

Chlamydial Lower Genital Tract Infection and Spontaneous Abortion

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ABSTRACT

Objective: Few previous studies have examined the role that acute *Chlamydia trachomatis* lower genital tract infection plays in the development of spontaneous abortion. This study evaluated cervical *C. trachomatis* infections among 52 women experiencing spontaneous abortion and 59 controls.

Methods: Pregnant women at less than 22 weeks of gestation who sought medical care in an emergency department in West Philadelphia were eligible for enrollment. Urine samples from enrolled women were tested for chlamydia using ligase chain reaction DNA amplification. All women were followed up to 22 weeks of pregnancy to ascertain the outcome of spontaneous abortion.

Results: The proportion of women with chlamydial infection was 3.8% among spontaneous abortion cases and 8.5% among controls. After adjustment for potentially confounding factors, there was no substantial difference in the rate of chlamydial infection between women with and without spontaneous abortion (odds ratio 1.8, 95% confidence interval 0.3-10.7).

Conclusion: These data do not support a relationship between acute *C. trachomatis* infection and the development of spontaneous abortion. *Infect. Dis. Obstet. Gynecol.* 6:8-12, 1998.

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KEY WORDS

miscarriage; pregnancy; sexually transmitted disease

Chlamydia trachomatis is among the most common sexually transmitted infections in industrialized nations.¹ Cervical infection during pregnancy can result in adverse birth outcomes; for example, *C. trachomatis* infection has been associated with premature rupture of membranes and resultant preterm delivery.²⁻⁴ However, the relationship between *C. trachomatis* and the most common adverse pregnancy outcome, spontaneous abortion, has yet to be thoroughly investigated.

The first concern that there might be a link between chlamydia species and spontaneous abortion was derived from the observation that *Chlamydia*

psittaci caused fetal wastage in bovine species and from case reports that farm women had spontaneous abortions after contact with an infected sheep or lamb.^{5, 6} However, contact with *C. psittaci* is rare among humans living outside of farming communities.

Subsequently, a handful of studies attempted to establish a relationship between serological evidence of past *C. trachomatis* infection and spontaneous abortion. These investigations were conducted among women who had experienced recurrent pregnancy loss or who had an unsuccessful implantation during in vitro fertilization.⁷⁻¹¹ The

Grant Sponsor: NIDA; Grant Number: 5 RO1 DA08252-02.

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results were mixed. However, several studies found that more women who had recurrent abortions or a pregnancy loss during in vitro fertilization had high IgG antibody titers to *C. trachomatis*.

These studies were limited in several respects. First, their generalizability is restricted because women who have recurrent spontaneous abortions represent a small subset of all women who experience spontaneous abortion. Second, none of the studies used the very sensitive DNA-based methods now available for detection of lower genital tract infections. Finally, most studies focused on past infection and did not evaluate current sexually transmitted infections; those that did found no acutely infected women.

Only one previous study to our knowledge evaluated the relationship between chlamydial antibodies and spontaneous abortion in a population of unselected pregnant women. That study found a higher rate of miscarriage in women with high chlamydial titers.¹²

We conducted a pilot study to examine the relationship between current *C. trachomatis* infection and randomly occurring spontaneous abortion. This study was designed to overcome previous study limitations by using subjects enrolled in a population-based, prospective study of spontaneous abortion and by using ligase chain reaction (LCR) to test for the presence of current bacterial sexually transmitted infections.

SUBJECTS AND METHODS

Subject Recruitment

This study used stored urine specimens and demographic data obtained from women enrolled in the Early Pregnancy Study between August 1995 and November 1996. The Early Pregnancy Study is a federally funded project designed to examine the role of lifestyle factors on pregnancy outcome, including the effects of drug abuse on spontaneous abortion. Women between the ages of 14 and 40 years who came to the emergency department at the hospital of the University of Pennsylvania were screened for pregnancy using a urine test. A woman was recruited if she was found to be pregnant, had a singleton intrauterine gestation at less than 22 weeks of gestation, and lived in the lower socioeconomic, inner-city neighborhood of West Philadelphia. Of the women recruited for participation, 96% consented to enrollment. About one third of

women came to the emergency department because of vaginal bleeding and about another third came with abdominal pain. The rest reported with a broad range of chief symptoms, including back pain, nausea or vomiting, trauma, upper respiratory symptoms, and urinary tract symptoms. Extensive demographic, medical, social, and lifestyle information was collected by in-person interview. In addition, hair, urine, and blood specimens were collected and stored. Investigators followed up with participants at 16 and 22 weeks of gestation for the primary purpose of determining early pregnancy outcomes, particularly spontaneous abortion. Follow-up rates were 86% at 16 weeks and 84% at 22 weeks. Anyone diagnosed with an ectopic pregnancy, molar pregnancy, or multiple gestation and any woman who electively terminated a pregnancy during the follow-up period was excluded from the study.

