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Repeated Measures of Cervicovaginal Cytokines during Healthy Pregnancy: Understanding "Normal" Inflammation to Inform Future Screening

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Abstract

Objective This study aimed to describe characteristics of cervicovaginal cytokines obtained during pregnancy from women who subsequently delivered at term.

Study Design We used repeated measures of 20 cervicovaginal cytokines, collected on average on a monthly basis, from the second to the ninth month of gestation among 181 term pregnancies in the Mexico City Pregnancy Research on Inflammation, Nutrition, & City Environment: Systematic Analyses cohort (2009–2014). Cytokines were quantified using multiplex assay.

Results Cytokine distributions differed more between than within cytokines. Across trimesters, cytokines interleukin (IL)-1Ra, IL-1α, and IL-8 consistently had high concentrations compared with other measured cytokines. Cytokine intraclass correlation coefficients ranged from 0.41 to 0.82. Spearman's correlation coefficients among cytokine pairs varied but correlation directions were stable; 95.3% of the 190 correlation pairs remained either negative or positive across trimesters. Mean longitudinal patterns of log-transformed cytokines from Tobit regression varied across but less within cytokines.

Conclusion Although mean concentrations of cervicovaginal cytokines among term pregnancies were high, they were largely stable over time. The high cytokine concentrations corroborate that pregnancy is associated with an active inflammatory state. These characterizations may serve as a baseline for comparison to other obstetric outcomes, which may be helpful in understanding deviations from normal gestational inflammation.

Keywords

- appropriate statistical methods
- cytokines
- inflammation
- patterns
- longitudinal data
- pregnancy
- PRINCESA cohort
- Tobit regression
- term birth

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Inflammation is a normal part of human pregnancy; it is particularly needed for physiological events such as implantation and parturition.[1] Interestingly, parturition is associated with inflammatory states similar to those found in pathological conditions such as infection,[2] and this inflammation may occur in the absence of microorganisms (identified using culture and molecular methods) in the amniotic cavity, a condition known as sterile inflammation.[3] Additionally, other points in normal pregnancy are marked by controlled systemic inflammation.[4,5] However, inadequately regulated inflammation can lead to pathological states, including adverse pregnancy outcomes.[6] To date, there is limited characterization of the range of normal variation in inflammation biomarkers such as cytokines during a "healthy" pregnancy and inflammatory characteristics that constitute a pathologic departure from the norm have not been clearly elucidated.

The role of proinflammatory and anti-inflammatory cytokines in pregnancy—especially their potential influence on the length of pregnancy—is being increasingly evaluated. However, the dearth of longitudinal studies describing cervicovaginal cytokine patterns over the course of pregnancy[7] highlights the need for comprehensive studies describing the range of "normal" immunologic responses to pregnancy.

To fill these gaps, our objective was to characterize trajectories and concentrations of 20 cervicovaginal cytokines during pregnancy among women who delivered at term. We selected the cervicovaginal compartment as an acceptable proxy of intrauterine milieu conditions during pregnancy. Cytokines were selected from among those previously evaluated in pregnant women[8] to represent four important categories of cytokines, namely: proinflammatory, anti-inflammatory, chemokines, and growth factor.

Materials and Methods

This study uses data collected from pregnant women in Mexico City participating in the Pregnancy Research on Inflammation, Nutrition, & City Environment: Systematic Analyses (PRINCESA) cohort.[9] The study was approved by the Institutional Review Board from the University of Michigan and ethics committees from the Mexico City Health Ministry (Secretaría de Salud del Gobierno de la Ciudad de México), and the School of Medicine of the National Autonomous University of Mexico (Facultad de Medicina, Universidad Nacional Autonoma de México [UNAM]).

