High cervical IL-6 levels predict spontaneous preterm birth among women in western Kenya

Emily Begnel

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Committee:

Jennifer Slyker

Grace John-Stewart

Alison Drake

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Abstract

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Emily Begnel

Chair of Supervisory Committee:
Jennifer Slyker, PhD
Department of Global Health

BACKGROUND: Spontaneous preterm birth (sPTB) increases neonatal morbidity and mortality. Infection and subsequent inflammation of the genital tract have been identified as risk factors for sPTB; however, the role of cytokines in this relationship remains unclear.

OBJECTIVES: This study determined the association between genital cytokines and sPTB, identified cofactors for high genital cytokine levels, and evaluated the correlation between cervical and vaginal cytokine levels.

METHODS: We conducted a case control analysis nested in a cohort study in western Kenya. Cases were women who delivered preterm and were matched 1:1 by gestational age at time of genital swab collection to controls who delivered at or post-term. Genital cytokine levels and diagnoses of genital infections were ascertained in pregnancy in cervical and vaginal swab swabs. Conditional logistic regression was used to determine the association between levels of genital interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α) and sPTB. Linear regression was used to determine the association between

genital infections and cytokine concentrations during pregnancy, and the relationship between vaginal and cervical cytokines.

RESULTS: Among 86 cases and 86 matched controls, IL-1, IL-6, and TNF-α levels were significantly higher in the cervix than the vagina ($p<0.001$ for each); levels for all assessed cytokines were correlated between compartments ($p<0.005$ for each). Odds of sPTB were increased by 54% for each 1 $\log_{10}$- increase in cervical IL-6 level (OR 1.54, 95% CI: 1.00 – 2.38, but not other cervical or vaginal cytokines. High cervical IL-6 concentrations during pregnancy were associated with a concurrent diagnosis of trichomonas ($\beta=0.67$, 95% CI: 0.37-0.97) and abnormal vaginal discharge ($\beta=0.84$, 95% CI: 0.55-1.14). Trichomonas, bacterial vaginosis, and abnormal vaginal discharge were each associated with an inflammatory vaginal milieu with elevated IL-1, IL-6, and TNF-α ($p<0.005$ for all).

CONCLUSION: Our findings suggest that trichomonas infection may mediate risk of sPTB through IL-6 release, and warrants further studies to explore this mechanistic link and potential interventions.

Introduction

Preterm birth (PTB), defined as birth before 37 weeks' gestation, continues to be one of the most common adverse birth outcomes worldwide. An estimated 15 million infants are born preterm each year[1], over half of which occur in Africa and South Asia.[2] Rates of PTB have remained constant over the past several decades, accounting for a substantial portion of infant and child morbidity and mortality. Infants born preterm have a higher risk of neonatal infection and death than infants born at or post-term, as well as increased risk of long-term complications and chronic disease.[3,4] Consequently, PTB imposes substantial emotional and financial burden on families and health care systems, especially in low-resource settings where the risk of maternal and child mortality is high and healthcare resources to support preterm infants are limited.[4]

Previous studies have identified many epidemiologic risk factors for PTB, including low socioeconomic status, maternal and fetal infection, uterine anomalies, adolescence or advanced maternal age, stress, and tobacco use, among others.[5] Infection, in particular, appears to be a strong predictor of PTB and research has linked this risk factor to inflammation of the upper and lower genital tract.[6,7] Sexually transmitted infections (STIs), yeast infection, and bacterial vaginosis (BV) are major causes of genital tract inflammation and are associated with increased risk of PTB, often regardless of treatment[6,8]. Although data specific to women in sub-Saharan Africa are limited, a longitudinal study of women in western Kenya found that having trichomonas, chlamydia, syphilis, gonorrhea, and/or HIV during pregnancy was associated with PTB (*Ravindran, manuscript in preparation*).

One specific mechanism by which genital infections may lead to PTB is through cytokine-mediated initiation of labor. Levels of inflammatory cytokines naturally increase around 37 weeks' gestation in response to fetal signals[9], stimulating prostaglandin production in gestational tissues that subsequently leads to the cervical ripening, membrane rupture, and contractions involved in labor.[10] There is evidence that inflammation from cervicovaginal, uterine,

or amniotic infections may prematurely elevate these cytokine levels and initiate labor processes before 37 weeks.[11,12]

To date, the majority of research studies have focused on four proinflammatory cytokines: interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α). These cytokines are directly involved in the initiation and progression of labor processes in both term and preterm delivery[10,13] and have been previously studied in relation to PTB. Although all have been found to be significantly associated with PTB, IL-1 and IL-6 are more frequently found to be associated with PTB than IL-8 and TNF-α.[14–18] These inconsistencies may in part stem from differences in specimen use (vaginal swabs, cervical swabs, or cervicovaginal lavage), uncontrolled confounding, and differences in source populations and inclusion/exclusion criteria. Additionally, existing studies have not assessed African women where a large proportion of the population experiences epidemiologic risk factors for PTB such as bacterial vaginosis and sexually transmitted infections (STIs).[19,20]