The present analysis involved a nested case-control design in which 60 women who experienced a spontaneous abortion (cases) were chosen at random from all the study participants who experienced spontaneous abortion at or before 22 weeks of gestation. Sixty women (controls) were chosen at random for comparison from the group of participants who had uncomplicated pregnancies that continued past 22 weeks of gestation.

Definition of Outcome

The outcome of spontaneous abortion was based on either the occurrence of that event at the time of ascertainment in the emergency department or on subjects' reports of that event during follow-up. For 61% of participants, reported spontaneous abortions were validated by medical record review and/or by histologic review, conducted by a single reference pathologist. We used a standard definition of spontaneous abortion recommended by the World Health Organization, i.e., fetal loss occurring at or before 22 weeks gestation. Gestational dating was based on recall of last menstrual period. Such dating is reasonably accurate; it has been estimated that 90–93% of women can accurately date their last menstrual period within two weeks.¹³ In the Early Pregnancy Study as a whole, among the 373 women who had a crown-rump length estimated by ultrasonography, the correlation to gestational age dating by last menstrual period was 0.60.

Measure of Exposure

Stored urine, collected at the initial visit and then frozen at -27°C for less than 18 months, was assayed for *C. trachomatis* and *Neisseria gonorrhoeae* using the Abbott LCR Probe systems. Ligase chain reaction testing has been shown to be an accurate measure of chlamydial cervicitis in several previous studies.¹⁴⁻¹⁹ These systems use amplification of portions of the DNA which are highly conserved among *C. trachomatis* and *N. gonorrhoeae* species but are not found in other species. In the DNA amplification step, the prepared sample is added to the LCR reaction mixture consisting of several oligonucleotide probes, thermostable ligase and polymerase, and individual nucleotides in buffer. The oligonucleotide probes are designed to hybridize to complementary single-stranded *C. trachomatis* or *N. gonorrhoeae*. Samples were considered contaminated if they met any of the criteria for rejection outlined in the Abbott LCx Probe guidelines.²⁰ These included an inappropriate volume of urine collected, urine not transported or stored at proper temperature, leaky urine containers, or specimens that are moderately bloody or grossly mucoid.

Covariates

At the baseline interview, subjects were asked for information, including age, race, education, last menstrual period, obstetric history, and smoking. Hair was tested for cocaine use via a radioimmunoassay protocol developed and used extensively by PSYCHEMEDICS, Culver City, CA.^{21, 22} Each of these variables was evaluated as a potential confounding factor, associated with both risk of sexually transmitted infection and risk of spontaneous abortion.

Statistical Analysis

Descriptive characteristics were assessed using means for continuous variables and frequencies for interval variables. The proportion of cases and controls with infection was determined. Logistic regression models were used to adjust for potentially confounding factors in assessing the relationship between *C. trachomatis* and spontaneous abortion. Adjusted odds ratios (with 95% confidence limits) were derived from these logistic regression models. In these models, case or control status was the dependent variable and infection status the main independent variable with covariates determined on

the basis of significance in univariate models or on the basis of biologic plausibility.

RESULTS

Of the 60 cases with spontaneous abortion and the 60 controls with uncomplicated pregnancies who were randomly selected from the Early Pregnancy Study, 52 cases and 59 controls had complete interview data and an available frozen urine specimen. Thus, 52 cases and 59 controls were included in the analysis.

Cases and controls were predominantly young and African-American (Table 1). In both cases with spontaneous abortion and controls, almost 40% had not graduated from high school. The mean gestational age at entry into the study was 10.7 weeks for cases and 11.5 weeks for controls. Mean gravidity was between 2.0 and 2.5, and the mean number of miscarriages was around 0.5. Overall, one third of women had at least one previous miscarriage, and 7.2% had two or more miscarriages in the past. Over a quarter of women in both groups had evidence of cocaine use in the past three months by hair analysis. Among cases, 34.6% were current smokers and among controls, 18.6% were current smokers. Five (8.5%) control women and two (3.8%) case women had detectable *C. trachomatis* in a urine specimen. There was no significant difference between infection rates in these two groups. Omitting samples that could have possibly been contaminated, 5.1% of control women and 4.8% of case women were infected with *C. trachomatis*. *N. gonorrhoeae* was not detected among any of the women studied.

A multiple logistic regression model including age, gestational age, chlamydia result, current cigarette use, detection of cocaine in hair, and previous miscarriage confirmed a lack of association between *C. trachomatis* infection and spontaneous abortion (Table 2). The adjusted odds ratio for chlamydial infection in relation to spontaneous abortion was 1.8 (confidence interval 0.3-10.7). None of the other variables in the model significantly predicted spontaneous abortion.