Pregnant women were enrolled in the study during either their first or second trimester of pregnancy. Samples were obtained at enrollment and approximately monthly thereafter. Participants 18 years or older who lived and/or worked in metropolitan Mexico City (Mexico City and surrounding areas), and provided written informed consent, were enrolled in the study between 2009 and 2014. All participants agreed to attend monthly prenatal visits and all had singleton pregnancies. Clinical samples (blood, urine, cervicovaginal exudates) and demographic information were collected during monthly visits. Additionally, participants were eligible if they did not present with any medical or obstetric complications; those who subsequently developed complications were referred to another hospital for appropriate prenatal care.

Gestational age was calculated using infant's birth date and the first day of last menstrual period based on trustworthy (98.9% of participants) and exact (99.4% of participants) recall from the earliest available visit record or screening. Trustworthy and exact recall were assessed by asking participants, "Is your last menstrual period date a trustworthy recall?" and "Is your last menstrual period date an exact recall?" respectively. These analyses were limited to the 181 participants who delivered at term and for whom data from at least one visit (first or follow-up visit) were available at the time of data analysis.

We quantified cytokines from cervicovaginal samples collected at each monthly visit; samples were stored at −80°C until processing. Study participants were enrolled during their first or second trimester of pregnancy; samples were obtained at enrollment and approximately monthly thereafter. Therefore, initial samples were from the first trimester for some participants and second trimester from other participants. All samples obtained prior to when each participant was in labor were tested for cytokines. Samples were collected using a Dacron swab that was rotated for 10 seconds in the cervicovaginal section of the reproductive tract, the tip submerged, and washed repeatedly in a solution of phosphate buffered saline added with a cocktail of protease inhibitors (Sigma). Infection status was evaluated based on identification of microorganisms by culture count and clinical characteristics during genital and pelvic examinations. We assigned points according to these characteristics and classified patients as infected or noninfected using a cutoff in the sum of points. *Lactobacillus* spp. abundance was categorized as absent (three points), scarce (two points), intermediate (one point), and abundant (zero point). Other microorganism abundance was classified as absent (zero point), scarce (one point), intermediate (two points), and abundant (three points). Patients with three points or less were classified as noninfected; patients with four or more points were classified as infected. The opposite classification between *Lactobacillus* spp. and other microorganisms reflects different effects that these two groups of organisms have on lower reproductive health.[10,11] Identified microorganisms included *Streptococcus* spp., *Staphylococcus* spp., *Haemophilus* spp., *Gardnerella vaginalis*, *Escherichia coli*, *Staphylococcus aureus*, *Bifidobacterium* spp., *Proteus* spp., *Bacillus* spp., *Streptococcus agalactiae*, *Candida albicans*, and *Neisseria gonorrhoeae*.

Clinical cervical vaginal infection was based on the presence of abnormal vaginal discharge and reported itching, burning, or malodor (one point). Isolated symptoms and normal discharge were assigned zero point. A summary score which incorporated both microbiology and symptom scores was used to determine final infection status.

A total of 20 cytokines were quantified in monthly cervicovaginal samples using the MAP human cytokine/chemokine magnetic bead panel kit (Millipore Corporation, Billerica, MA).[12] Cytokines quantified were: eotaxin, interferon gamma (IFN-γ), IL-10, IL-12p40, IL-12p70, IL-17, IL-1Ra, soluble IL-2 receptor α (sIL-2Rα), IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IFN-γ-inducible protein (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1α), macrophage inflammatory protein-1 β (MIP-1β), tumor necrosis factor α (TNF-α), and vascular endothelial growth factor (VEGF).

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Analyses were done in the laboratories of UNAM or its affiliate, the National Institute of Genomic Medicine, using previously frozen samples following the published protocol of the manufacturer.[12] Analyses were done in duplicate for every sample, and standard controls were run.

First-time thawed samples were briefly vortexed and centrifuged for 20 to 30 seconds, and 25 µL of thawed cervicovaginal exudate samples were added to each well. Twenty-five µL of previously mixed beads were added to each well and 200 µL of wash buffer was added to all wells before the addition of 25 µL of detection antibodies to each well. Twenty-five µL streptavidin–phycoerythrin were subsequently added to the wells, washed twice, and then run on MAGPIX with XPONENT software for analysis.