In addition to evidence linking elevated cytokine levels and PTB, there is a need to understand the upstream physiological mechanisms that initiate cytokine-mediated pathways implicated in preterm labor. Infection is most frequently cited as a cause of PTB, and may directly influence cytokine levels in reproductive tissue. For example, previous studies have noted that BV and STIs (such as gonorrhea, chlamydia, trichomonas) are associated with higher genital cytokine levels among pregnant women.[21,22]

Additionally, many studies have used maternal serum or amniotic fluid samples to measure cytokine levels. Although amniotic fluid sampling provides ascertainment of cytokine levels in the fetal environment, it does not necessarily reflect the inflammatory processes occurring in the mother's body. Similarly, maternal serum samples provide systemic cytokine levels and may not capture changes specific to the reproductive tissues. Sampling of cervical and vaginal fluids better captures immune changes occurring in the reproductive tissues to

provide insight into the mechanisms of PTB. Further investigation of the relationship between cytokines and PTB using these methods is necessary to understand the pathway by which infection and inflammation can influence PTB and to focus interventional efforts.

To better understand the relationship between STIs, genital cytokines, and PTB, we assessed the relationship between inflammatory cytokines IL-1, IL-6, IL-8, and TNF-α and PTB among women in western Kenya. We also determined cofactors for high genital cytokine levels to inform mechanisms by which genital infections may influence the risk of PTB. Finally, we assessed the correlation between cytokine levels in vaginal and cervical swab samples to determine the preferred specimen for identifying women at risk of PTB.

Methods

Study Population

This nested case-control study was conducted with data from the Mama Salama Study (MSS), a prospective cohort study conducted to determine predictors of HIV incidence among pregnant and postpartum women.[23] The study was conducted in the Nyanza region of rural western Kenya where HIV prevalence exceeds 20%. Women seeking antenatal care (ANC) at between May 2011 and December 2014 and followed during pregnancy through nine months postpartum. Women were initially eligible to participate in the study if they were between 28 and 38 weeks gestation as calculated by last menstrual period (LMP), but eligibility was expanded in June 2011 to include women 14-28 weeks gestation, in November 2011 to include women >36 weeks gestation, and in March 2012 to include women <14 weeks gestation if they had a positive urine pregnancy test.

Ethics statement

The study protocol was reviewed and approved by the University of Washington Institutional Review Board (IRB) and the Kenyatta National Hospital Ethics and Research Committee (ERC).

Study design and sample size/power

For this analysis, we selected a subset of women from the MSS cohort of 1,304 women. Inclusion criteria for selection were: live birth via vaginal delivery or emergency Cesarean section, and availability of both a cervical and vaginal sample collected at enrollment. Women with preeclampsia, multiple births, or HIV-1 acquisition during the study period were excluded.

Cases were defined as women who had a spontaneous preterm birth (sPTB) at ≥28 weeks and <34 weeks gestation. Controls were defined as women who gave birth at ≥37 weeks and <45 weeks gestation. Women delivering between 34-37 weeks were excluded to reduce misclassification of sPTB. After cases were selected, controls were matched 1:1 by gestational age at time of cervical sample collection.

The study was powered to assess the relationship between cervical cytokine levels classified as high/low (above and below the cohort median) and odds of sPTB, assuming 30% of controls had a high cytokine level and a correlation between matched cases and controls of 0.20. Assuming a 2-sided test using conditional logistic regression, the minimum odds ratio detectable at 80% power is 2.03.

Collection of Demographic and Clinical Characteristics

At enrollment, data on sociodemographic characteristics, pregnancy history, vaginal bleeding, any vaginal washing or drying practices, and smoking status were collected using a survey administered by study clinicians. In addition, women received a pelvic exam and were tested for trichomoniasis, chlamydia, gonorrhea, syphilis, bacterial vaginosis (BV), yeast infection, and cytomegalovirus (CMV). Presence of genital warts or ulcers, abnormal discharge, or cervical inflammation was also recorded. Follow-up study visits were scheduled at 20, 24, 32, and 36 weeks gestation if these visits were >4 weeks after enrollment. Gestational age was determined by ultrasound when available, or by LMP if not ultrasound was not performed. If

LMP was unknown and ultrasound had not been performed, gestational age was estimated by fundal height.

Specimen collection & measurement of genital cytokines

Cervical swabs were obtained by study nurses during pelvic examinations using Dacron swabs to measure cervical cytokine levels, Aptima swabs to test for STIs using molecular methods, and sterile cotton swabs to test for STIs using Gram stain techniques. Aptima swabs were gently rotated in the endocervical canal for 10-30 seconds, sterile cotton swabs were rotated twice in the cervical os, and Dacron swabs were rotated three times in the cervical os. Vaginal swab samples were self-collected by participants. A clinician provided each woman with two sterile cotton swabs to collect vaginal fluids. The participant was instructed to insert the tip of each swab 1-2 inches into the vagina, rotate the swab once in a full circle, and wait 15 seconds before removing the swab and returning it to the study nurse. Cytokines were tested using commercially-available multiplex immunoassays (Meso Scale Discovery V-Plex platform; Rockville, MD) to measure levels of IL-1, IL-6, IL-8, and TNF-α from a 25 µl specimen.

Statistical Analysis

All analyses were conducted in Stata (version 14.2) and used 2-sided tests with α=0.05. We constructed separate conditional logistic regression models to measure the association between $\log_{10}$-transformed levels of each cytokine (IL-1, IL-6, IL-8, and TNF-α) and sPTB. Cervical and vaginal cytokine levels were evaluated separately for all statistical tests.

