Cytokines, Pregnancy, and Bacterial Vaginosis: Comparison of Levels of Cervical Cytokines in Pregnant and Nonpregnant Women with Bacterial Vaginosis

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Background. Pregnancy has been considered to be a time of relative immune compromise. Lower-genital-tract immune response appears to be influenced by pregnancy. The objective of this study was to compare, in pregnant versus nonpregnant women, endocervical proinflammatory-cytokine expression in response to bacterial vaginosis.

Methods. Endocervical levels of interleukin (IL)–1β, IL-6, and IL-8 in 99 pregnant and 99 nonpregnant women, all with bacterial vaginosis and without concurrent sexually transmitted infections, were assessed by ELISA. Vaginal flora was characterized on the basis of quantitative vaginal cultures.

Results. Women in the 2 groups differed with respect to smoking status and microbiological constituents responsible for bacterial vaginosis. When the data were stratified by these potential confounders, the levels of all 3 proinflammatory endocervical cytokines were significantly higher in pregnant women than in nonpregnant women.

Conclusions. The proinflammatory cytokine milieu in the cervix is enhanced in pregnant women with bacterial vaginosis, compared with that in nonpregnant women. The notion of pregnancy as an immune-compromised state may be anatomically compartment specific.

Cytokines are a group of intercellular proteins made by various immune cells that are involved in the generation and maintenance of an immune response. They are induced by specific stimuli and are responsible for the differentiation of many cell types, leading to a complex network of immune activities that may ultimately lead to eradication of invading organisms [1]. Infection in the female genital tract, either clinically apparent or subclinical, has been linked to important adverse reproductive sequelae, such as tubal-factor infertility and preterm birth, each imposing an enormous burden on women and society [2–4]. In an effort to delineate the immune response to lower-genital-tract infection, numerous studies focusing on the lower-genital-tract endocervical cytokine milieu in infected and uninfected women have been undertaken [5–13].

Investigations into the impact that pregnancy has on lower-genital-tract immunity (via lower-genital-tract cytokine response) have yielded conflicting results. In their study of women without genital-tract infections, Kutteh and Franklin reported that, compared with those in nonpregnant women, the levels of endocervical interleukin (IL)–1β in pregnant women were markedly increased [6]. In contrast, in a study of women without prevalent infections, Donders et al. failed to confirm...
these findings when they compared the levels of 3 different cytokines—IL-1β, IL-6, and IL-8—in pregnant women versus those in nonpregnant women [7].

For many years, bacterial vaginosis has been considered to be a common but innocuous condition. Recently, there has been increased interest in bacterial vaginosis, as a result of numerous publications linking it to many adverse reproductive sequelae. Associations between bacterial vaginosis and preterm delivery and intrauterine infection [4, 14], postpartum endometritis [15], pelvic inflammatory disease [3, 16], and postprocedural gynecologic infection [17] have been demonstrated. The mechanisms linking bacterial vaginosis to these adverse reproductive sequelae have not been fully identified, but local immune response is hypothesized to be vital. Despite the notion that bacterial vaginosis is a noninflammatory condition, evidence exists that demonstrates altered levels of certain proinflammatory cytokines in women with bacterial vaginosis [5, 18, 19]. A direct comparison of endocervical proinflammatory cytokines in pregnant women with bacterial vaginosis versus those in nonpregnant women with bacterial vaginosis has not been done. The aim of the present study was to directly compare the levels of 3 proinflammatory cytokines by using identical specimen-collection and cytokine-measurement methodology. On the basis of Kutteh and Franklin’s data on pregnant women without infection, we hypothesized that, compared with nonpregnant women with bacterial vaginosis, pregnant women with bacterial vaginosis would have increased levels of IL-1β, IL-6, and IL-8.