Per the manufacturer's protocol, the minimum detectable concentration in pg/mL, intra-assay coefficient of variation (CV) %, and interassay CV %, respectively for each cytokine are shown in ►**Supplementary Appendix 1** (available in the online version). Observations below the limit of detection (LOD) were transformed as LOD/√2 for analysis. In addition, some samples had cytokine concentrations that exceeded the upper LOD of the equipment and were quantified as more than 10,000 pg/mL. For statistical analyses, these right censored observations were transformed by adding 10 pg/mL.

Statistical analyses were performed using SAS Statistical Software version 9.3 (SAS Institute Inc. Cary, NC) and R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics on demographic and obstetric/gynecologic characteristics were generated. Probability of infection for each trimester was determined by calculating the mean across all participants for the number of infections per trimester per participant/number of visits per trimester per participant. The distribution of each cytokine was examined using histograms and q–q plots. We compared ranks (Kruskal–Wallis' test) for each of the 20 cytokines by body mass index (BMI) categories using (1) the four standard BMI categories and (2) a second two-group classification format which grouped underweight and normal in one category and overweight and obese in another category.

We calculated select percentiles of each cytokine by trimester. In addition to having censored observations, cytokine concentrations were not normally distributed. Therefore, we used empirical cumulative distribution functions (ECDFs) to graphically represent the distributions for each cytokine by select months of gestation. The *cenfit* function from the NADA package in R was used to generate ECDF plots.[13]

Spearman's correlations were calculated for pairs of cytokines for each trimester, using the LOD/√2 substitution for values below the LOD, and the upper LOD plus 10 pg/mL (10,010 pg/mL) substitution for values above the upper LOD. Correlations were graphically illustrated using heat maps.[14] We utilized only one observation per woman for each trimester to eliminate the influence of correlation due to repeated measurements, so Spearman's correlation coefficients were computed using cytokine concentrations from the gestation month with the highest number of observations for each trimester. To enhance interpretability of the heat maps, the ordering of cytokines on the axes was based on functional groups. The *p*-values that assessed the statistical significance of pairwise correlations were adjusted to control the false discovery rate.[15]

We also calculated intraclass correlation coefficients (ICCs) for each cytokine. Conventional methods used to calculate ICCs were not appropriate due to censored observations and a lack of normality even after log transformation. To address lack of normality which is important for ICC calculations based on censored data, we applied the Box–Cox transformation[16] to the cytokine with the lowest percent of observations below the LOD, and identified that an inverse fourth root transformation yielded distributions closer to the normal distribution; this transformation was deemed appropriate and applied to all cytokines. To account for the inverse fourth root transformation, we adapted a SAS NLMIXED model for repeated measures, which uses log-normally distributed data, to estimate ICCs[17]; this procedure uses maximum likelihood estimation to fit mixed effects models and accounts for left censoring of log-normally distributed data.

Finally, we characterized average cytokine patterns over time in women who delivered at term using Tobit regression (Survival package in R).[18] Tobit regression is a parametric regression which properly accounts for both left and right censoring[19] and may be used for data with repeated measures.[20] Participants were clustered by identification number and the models included a set of variables representing a natural cubic spline parameterization of gestational weeks at

Table 1 Demographic and obstetric characteristics of Mexican women in the PRINCESA cohort delivering term births, 2009 to 2014 ($N = 181$)