Linear regression was used to assess the association between genital infections (BV, STIs) and $\log_{10}$-transformed cytokine concentrations. Since all eligible cases were selected, cases were assigned a sampling weight of 1, and controls were assigned weights according to their probability of selection within each gestational age stratum. The Bonferroni-Holm correction was used to adjust the p-value threshold for multiple testing.

Linear regression was used to assess the strength and direction of the linear relationship between vaginal and cervical cytokine levels, treating these as continuous $\log_{10}$-transformed variables. Paired t-tests were used to assess equivalence between mean cervical and vaginal cytokine levels.

Results

Population Characteristics

We identified 86 women in the MSS cohort that had a sPTB and selected 86 matched controls, from 615 eligible women who did not experience sPTB. Controls did not differ significantly from non-selected eligible controls for any cofactors assessed (data not shown).

Cases and controls had similar demographic and health characteristics, although cases were younger (p=0.002), less likely to be married (p=0.01), more likely to have been diagnosed with chlamydia (p=0.003), and less likely to report vaginal washing or drying practices during pregnancy (p=0.02) at enrollment (Table 1); these variables were also predictors of sPTB in the cohort (*Ravindran, manuscript in preparation*). There was a trend for higher prevalence of trichomonas (11% vs 3.5%) and abnormal vaginal discharge (28% vs 17%) in the case-control subset; these were associated with the risk of sPTB in the larger cohort (*Ravindran, manuscript in preparation*). Prevalence of BV and yeast were high with each affecting ~30% of cases and controls; and did not differ significantly between cases and controls. Abnormal vaginal discharge was reported by 38 women, 55% of whom were concurrently diagnosed with BV.

Cytokine levels and sPTB

The associations between IL-1, IL-6, IL-8 and TNF-α levels and the odds of sPTB were determined using data from cervical and vaginal swabs collected at enrollment during pregnancy at median gestation age of 23 weeks. The odds of sPTB were increased by 54% for each 1 $\log_{10}$- increase in cervical IL-6 (OR 1.54, 95% CI: 1.00 – 2.38; Table 2). Similarly, if

cervical IL-6 levels were at or above the population median of 1.01 log_{10}-units, a woman had a 59% greater odds of sPTB, although this association was not statistically significant (OR 1.59, 95% CI: 0.87-2.91). No other cervical or vaginal cytokines were significantly associated with sPTB.

Cofactors associated with high cytokine levels

High cervical IL-6 level was associated with trichomonas (β=0.67, 95% CI: 0.37-0.97) and abnormal discharge (β=0.84, 95% CI: 0.55-1.14; Table 3). Both trichomonas and abnormal discharge were also associated with high cervical IL-8 concentrations (β=1.32, 95% CI: 0.95-1.69 and β=0.82, 95% CI: 0.36-1.27, respectively) but not with cervical IL-1 or TNF-α concentrations. Chlamydia and BV were associated with higher cervical IL-1 concentrations, and yeast was not correlated with any cervical cytokines.

Trichomonas, BV, and abnormal discharge were significantly associated with vaginal concentrations of all cytokines evaluated, but yeast infection was not associated with any vaginal cytokines. Despite correlation with cervical IL-1 concentrations, chlamydia was not significantly correlated with vaginal IL-1.

Demographic factors (including age, education, employment, marital status) and clinical history (including parity, complications with last pregnancy, short inter-pregnancy interval, vaginal bleeding, and diagnosis with gonorrhea, CMV, genital ulcers, or genital warts) were not associated with high cervical or vaginal cytokine levels (data not shown).

Correlation between cervical and vaginal cytokines

Cervical and vaginal cytokine levels were positively and significantly correlated for all cytokines evaluated (Figure 1). Mean cervical cytokine levels were higher than vaginal levels for IL-1, IL-6, and TNF-α (all $p < 0.001$), but not for IL-8 ($p=0.7$; Table 4).

Discussion

In the MSS Cohort, trichomonas, chlamydia and vaginal discharge were associated with the risk of sPTB. In this nested analysis, we found that higher levels of IL-6 in cervical fluid were also associated with increased odds of sPTB; in turn, trichomonas and abnormal vaginal discharge were strongly associated with higher levels of cervical IL-6. Together, these data suggest that trichomonas infection may mediate an increased risk of sPTB through a pathway involving IL-6 release, and warrant further studies to explore this mechanistic link and potential interventions.