PATIENTS AND METHODS

Patients. The present study is a secondary analysis of 2 previously published data sets derived from 2 different studies evaluating treatment of bacterial vaginosis for 2 different endpoints [10, 20]. In brief, the first study included only pregnant women and correlated cytokine response to therapeutic cure using systemic versus topical metronidazole, and the second study included nonpregnant women in an evaluation for the detection of anaerobic bacterial resistance after use of 2 different intravaginal therapies for bacterial vaginosis. The present study compared data from these 2 studies, using 99 pregnant women enrolled during early pregnancy (median gestational age, 13 weeks) and 99 nonpregnant women. Women 18–45 years of age were eligible for participation in both studies. To exclude the possible presence of coinfection, all women were tested for sexually transmitted pathogens, including T. vaginalis, N. gonorrhoeae, and C. trachomatis; trichomoniasis was excluded by culture, and N. gonorrhoeae and C. trachomatis were excluded by nucleic acid–amplification testing. Any woman with a known active infection with any of the above sexually transmitted agents, as well as any woman with active herpes simplex lesions at the screening visit, was excluded from participation. All patients gave written informed consent, and the protocols were approved by the institutional review board of Magee-Womens Hospital of the University of Pittsburgh Medical Center.

All women enrolled in both studies were symptomatic for bacterial vaginosis. Entry criteria for both studies included (1) clinically diagnosed bacterial vaginosis, defined as ≥3 of 4 Amsel’s criteria (>20% clue cells, homogenous vaginal discharge, elevated pH [i.e., ≥4.7] of vaginal discharge, and release of a fishy amine odor when 10% KOH was added to vaginal fluid) and (2) Gram-stain scoring consistent with altered vaginal flora and/or bacterial vaginosis (Nugent score, ≥4) based on criteria developed by Amsel et al. and Nugent et al. [21, 22].

At enrollment, in both studies, women completed standardized questionnaires including inquiry into demographics and medical, reproductive, and sexual history. All women then underwent thorough gynecological examinations, with collection of vaginal swabs for Gram-stain scoring as well as for quantitative vaginal cultures, in accordance with methods described elsewhere [23]. All microbiologic methods and investigations were performed in the same laboratory and used standardized protocols.

Cytokine analysis. From all women, endocervical-swab specimens were collected, before any treatment, for evaluation of levels of 3 cytokines—IL-1β, IL-6, and IL-8. Comparison of these pretreatment levels provided the bases for our analyses. Specimens for cytokine analysis were collected by use of 2 Dacron (Fisherbrand; Fisher Scientific) swabs, at the time of the gynecological examination. The swabs were placed in the endocervix one at a time and, to achieve saturation, were left there for 10 s. Each swab was then placed in a 2-mL microtube containing 400 μL of PBS (Mediatech) and was stored at −20°C.

Before cytokine analysis, each specimen was thawed at room temperature and then, along with the specimen diluent, was placed within a spin-X centrifuge filter unit (Costar) and was centrifuged at 12,128 g for 20 min. Aliquots of the filtered solution were stored at −20°C until the assays were performed. Commercially available cytokine kits (Quantikine; R&D Systems) were used to determine the levels of cytokines IL-1β, IL-6, and IL-8. In brief, standards/specimens were added to a precoated, 96-well microtiter plate and were incubated for 1–2 h. After incubation, the plate was washed 3 times, 200 μL of conjugate was added to each well, and the plate was again incubated for 1–2 h. The plate was washed 3 times, and then 200 μL of substrate solution was added to each well and was incubated for 20 min. The reaction was stopped, and the optical density of each well was determined by use of a microplate reader, at 450 nm. The standards were used to generate a standard curve from which the unknown values were quantified. The minimum detectable levels of IL-1β, IL-6, and IL-8 are <1

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The demographic, behavioral, and microbiologic characteristics of women in the study are presented in Table 1. Nonpregnant women were slightly older, more likely to be smokers, and more likely to have pigmented anaerobic gram-negative rods. The 2 groups were nearly identical in the percentages of women with anaerobic gram-negative rods, anaerobic gram-positive cocci, *Gardnerella vaginalis*, and *Mycoplasma hominis* present in the vagina.