Age (y)	***N* (%)**
< 20	34 (18.8)
20–35	127 (70.2)
> 35	20 (11.1)
Pre-pregnancy BMI	***N* (%)**
< 18.5 kg/m^2	9 (5.0)
18.5–24.9 kg/m^2	60 (33.2)
25–29.9 kg/m^2	59 (32. 6)
≥ 30 kg/m^2	31 (17.1)
Missing	22 (12.2)
Parity	***N* (%)**
Nulliparous	51 (28.2)
Parous	99 (54.7)
Missing	31 (17.1)
Reproductive tract infection	**Probability of infection[a]**
Trimester 1	0.31
Trimester 2	0.37
Trimester 3	0.28

Abbreviations: BMI, body mass index; PRINCESA, Pregnancy Research on Inflammation, Nutrition, & City Environment: Systematic Analyses.
[a]Mean of (number of infection per trimester/number of visits for each participant per trimester).

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the time of the *i*th observation in the *j*th woman. A working independence assumption was used to fit the model, and robust standard errors of model coefficients were computed. We used the estimated parameters to construct plots that illustrate mean cytokine patterns during the course of pregnancy. We also considered the sensitivity of results to not adjusting for infection status.

Results

Cytokines were quantified from samples obtained from the end of second month to the end of pregnancy among 181 women who were not actively in labor. The median gestational age at enrollment was 12.4 weeks (range 7–25.4 weeks) and the median number of visits over the course of pregnancy was four visits (range 1–8 visits). ►Supplementary Appendix 2 (available in the online version) shows a distribution of visits by gestational month. Women were primarily in the 20 to 35 age group (70%) and more than half (54.7%) had at least one previous child. Most participants were either normal weight (33.2%) or overweight (32.6%) prior to pregnancy. Mean ranks from Kruskal–Wallis' tests were not different across BMI categories for the 20 cytokines evaluated (data not shown). The probability of reproductive tract infection was highest in the second trimester (0.37) and decreased in the third trimester (0.28) (►Table 1).

The percentage of observations below the lower LOD and above the upper LOD for each cytokine varied across pregnancy with no consistent pattern in the monthly percentages (data not shown). Most cytokines had 10 to 30% of values below the lower LOD; exceptions were IP-10, MCP-1, MIP-1β, and IL-8 with less than 5%, and IL-4 and IL-12p40 with more than 40% below the lower LOD. For the majority of cytokines, less than 5% of the values were above the upper LOD, including five cytokines (IL-2, TNF-α, IL-12p70, IL-17, and IFN-γ) with no observations above the upper LOD (►Supplementary Appendix 3, available in the online version). Cytokine concentrations were not normally distributed, so log-transformed values or inverse-fourth-root–transformed values were used in analyses that required a normal distribution and indicated when this

Table 2 Distribution of cervicovaginal cytokine concentrations (in pg/mL) in term births by trimester (number of samples = 902), PRINCESA cohort, 2009 to 2014

	First trimester (N^a = 88)			Second trimester (N = 443)			Third trimester (N = 371)		
Cytokine	**P50**	**P75**	**P95**	**P50**	**P75**	**P95**	**P50**	**P75**	**P95**
Anti-inflammatory									
sIL-2Rα	14	44	1,840	22	51	921	20	54	1,436
IL-4[b]	10	57	1,941	8	54	1,699	10	122	1,746
IL-10	5	14	98	5	17	412	5	18	382
IL-12p40	9	24	111	10	25	634	11	29	988
IL-1Ra	4,219	7,043	10,010	4,087	7,654	10,010	4,674	9,178	10,010
Proinflammatory									
IL-1α	1,162	2,799	10,010	784	2,310	10,010	690	2,486	10,010
IL-1β	82	1,011	4,285	47	871	9,750	44	538	10,010
IL-2	3	10	41	3	12	163	3	14	230
IL-6	11	31	344	11	28	534	10	22	1,905
IL-8[b]	2,440	10,010	10,010	1,442	7,189	10,010	1,408	10,010	10,010
TNF-α	3	10	321	4	11	312	4	12	498
IL-12p70[b]	1	5	249	1	6	278	1	8	233
IL-17	1	4	80	2	6	165	2	6	186
IP-10	154	612	10,010	248	648	10,010	254	619	10,010
IFN-γ[b]	1	11	408	3	13	358	3	15	575
MCP-1	102	402	4,563	135	428	7,363	143	568	8,371
MIP-1α	14	44	1,886	10	45	3,455	8	48	2,861
MIP-1β	25	67	1,106	26	67	1,754	28	74	1,945
Eotaxin	16	30	401	21	37	1,255	22	39	2,001
VEGF	145	318	10,010	133	251	10,010	128	254	10,010