Although the physiological pathways of parturition are not fully understood, proinflammatory cytokines, including IL-6, have been recognized as a key component in this process. Specifically, release of cytokines from uterine and fetal tissue increases with gestational age to promote prostaglandin production, which promotes cervical ripening, increased contractility of uterine muscles, and rupture of fetal membranes.[10,11] While cytokine release is thought to occur naturally due to uterine distention and fetal lung development, fetal membranes can also release cytokines as part of an immune response to infection.[6,11] Macrophages in the membrane tissue release proinflammatory cytokines, which can start the parturition processes prematurely. However, the individual cytokines involved in premature and normal parturition processes remain unclear and further investigation is needed to identify the specific role of IL-6.[10,11]

IL-6 has previously been associated with sPTB in other cohorts, although no study to date has concurrently assessed the role of STIs, cytokines and sPTB. A systematic review by Wei et al. found nine studies that examined the association between IL-6 levels and sPTB among women in the United States, Italy, Spain, and Korea. Four of these collected samples from cervicovaginal fluid, all of which found significantly greater odds of sPTB among women with higher IL-6 levels (pooled OR 3.05, 95% CI: 2.00-4.67). Similar to our study, three of the studies that assessed the odds of sPTB among women with elevated IL-1, IL-8, and TNF-α

levels did not find any significant associations.[15] Other recent studies have also found higher IL-6 levels to be consistently associated with sPTB in various biological compartments (maternal, newborn, and umbilical cord serum; amniotic fluid; and cervicovaginal fluid).[14,24,25] However, the findings for IL-1, IL-8, and TNF-α are mixed—some studies show these cytokines to be significantly associated with sPTB, while others do not indicate higher levels increase risk of sPTB.[14]

To our knowledge, only one other study has examined the association between cytokines and sPTB in women from sub-Saharan Africa. Abrams et al. examined the association between maternal, placental, and cord serum cytokine levels and sPTB among women in Malawi. African women warrant unique consideration in this context due to their very high rate of BV, sPTB, and evidence that individuals of African origin may have higher constitutive levels of immune activation and inflammation compared to individuals of European descent.[26,27] Their results were similar to ours—they found maternal IL-6 levels to be significantly associated with sPTB (OR 3.7, 95% CI: 1.1-12.7) but not IL-8 (OR 5.4, 95% CI: 0.3-86.4) or TNF-α levels (OR 1.6, 95% CI 0.5-5.0). However, they did not measure IL-1 levels. Interestingly, IL-8 was a significant cofactor when measured via placental and cord serum, suggesting that the biologic compartment assessed can influence the detected association.[28] This possibility is supported by our finding that cervical and vaginal samples did not predict the same odds of sPTB.

Trichomonas, BV, and abnormal discharge were strongly correlated with high vaginal levels of all the cytokines measured, which reflects the profound inflammatory response they evoke in vaginal tissue. However, these were not as universally associated with cervical cytokines. Trichomonas and abnormal vaginal discharge were associated with elevated IL-6 and IL-8 levels, while BV and chlamydia were associated with cervical IL-1 levels. It thus appears that specific infections differentially affect the cervical and vaginal tissues. STIs and BV, have been shown to increase cervicovaginal levels of IL-1, IL-6 and IL-8 in non-pregnant women[29],

which is consistent with our results. Yeast infection was not associated with higher vaginal or cervical cytokine levels in our cohort, even though it elicits vaginal inflammation.[30]

Sampling of vaginal fluids can be done without speculum, which makes it less expensive, less invasive, and generally easier to perform in pregnant women. We thus evaluated whether assessment of these two compartments was equivalent for cytokine measurements. Although vaginal and cytokine levels were highly correlated, cervical levels tended to be higher than vaginal levels and although cervical IL-6 was associated with sPTB vaginal levels were not. Our findings are consistent with the composition of the mucosal tissue at the two sites. IL-6 and TNF-α are primarily made by lymphocytes and macrophages and IL-1 is made by macrophages and dendritic cells, so it is expected that a higher concentration of these cytokines was found in the cervical fluids as these cells are present in greater numbers and closer to the surface. In contrast, IL-8 is made by epithelial cells as well as macrophages, and would thus be expected to be found at high concentrations in both the vagina and cervix.

These results suggest that cervical and vaginal cytokine samples are not interchangeable and that assessment of cervical cytokines may more accurately predict sPTB risk. To date, few studies have examined whether cytokine levels vary by type of sampling method. A study by Gennaro et al. found high correlation between cervical and vaginal IL-1 and IL-6 levels, but not TNF-α levels.[31] Another study by Yavari et al. conducted a similar analysis to compare IL-6 levels in serum and cervicovaginal fluids, but found that the two measures did not correlate significantly.[32] In general, many studies have suggested that IL-6 levels measured in amniotic fluid can be used as a biomarker to predict sPTB, either alone or in combination with other measures such as cervical length[24,33–35]. However, others state there is a need for further research and careful consideration when using cytokine levels as a diagnostic tool as there are still concerns about the timing of sampling, the type of biological sample (serum from mother,

umbilical cord, placenta or infant; amniotic fluid; cervical mucus; or vaginal fluids), the sensitivity and specificity of available testing methods, and the role of various infections.[36–38]

Our study has many strengths and a few important limitations to note. First, concurrent assessment of STIs, BV, and physiologic genital inflammation allowed us to assess the intersection of STI, cytokines and sPTB. Correlates of cytokine levels were determined for the case-control subset using linear regression. Measures of association were very similar when cases and controls were assessed separately (data not shown); in the final analysis we combined cases and controls, and accounted for probability of selection by weighting in our regression model. Combining groups in this way increased statistical power and precision and reduced the number of statistical tests. Drawing from a large existing cohort, we had a large sample of women which allowed us to match by gestational age; since cytokine levels increase closer to parturition, it was important to adequately control for this important confounder. We excluded cases close to 37 weeks that would likely include misclassified outcomes if included; however, this limits generalizability in that the results of this analysis may not apply to births that are near term. It is also possible that gestational age at enrollment was inaccurately dated due to reliance on self-report of last menstrual period. Similarly, although ultrasound and fundal height were used to ascertain gestational age at follow-up visits and birth, these measures were not always available for every participant.