The 2 groups’ pretreatment levels of cytokines IL-1β, IL-6, and IL-8 are shown in Table 2. For each endocervical cytokine, the geometric mean values in the pregnant women were statistically significantly higher (P < .001) than those in the nonpregnant women. To account for potential relevant differences between the 2 groups, the same comparison was done after the data were stratified by smoking status and the presence of pigmented anaerobic gram-negative rods. Pregnant women consistently had significantly higher levels of each endocervical cytokine, across all strata (Table 2). Overall, smokers and non-smokers did not differ with respect to concentrations of endocervical cytokines (data not shown). In addition, multivariable linear regression analysis demonstrated that age, smoking status, and microbiologic makeup were not associated with

### Table 1. Demographic, behavioral, and microbiologic characteristics of women in the study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nonpregnant women (n = 99)</th>
<th>Pregnant women (n = 99)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years</td>
<td>25</td>
<td>22</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Race</td>
<td>.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>30 (30)</td>
<td>34 (34)</td>
<td></td>
</tr>
<tr>
<td>Nonwhite</td>
<td>68 (69)</td>
<td>65 (66)</td>
<td></td>
</tr>
<tr>
<td>Unmarried</td>
<td>89 (90)</td>
<td>83 (86)</td>
<td>.4</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>55 (56)</td>
<td>39 (39)</td>
<td>.03</td>
</tr>
<tr>
<td>Anaerobic gram-negative rods</td>
<td>99 (100)</td>
<td>98 (99)</td>
<td>1.0</td>
</tr>
<tr>
<td>Pigmented</td>
<td>88 (89)</td>
<td>65 (66)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Nonpigmented</td>
<td>95 (96)</td>
<td>97 (98)</td>
<td></td>
</tr>
<tr>
<td>Anaerobic gram-positive cocci</td>
<td>88 (89)</td>
<td>86 (88)</td>
<td>.8</td>
</tr>
<tr>
<td><em>Gardnerella vaginalis</em></td>
<td>96 (97)</td>
<td>98 (99)</td>
<td>.6</td>
</tr>
<tr>
<td><em>Mycoplasma hominis</em></td>
<td>72 (73)</td>
<td>70 (71)</td>
<td>.9</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of women, unless otherwise indicated.

* Generated on the basis of Fisher’s exact test.

† From Mann-Whitney U test.

### Table 2. Geometric mean of log10-transformed cytokine levels in pregnant and nonpregnant women, stratified by smoking and bacterial subpopulations.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Overall</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>3.4 ± 0.5</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>IL-6</td>
<td>3.2 ± 0.5</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>IL-8</td>
<td>4.7 ± 0.4</td>
<td>4.3 ± 0.7</td>
</tr>
</tbody>
</table>

**NOTE.** IL, interleukin.

* Generated on the basis of Student’s t test.
cytokine response. The only factor that was independently associated with cytokine response was pregnancy status \((P < 0.001;\) data not shown).

To ensure that the increase noted in the levels of endocervical cytokines in pregnant women was not a function of concentration of each bacterial constituent, the same analysis of baseline levels of cytokines was performed after the data were stratified by each bacterial constituent on a concentration basis \(\) (data not shown). For all 3 cytokines, in every category of concentration of each bacterial constituent, pregnant women had statistically significantly increased levels.

**DISCUSSION**

The present study confirms Kutteh and Franklin’s earlier study reporting increased levels of IL-1\(\beta\) in noninfected pregnant women versus nonpregnant women—and it extends these findings, to demonstrate that, compared with nonpregnant women and it extends these findings, to demonstrate that, compared with nonpregnant women versus nonpregnant women. Kutteh and Franklin’s findings, suggesting that, like IL-1\(\beta\), IL-6 and IL-8 also are increased in pregnant women with bacterial vaginosis versus nonpregnant women with bacterial vaginosis.