Abbreviations: IFN-γ, interferon gamma; IL, interleukin; IP-10, IFN-γ-inducible protein; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1 α MIP-1β, macrophage inflammatory protein-1 β; sIL-2Rα, soluble IL-2 receptor α; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.
[a]N = number of samples.
[b]More than 50% of observations missing.

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was the case; all other analyses used nontransformed cytokine values.

The distributions of cytokine values in their natural scale differed across cytokines and across trimesters (►Table 2). Three cytokines (one anti-inflammatory [IL-1Ra] and two proinflammatory [IL-1α, and IL-8]) consistently had high concentrations across trimesters and compared with other cytokines for the study-specific median, 75th, and 95th percentiles, while IP-10 and VEGF consistently had high concentrations at the 95th percentile across pregnancy but substantially lower concentrations at the 50th and 75th percentiles compared with IL-1Ra, IL-1α and IL-8. Similarly, plots for all 20 cytokines for the selected gestational months (months 3, 5, and 7, ►Supplementary Appendix 4, available in the online version), showed differences in ECDFs across cytokines, but ECDFs did not differ across time for most cytokines.

Many of the cytokines exhibited good reproducibility (defined as ICCs between 0.60 and 0.74)[21] over the course of pregnancy (►Table 3). Across all cytokines, ICCs ranged from 0.41 to 0.82 (which included the following three reproducibility categories [ranges], namely: fair [0.40–0.59], good [0.60–0.74], and excellent [≥ 0.75]).[21] Correlation heat maps for month 3 (►Fig. 1), and months 5 and 7 (►Supplementary Appendix 5, available in the online version) and the 570 Spearman's correlation coefficients (►Supplementary Appendix 6, available in the online version) among all possible pairs of the 20 cytokines illustrate that the magnitude of correlation coefficients varied over time, but the direction of correlation was stable over the course of pregnancy; 95.3% of the 190 correlation pairs remained either negative or positive at the three points evaluated in pregnancy.

The mean longitudinal patterns of log-transformed cytokines adjusted for lower reproductive tract infection status varied across cytokines, but little variability was observed within cytokines (►Fig. 2, ►Supplementary Appendix 7, available in the online version). Mean levels of IL-1Ra, IL-1α, and IL-8 were high throughout pregnancy compared with other cytokines evaluated. IL-1Ra, IL-1α, and IL-8 were among the few cytokines that exhibited subtle changes over time; specifically, IL-1Ra and IL-1α exhibited a pattern of slight decrease toward the end of the first trimester of pregnancy followed by a slight increase late in the second trimester (around week 25) and a decrease by the end of pregnancy. IL-8 had a gently decreasing trend beginning at the 30th week of gestation. Lower reproductive tract infection did not appear to substantially influence mean cytokine patterns, as unadjusted patterns (data not shown) did not differ from patterns adjusted for infection (shown in ►Fig. 1 and ►Supplementary Appendix 7, available in the online version).

