal age at the time of sample provision