Pregnancy has been referred to as a state of relative immune compromise. This notion has been related both to demonstration of depression of certain aspects of cell-mediated immunity and to clinical observations of an increased severity of numerous infectious conditions in pregnant women \([24]\). The results of the present study call into question the notion of pregnancy as being a state of immune compromise, given that the pregnant women had a significantly higher endocervical cytokine response to the same stimuli \(\) (bacterial vaginosis). Bacterial vaginosis is a polymicrobial condition, and this difference persisted regardless of the precise microbiologic makeup of the women. It is recognized that the 2 groups may have differed with respect to noncultivated microorganisms. One explanation for the finding of a heightened level of cervical cytokines in the endocervical fluid of pregnant women is that immunity is compartment specific—and that an immune response in the lower genital tract may be independent or not fully representative of systemic response. This apparently heightened lower-genital-tract immunity during pregnancy may also provide an explanation of the recent intriguing findings of high rates of spontaneous clearance of \textit{Chlamydia trachomatis} infection and bacterial vaginosis that have been reported in asymptotically infected pregnant women \([25, 26]\).

Other studies have investigated the lower-genital-tract immune response to bacterial vaginosis in either pregnant women only or nonpregnant women only. Platz-Christensen et al. have demonstrated that levels of IL-1\(\alpha\) are significantly higher in pregnant women with bacterial vaginosis than in pregnant control women without bacterial vaginosis \([18]\). Subsequent investigations have also demonstrated increased levels of IL-1\(\beta\) and IL-8 in pregnant women with bacterial vaginosis, whereas increased levels of IL-6 have not been consistently reported \([5, 19]\). Spandorfer et al. documented increased levels of both IL-1\(\beta\) and IL-8 in pregnant women with bacterial vaginosis \([27]\). The present study is unique in that it directly compares the expression of these cytokines in pregnant women versus nonpregnant women, all with bacterial vaginosis.

The role that the lower-genital-tract immune response to infectious challenge plays, via local expression of cytokines, as a correlate to preterm birth is also the topic of numerous ongoing investigations. Simhan et al. have demonstrated that underexpression of endocervical cytokines during early pregnancy
(as demonstrated by lower median values of IL-1β, IL-6, and IL-8) is a marker for subsequent clinical chorioamnionitis [9], which suggests that there is a predisposition to upper-genital-tract infection in pregnancy in a subset of women with an “inadequate” endocervical cytokine milieu. In a series of recent publications, Genc et al. have demonstrated important genetic predispositions (via genetic polymorphisms in receptors involved in cytokine expression and effects) that predispose to an altered response to pathogenic vaginal flora that correlate with the risk of preterm birth [11–13]. These aforementioned and other ongoing investigations will continue to elucidate both the role played by lower-genital-tract immunity and its relation to the disproportionate rates of preterm birth seen in different cohorts of women. In the present study, we did not investigate the women’s genotypes (and therefore genetic predispositions) with respect to cytokine production. Although it has been demonstrated that there can be significant person-to-person variability in the expression of endocervical cytokines in response to infection, the present study’s comparison of the mean values for large groups of pregnant women and nonpregnant women is unlikely to be significantly influenced by patient-specific variation.

The effects of smoking has on the expression of cervical cytokine expression and on lower-genital-tract immunity has been investigated by others, with differing results. Simhan et al. demonstrated an increase in the concentration of the anti-inflammatory cytokines IL-4, IL-10, and IL-14 in the cervices of women who smoked, with a concomitant increase in certain proinflammatory cytokines [28], which was hypothesized to potentially contribute to the increased risk of preterm birth in gravid smokers. In contrast, Scott et al. demonstrated a decreased concentration of mRNA of both IL-4 and IL-10 in women who had smoked during the preceding 24 h [29]. The present study failed to find a relationship between smoking and differences noted in the 3 proinflammatory cytokines measured. Determination of the precise role that smoking plays with respect to lower-genital-tract immunity awaits further investigation.

The findings of present study suggest that lower-genital-tract immunity in response to infectious challenge, as assessed on the basis of endocervical cytokine expression, is influenced by pregnancy; in response to the same microbiologic stimuli (i.e., bacterial vaginosis), pregnant women produced an endocervical cytokine response that was ~2-fold higher than that produced by nonpregnant women. Moreover, the heightened immune reactivity in the lower genital tract during pregnancy contradicts the paradigm of pregnancy as an immune-compromised state and suggests that immunity may be compartment specific. Given the pivotal role that lower-genital-tract immunity plays in reproductive health, continued investigation is warranted, to further delineate the factors mediating response to infectious challenge.

References