Table 3 Intraclass correlation coefficients of cervicovaginal cytokine concentrations in term births ($N = 181$), PRINCESA cohort, 2009 to 2014

Cytokine	Inverse fourth root
Anti-inflammatory	
IL-1Ra	0.67
IL-4	0.75
IL-10	0.64
sIL-2Rα	0.71
IL-12p40	0.79
Proinflammatory	
IL-1α	0.68
IL-1β	0.64
IL-2	0.62
IL-6	0.50
IL-8	0.41
TNF-α	0.62
IL-12p70	0.79
IL-17	0.65
IP-10	0.50
IFN-γ	0.78
MCP-1	0.45
MIP-1α	0.56
MIP-1β	0.58
Eotaxin	0.82
VEGF	0.45

Abbreviations: IFN-γ, interferon gamma; IL, interleukin; IP-10, IFN-γ-inducible protein; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1 α; MIP-1β, macrophage inflammatory protein-1 β; PRINCESA, Pregnancy Research on Inflammation, Nutrition, & City Environment: Systematic Analyses; sIL-2Rα, soluble IL-2 receptor α; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

Discussion

We used longitudinal data to describe characteristics of individual and pairs of 20 cervicovaginal cytokines among 181 women in the PRINCESA cohort who delivered at term without major obstetrical and medical complications. Our results suggest that "normal" immunologic state of pregnancy encompasses a range of inflammation levels that are not necessarily indicative of pathology.

Infection is a known stimulant of immune activity, so the finding that mean cervicovaginal patterns were unaffected by infection was unexpected. However, previous studies have had inconsistent results (reviewed in Mitchell and Marrazzo[22]). Perhaps because pregnancy encompasses periods of varying severity of inflammation including concentrations that may be reflective of pathology,[1] infection did not further influence inflammation levels in this population. It is also possible that in the context of a strict monthly prenatal follow-up of women and treatment of infections, participants did not reach an advanced stage of inflammation.

Additionally, cytokines in this study exhibited fair to excellent reproducibility, suggesting that, on average, cytokines are stable over time among women who will deliver at term. Notably, four of the six cytokines with ICCs indicating fair reproducibility were chemokines IL-8, IP-10, MCP-1, and

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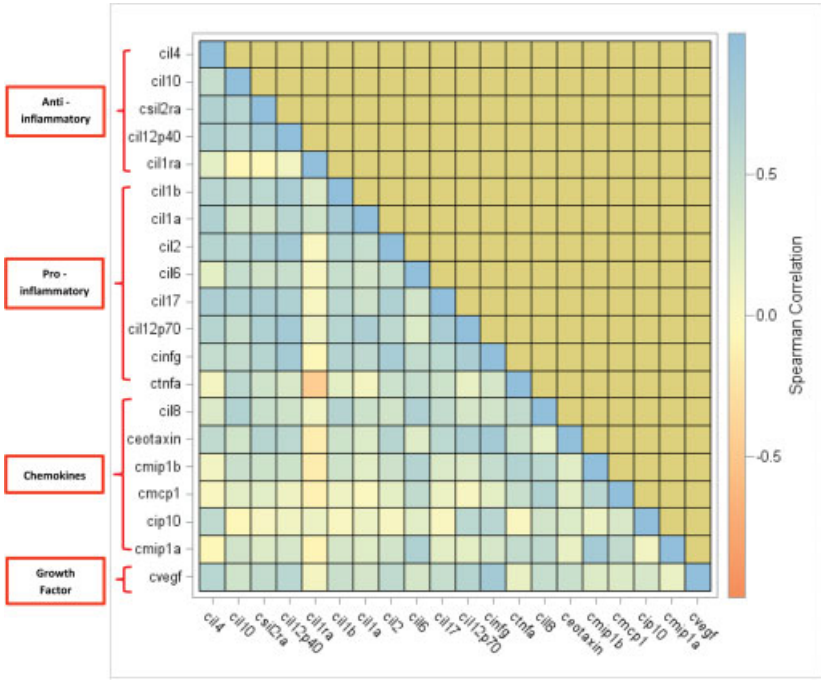

Fig. 1 Heat map of Spearman's correlation coefficients for cervicovaginal cytokines at month 3 of gestation. Darker colors indicate stronger negative (tan) or positive (blue) correlations. The c-prefix denotes cervicovaginal cytokine.