Our study also made the assumption that early cytokine levels would be reflective of cytokine levels closer to birth, which would be most relevant to the actual biological processes involving onset of labor; however, since we did not test later levels we cannot validate this assumption. STIs were treated when detected, so some of the inflammatory impact of these may have “washed out” by the time of labor.

Overall, this study contributes to our knowledge of the association between cervical and vaginal cytokine levels and sPTB. Given our finding that cervical IL-6 levels are associated with greater odds of sPTB, future studies of sPTB mechanisms should examine cytokine-mediated

pathways involving IL-6 to determine whether interventions along this pathway could reduce the risk of sPTB.

References

1. World Health Organization (WHO). Born Too Soon. 2012.
2. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: A systematic analysis and implications. *Lancet*. 2012;379(9832):2162-2172. doi:10.1016/S0140-6736(12)60820-4.
3. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet*. 2014;(385):430-440. doi:10.1016/S0140-6736(14)61698-6.
4. Frey HA, Klebanoff MA. The epidemiology, etiology, and costs of preterm birth. *Semin Fetal Neonatal Med*. 2016;21(2):68-73. doi:10.1016/j.siny.2015.12.011.
5. Goldenberg RL, Culhane JF, Iams JD, Romero R. Preterm Birth 1: Epidemiology and Causes of Preterm Birth. *Obstet Anesth Dig*. 2008;29(1):6-7. doi:10.1097/01.aoa.0000344666.82463.8d.
6. Nadeau HCG, Subramaniam A, Andrews WW. Infection and preterm birth. *Semin Fetal Neonatal Med*. 2016;21(2):100-105. doi:10.1016/j.siny.2015.12.008.
7. Cappelletti M, Della Bella S, Ferrazzi E, Mavilio D, Divanovic S. Inflammation and preterm birth. *J Leukoc Biol*. 2016;99(1):67-78. doi:10.1189/jlb.3MR0615-272RR.
8. Cunnington M, Kortsalioudaki C, Heath P. Genitourinary pathogens and preterm birth. *Curr Opin Infect Dis*. 2013;26(3):219-230. doi:10.1097/QCO.0b013e328360dc31.
9. Mendelson CR. Minireview: fetal-maternal hormonal signaling in pregnancy and labor. *Mol Endocrinol*. 2009;23(7):947-954. doi:10.1210/me.2009-0016.
10. Keelan JA, Blumenstein M, Helliwell RJA, Sato TA, Marvin KW, Mitchell MD. Cytokines, prostaglandins and parturition - A review. *Placenta*. 2003;24(SUPPL. A):2-6. doi:10.1053/plac.2002.0948.
11. Ravanos K, Dagklis T, Petousis S, Margioula-Siarkou C, Prapas Y, Prapas N. Factors implicated in the initiation of human parturition in term and preterm labor: a review. *Gynecol Endocrinol*. 2015;31(9):679-683. doi:10.3109/09513590.2015.1076783.
12. Vrachnis N, Karavolos S, Iliodromiti Z, et al. Impact of mediators present in amniotic fluid on preterm labour. *In Vivo (Brooklyn)*. 2012;26(5):799-812. doi:26/5/799 [pii].
13. Romero R, Espinoza J, Gonçalves L, Kusanovic J, Friel L, Hassan S. The role of inflammation and infection in preterm birth. *Semin Reprod Med*. 2007;25(1):21-39. doi:10.1055/s-2006-956773.
14. Lyon D, Cheng C-Y, Howland L, et al. Integrated Review of Cytokines in Maternal, Cord, and Newborn Blood: Part I--Associations With Preterm Birth. *Biol Res Nurs*. 2010;11(4):371-376. doi:10.1177/1099800409344620.
15. Wei SQ, Fraser W, Luo ZC. Inflammatory cytokines and spontaneous preterm birth in asymptomatic women: a systematic review. *Obs Gynecol*. 2010;116(2 Pt 1):393-401. doi:10.1097/AOG.0b013e3181e6dbc0.
16. Paternoster DM, Stella A, Gerace P, et al. Biochemical markers for the prediction of spontaneous pre-term birth. *Int J Gynecol Obstet*. 2002;79(2):123-129.

doi:10.1016/S0020-7292(02)00243-6.