DISCUSSION

Like previous studies, the current small study did not reveal any differences in the prevalence of chlamydial cervicitis between women who experi-

TABLE 1. Means (range) and frequencies of selected descriptive characteristics among 52 cases with spontaneous abortion and 59 controls

| | Cases | Controls |
|---------------------------------|--------------|--------------|
| Age (years) | 23.5 (15–32) | 23.0 (14–32) |
| Race | | |
| White | 5.8% | 0% |
| Black | 88.5% | 88.1% |
| Other | 5.7% | 11.9% |
| Gestational age (weeks) | 10.7 (4–23) | 11.5 (4–23) |
| Education | | |
| Not high school grad | 36.5% | 40.7% |
| High school grad or above | 63.5% | 59.3% |
| Parity | 1.1 (0–4) | 1.1 (0–5) |
| Gravidity | 2.5 (0–8) | 2.0 (0–9) |
| Miscarriages | 0.5 (0–4) | 0.4 (0–5) |
| Current smoker | 34.6% | 18.6% |
| Cocaine detected in hair | 28.8% | 30.5% |
| <i>C. trachomatis</i> infection | 3.8% | 8.5% |
| <i>N. gonorrhoeae</i> infection | 0% | 0% |

TABLE 2. Logistic regression analysis predicting spontaneous abortion

| Variable | Odds ratio | 95% confidence interval |
|---------------------------------------|------------|-------------------------|
| Age of patient | 1.0 | 0.9–1.1 |
| Gestational age | 1.0 | 1.0–1.0 |
| Chlamydia infection | 1.8 | 0.3–10.7 |
| Current cigarette smoker | 0.5 | 0.2–1.4 |
| Cocaine use detected by hair analysis | 0.6 | 0.1–2.6 |
| Previous miscarriage | 0.7 | 0.3–1.7 |

enced spontaneous abortions and women who had uncomplicated pregnancy.

The notion that sexually transmitted infections might relate to spontaneous abortion is biologically plausible. Chlamydia and gonorrhea have been linked to other adverse outcomes in pregnancy, particularly chorioamnionitis, premature rupture of membranes, and low birthweight.^{2–4} Three proposed mechanisms have been postulated by which current or past infection with *C. trachomatis* might cause adverse pregnancy outcomes, including spontaneous abortion. These proposed mechanisms are that the placenta and membranes might be adversely affected by: 1) infection resulting in endometrial damage, 2) an immune response to an epitope shared by a chlamydia and a fetal antigen, or 3) cytokine release by the inflammatory response to genital infection.

Past studies have focused on the relationship between past chlamydial infection and spontaneous abortion but have not examined the impact

from current infection. Quinn et al.⁹ first suggested that there might be a positive relationship between levels of *C. trachomatis* antibody and recurrent spontaneous abortion. However, none of the study subjects had an active cervical chlamydial infection. In a similar study, Witkin et al.¹¹ investigated the relationship between high-titer IgG antibodies to *C. trachomatis* and recurrent spontaneous abortion in a population of women with unexplained infertility. Women with three or four recurrent spontaneous abortions had the highest levels of IgG antibodies to *C. trachomatis*. They concluded that a chronic silent chlamydia infection can increase susceptibility to first trimester abortion. Again, none of the subjects had positive chlamydia culture tests.

Only two previous studies examined nonrecurrent spontaneous abortions. Osser et al.⁸ compared antibody rates in women with at least one miscarriage as compared to pregnant women without complications, matched for age. Chlamydial IgG antibodies were not associated with miscarriage in the index pregnancy; however, the investigators did find a tendency towards a higher frequency of IgG antibodies and previous miscarriages. There was no attempt to examine current infection. Tadmor et al.¹² examined chlamydial IgG and IgA among pregnant women and found that a higher proportion of women with high titers had a previous miscarriage.

Although there were few women with acute infections in the current study, it is the first to examine the association between acute chlamydial cervicitis and spontaneous abortion in an unselected population of pregnant women. Strengths of this study are its prospective design, generalizability, and sensitive methods for detection of infection. The major weaknesses of the study are its small sample size and the limited number of lower genital tract infections evaluated. For example, there was no attempt to evaluate bacterial vaginosis in our design. Also, the mean gestational age at entry into the study was 10–11 weeks, so early spontaneous abortions would have been missed by this study.

Further prospective studies in larger populations of women will be needed to completely exclude the possibility of a relationship between lower genital tract infection and spontaneous abortion. In this pilot investigation, the sample size

tested provided detection of only a four-fold increase in risk given 80% power and a two-sided $\alpha = 0.05$. A case-control study of about 1800 women (900 cases of spontaneous abortion and 900 controls) would be needed to detect as statistically significant a 50% increase in lower genital tract chlamydial infection with 80% power.

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