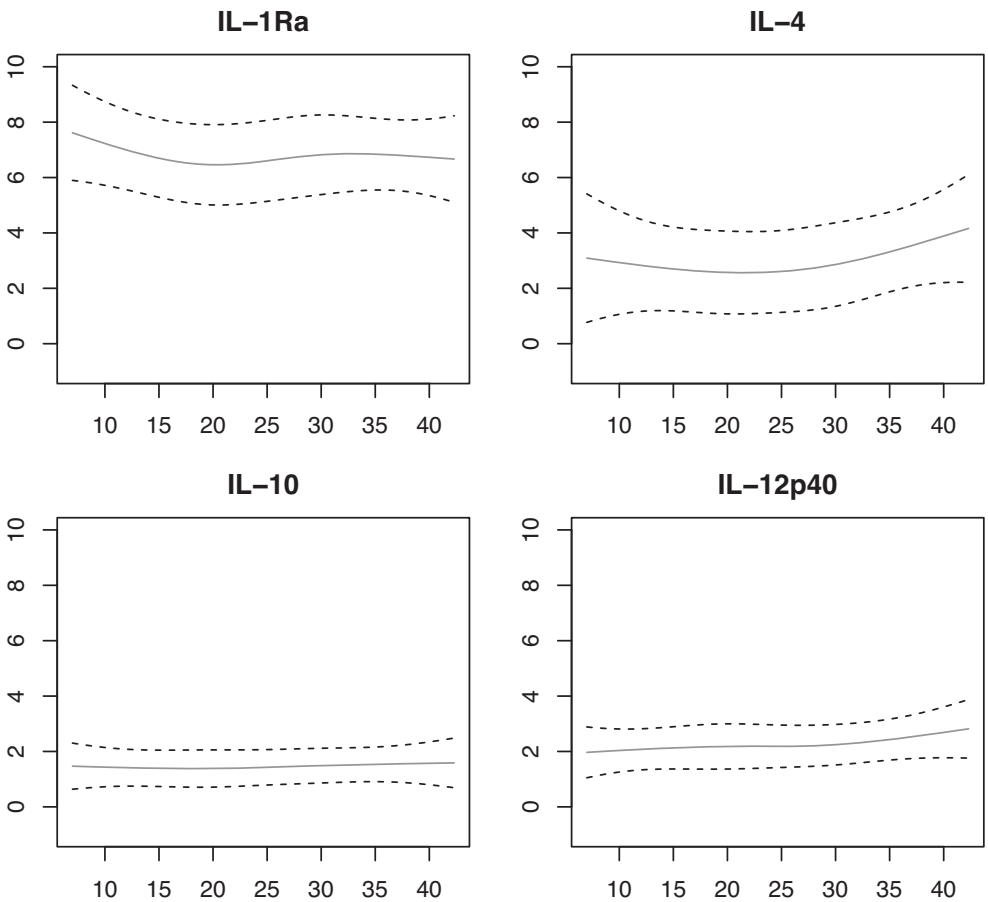

Fig. 2 Infection adjusted patterns of IL-1Ra, IL-4, IL-10, and IL-12p40 (Tobit regression models). *Y*-axis denotes log-transformed cytokine concentrations (in pg/mL) and the *X*-axis indicates gestational weeks. IL, interleukin.

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MIP-1α (the two exceptions were IL-6 and VEGF). A fair category indicates that these cytokines exhibited some degree of variability within participants across the study sample and may be reflective of frequent expression of chemokines for their many different roles, which include placental development, immune efficiency and tolerance, and cellular differentiation.[23] Although stability in mean cytokine levels was corroborated by a summary within-participant measure for most cytokines, fair reproducibility seen particularly in chemokines suggests that, possibly due to their physiologic roles, caution and further investigation may be needed when evaluating chemokines.