17. Tanaka Y, Narahara H, Takai N, Yoshimatsu J, Anal T, Miyakawa I. Interleukin-1beta and interleukin-8 in cervicovaginal fluid during pregnancy. *Am J Obstet Gynecol*. 1998;179(3 I):644-649. doi:10.1016/S0002-9378(98)70058-4.
18. Jun JK, Yoon BH, Romero R, et al. Interleukin 6 determinations in cervical fluid have diagnostic and prognostic value in preterm premature rupture of membranes. *Am J Obstet Gynecol*. 2000;183(4):868-873. doi:10.1067/mob.2000.109034.
19. Jespers V, Crucitti T, Menten J, et al. Prevalence and correlates of bacterial vaginosis in different sub-populations of women in sub-Saharan Africa: A cross-sectional study. *PLoS One*. 2014;9(10). doi:10.1371/journal.pone.0109670.
20. Chico RM, Mayaud P, Mabey D, Ronsmans C. Prevalence of Malaria and Sexually Transmitted and Reproductive Tract Infections in Pregnancy in Sub-Saharan Africa A Systematic Review. 2013;307(19):2079-2086.
21. Cauci S, Culhane JF. Modulation of vaginal immune response among pregnant women with bacterial vaginosis by Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoeae, and yeast. *Am J Obstet Gynecol*. 2007;196(2):1-7. doi:10.1016/j.ajog.2006.08.033.
22. Basso B, Giménez F, López C. IL-1beta, IL-6 and IL-8 levels in gyneco-obstetric infections. *Infect Dis Obstet Gynecol*. 2005;13(4):207-211. doi:10.1080/10647440500240664.
23. Kinuthia J, Drake AL, Matemo D, et al. HIV acquisition during pregnancy and postpartum is associated with genital infections and partnership characteristics. *AIDS*. 2015;29(15):2025-2033. doi:10.1097/QAD.0000000000000793.
24. Kim A, Lee ES, Shin JC, Kim HY. Identification of biomarkers for preterm delivery in mid-trimester amniotic fluid. *Placenta*. 2013;34(10):873-878. doi:10.1016/j.placenta.2013.06.306.
25. Jung EY, Park JW, Ryu A, Lee SY, Cho SH, Park KH. Prediction of impending preterm delivery based on sonographic cervical length and different cytokine levels in cervicovaginal fluid in preterm labor. *J Obstet Gynaecol Res*. 2016;42(2):158-165. doi:10.1111/jog.12882.
26. Menon R, Fortunato SJ, Edwards DRV, Williams SM. Association of genetic variants, ethnicity and preterm birth with amniotic fluid cytokine concentrations. *Ann Hum Genet*. 2010;74(2):165-183. doi:10.1111/j.1469-1809.2010.00562.x.
27. Menon R, Dunlop AL, Kramer MR, Fortunato SJ, Hogue CJ. An overview of racial disparities in preterm birth rates: Caused by infection or inflammatory response? *Acta Obstet Gynecol Scand*. 2011;90(12):1325-1331. doi:10.1111/j.1600-0412.2011.01135.x.
28. Abrams ET, Milner DA, Kwiek J, et al. Risk factors and mechanisms of preterm delivery in Malawi. *Am J Reprod Immunol*. 2004;52(2):174-183. doi:10.1111/j.1600-0897.2004.00186.x.
29. Mitchell C, Marrazzo J. Bacterial vaginosis and the cervicovaginal immune response. *Am J Reprod Immunol*. 2014;71(6):555-563. doi:doi:10.1111/aji.12264.
30. Sobel JD. Vulvovaginal candidosis. *Lancet*. 2007;369(9577):1961-1971. doi:10.1016/S0140-6736(07)60917-9.
31. Gennaro S, Raftery N, Campbell D, Shults J. Cervical and vaginal cytokine determinations in pregnant women: methodologic issues. *Biol Res Nurs*. 2008;9(3):215-222. doi:10.1177/1099800407309281.
32. Yavari Kia P, Baradaran B, Shahnazi M, Asghari Jafarabadi M, Khaze V, Pourasad

Shahrak S. Maternal Serum and Cervicovaginal IL-6 in Patients with Symptoms of Preterm Labor. *Iran J Immunol*. 2016;13(3):229-236.

33. Romero R, Yoon BH, Kenney JS, Gomez R, Allison AC, Sehgal PB. Amniotic-Fluid Interleukin-6 Determinations Are of Diagnostic and Prognostic Value in Preterm Labor. *Am J Reprod Immunol*. 1993;30(2-3):167-183.
34. Chaemsaithong P, Romero R, Korzeniewski SJ, et al. A Rapid Interleukin-6 Bedside Test for the Identification of Intra-amniotic Inflammation in Preterm Labor with Intact Membranes. *J Matern Neonatal Med*. 2015;7058(September):1-30. doi:10.3109/14767058.2015.1006620.
35. Chandiramani M, Seed PT, Orsi NM, et al. Limited Relationship between Cervico-Vaginal Fluid Cytokine Profiles and Cervical Shortening in Women at High Risk of Spontaneous Preterm Birth. *PLoS One*. 2012;7(12):1-11. doi:10.1371/journal.pone.0052412.
36. Goldenberg R, Goepfert A, Ramsey P. Biochemical Markers for the Prediction of Preterm Delivery. *Am J Obstet Gynecol*. 2005;192:S36-46. doi:doi:10.1016/j.ajog.2005.02.015.
37. Genc MR, Ford CE. The clinical use of inflammatory markers during pregnancy. *Curr Opin Obstet Gynecol*. 2010;22(2):116-121. doi:10.1097/GCO.0b013e3283374ac8.
38. Vousden N, Chandiramani M, Seed P, Shennan A. Interleukin-6 bedside testing in women at high risk of preterm birth. *J Matern Fetal Neonatal Med*. 2011;24(10):1301-1304. doi:10.3109/14767058.2011.558954.