In one of few longitudinal studies identified, Donders et al evaluated—among 92 women in Leuven, Belgium—whether six vaginal cytokines (IL-6, IL-8, IL-1β, IL-1Ra, leukemia inhibitory factor, and TNF-α) varied during the course of uncomplicated pregnancy ($n = 30$) and further compared trimester-specific cytokine distributions to nonpregnant participants ($n = 62$).[24] Cytokine concentration ranges were different in that study compared with ours, so comparison between the two studies focuses on patterns. Similar to our results, Donders et al reported a slight decrease in mean concentration of IL-1β between the first and second trimesters followed by a slight increase during the third trimester. IL-6 levels were low with an increase at the end of pregnancy in both studies, but Donders et al showed a steeper increase. Although greatly different numerically in terms of concentrations, patterns were similar for IL-8 until the very end of pregnancy where we saw a decrease not seen in Donders et al. This may reflect true differences between the studies or might be influenced by the availability of data up to 40 weeks in our study, whereas Donders et al collected data up to 38 weeks of gestation. Finally, mean levels of TNF-α were low, and IL-1Ra showed variability over time in both studies, but IL-1Ra patterns were different.

Higher expression of IL-10 and its receptor compared with proinflammatory cytokines in placental tissues has been reported,[25] but patterns similar to our findings have also been reported for cervical and cervicovaginal IL-10 cytokines. Kutteh and Franklin, in a study that evaluated the levels of cervical cytokines and immunoglobulins among 36 pregnant women with no known health problems, did not detect measurable levels of IL-10 in any of the three trimesters evaluated. However, among the same participants, mean levels of proinflammatory cytokine IL-1β were 4,261, 12,899, and 11,620 pg/mL during the first, second, and third trimesters, respectively. IL-6 was considerably lower (average 323 pg/mL) in the first and the second trimesters.[26] In another study of 63 asymptomatic women at risk for spontaneous preterm birth (due to a history of preterm birth risk factors), Amabebe et al reported similar patterns of low cervicovaginal IL-10 and higher proinflammatory cytokines among 25 and 33 term births at weeks 20 to 22 and 26 to 28 of gestation, respectively.[27] It is unclear why these differences exist between studies and may need to be investigated in appropriately designed future studies.

Information on correlations among cervicovaginal cytokines in term births is limited in published work; the few studies identified primarily focused on correlations between cytokines, at select points[28] and in the context of other factors.[29–31] Therefore, the current study fills a specific gap about the relationship between pairs of cervicovaginal cytokines during pregnancy.

The strengths of this study include: the use of appropriate statistical methods for censored data, and evaluation of pairwise correlations at multiple points in gestation; but future studies that utilize additional analytic approaches may help untangle the complexities of the inflammatory process.

One limitation is that the percentage of observations below the lower LOD was high—although comparable to other studies.[8,32] Another limitation is that only two participants had cytokine data for the second month of gestation, so our results represent a characterization of cytokines from the third month to the end of pregnancy prior to the start of labor and may not have captured potential variability of cytokines during implantation and labor. The inability to use the same set of participants over the course of pregnancy is a limitation of this study. In addition, the role of specific lower reproductive tract infections, such as bacterial vaginosis, on mean cytokine concentrations was not evaluated in this study. The use of LMP to estimate gestational age is another limitation to consider. Finally, the method used to diagnose lower reproductive tract infection has not been validated and may represent a limitation.

In conclusion, our findings highlight important characteristics of cervicovaginal cytokines during term pregnancy, and suggest that cytokine stability during pregnancy may be a useful characteristic to evaluate for potential differences between term and other obstetric outcomes. Additionally, the finding of very high cytokine levels in term pregnancies further corroborates that pregnancy—independent of pathology/infection—is associated with an inflammatory state. These results may serve as a baseline against which future studies involving other pregnancy outcomes may be compared.

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Conflict of Interest

None declared.

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