Table 1. Study population characteristics among cases and controls.

Maternal Characteristics	Cases (N=86)	Controls (N=86)	p-value
	Mean (±SD) or % (n)	**Mean (±SD) or % (n)**	
Age (years)	22 (±4.8)	24 (+5.6)	0.002
Years of education	8.5 (±2.0)	9.0 (±2.1)	0.1
Gestational age at time of cervical/vaginal swab sample (weeks)	22 (±4.8)	22 (±4.8)	1.0
Adolescent (aged <19 years)	28% (24)	19% (16)	0.1
Employed	37% (32)	44% (38)	0.4
Married	72% (62)	87% (75)	0.01
Number of previous live births			0.3
0	1.2% (1)	2.3% (2)	
≥ 1	62% (52)	70% (60)	
Complications with last pregnancy [a]	42% (36)	38% (33)	0.6
Last pregnancy within 6 months of conception of current pregnancy	3.5% (3)	1.2% (1)	0.3
Vaginal bleeding during pregnancy	0% (0)	0% (0)	--
Any STI, including BV	48% (41)	31% (27)	0.09
Any STI, excluding BV	11% (9)	4.7% (4)	0.07
BV	36% (31)	27% (23)	0.4
Trichomonas	11% (9)	3.5% (3)	0.07
Chlamydia	13% (11)	1.2% (1)	0.003
Gonorrhea	2.3% (2)	2.3% (2)	1.0
Syphilis	1.2% (1)	0% (0)	0.4
CMV	28% (24)	24% (21)	0.6
Genital ulcers	2.3% (2)	0% (0)	0.2
Genital warts	2.3% (2)	0% (0)	0.2
Yeast infection	29% (25)	30% (26)	0.9
Abnormal discharge	28% (24)	17% (15)	0.1
Cervicitis	4.7% (4)	2.3% (2)	0.4
Mucopus	2.3% (2)	1.2% (1)	0.6
Friable cervix	3.5% (3)	0% (0)	0.08
Cervical ectopy	0% (0)	1.2% (1)	0.3
Smoking during pregnancy	0% (0)	0% (0)	--
Any vaginal washing or drying during pregnancy [b]	48% (41)	65% (56)	0.02

[a] Complications defined as experiencing one or more of the following during the previous pregnancy: late pregnancy bleeding, miscarriage, fetal mal-presentation, or eclampsia.
[b] BV = bacterial vaginosis
[c] CMV = cytomegalovirus
[d] Vaginal washing includes washing with water, soap, antiseptics, detergent, or other substances. Vaginal drying includes placement of herbs/leaves, powders, cloth, or other material inside the vagina.

Table 2. Mean $\log_{10}$-transformed cervical and vaginal cytokine levels among cases and controls with odds ratios and 95% confidence intervals for the association between cytokine levels and sPTB.

	Cases Mean (±SD)	**Controls** Mean (±SD)	**p-value** [a]	**OR (95% CI)**
Cervical				
IL-1	2.82 (0.96)	2.79 (1.15)	0.832	1.03 (0.77-1.39)
IL-6	1.13 (0.79)	0.88 (0.78)	0.041	1.54 (1.00-2.38)
IL-8	3.12 (1.25)	2.98 (1.01)	0.449	1.11 (0.85-1.44)
TNF-α	0.84 (0.60)	0.91 (0.76)	0.515	0.89 (0.56-1.42)
Vaginal				
IL-1	1.74 (0.99)	1.59 (1.17)	0.408	1.14 (0.82-1.57)
IL-6	0.48 (0.69)	0.32 (0.63)	0.148	1.45 (0.87-2.40)
IL-8	3.13 (0.61)	2.98 (0.75)	0.174	1.40 (0.83-2.35)
TNF-α	0.30 (0.66)	0.18 (0.73)	0.267	1.35 (0.79-2.32)

[a] p-value for paired t-test.

Table 3. Linear regression coefficients and 95% confidence intervals for the associations between genital infection/STI cofactors and log_{10}-transformed cytokine concentrations.

Cofactor	Cervical IL-1	Cervical IL-6	Cervical IL-8	Cervical TNF-α	Vaginal IL-1	Vaginal IL-6	Vaginal IL-8	Vaginal TNF-α
Trichomonas (n=12)	0.28 (-0.28-0.83)	0.67 (0.37-0.97)*	1.32 (0.95-1.69)*	0.22 (-0.16-0.59)	1.17 (0.71-1.63)*	0.58 (0.40-0.77)*	0.65 (0.45-0.85)*	0.75 (0.51-1.00)*
Chlamydia (n=12)	0.66 (0.24-1.09)*	0.001 (-0.65-0.65)	-0.20 (-1.25-0.85)	0.07 (-0.33-0.48)	0.38 (-0.26-1.02)	0.27 (0.03-0.52)	0.17 (-0.26-0.61)	0.13 (-0.43-0.69)
BV (n=54)	1.00 (0.52-1.48)*	0.44 (0.05-0.82)	-0.02 (-0.51-0.47)	0.28 (-0.08-0.64)	1.49 (1.07-1.91)*	0.62 (0.40-0.84)*	0.68 (0.40-0.96)*	0.86 (0.60-1.13)*
Yeast infection (n=51)	0.45 (-0.08-0.97)	0.02 (-0.49-0.53)	0.12 (-0.35-0.60)	0.35 (-0.02-0.71)	0.10 (-0.51-0.72)	0.18 (-0.12-0.47)	0.14 (-0.21-0.50)	0.0002 (-0.38-0.38)
Abnormal discharge (n=39)	0.63 (0.09-1.16)	0.84 (0.55-1.14)*	0.82 (0.36-1.27)*	0.29 (-0.11-0.68)	1.11 (0.49-1.74)*	0.45 (0.15-0.74)*	0.56 (0.24-0.88)*	0.75 (0.43-1.07)*

*Statistically significant after Bonferroni-Holm adjustment for multiple testing.

Table 4. Mean cervical and vaginal cytokine levels among study participants.

Cytokine	Cervical Mean (±SD)	Vaginal Mean (±SD)	p-value[a]
IL-1	2.80 (±1.06)	1.67 (±1.08)	<0.0001
IL-6	0.98 (±0.79)	0.41 (±0.67)	<0.0001
IL-8	3.02 (±1.14)	3.05 (±0.68)	0.7
TNF-α	0.86 (±0.70)	0.24 (±0.69)	<0.0001

[a] p-value for paired t-test.

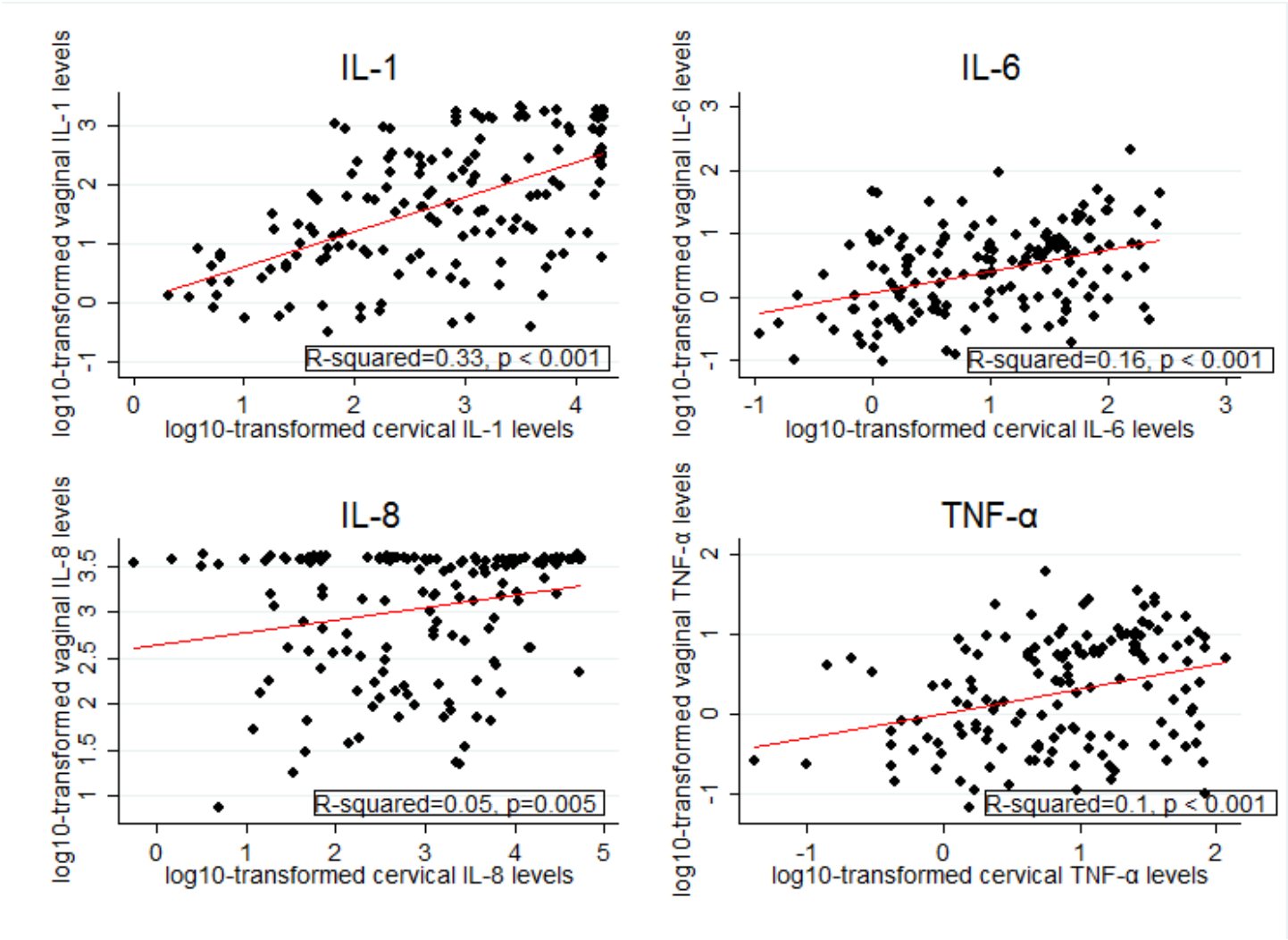

Figure 1. Comparison of cervical and vaginal cytokine levels.