Decreased Proinflammatory Cytokines in Cervicovaginal Fluid, as Measured in Midgestation, are Associated with Preterm Delivery

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Problem
The main aim of this study was to investigate the relationship between selected proinflammatory cytokines [interleukin IL-1 alpha (IL-1α), IL-1 beta (IL-1β), IL-6 and IL-8] concentrations in cervicovaginal fluid, as measured in midgestation, and the risk of subsequent preterm delivery.

Method of study
Cervicovaginal fluids were obtained from a cohort of 114 pregnant women at 22–34 weeks' gestation and analyzed for the concentrations of IL-1α, IL-1β, IL-6 and IL-8 using enzyme-linked immunosorbent assay technique. Lower genital tract microbiology was diagnosed using Gram stain method and by culture.

Results
Mean gestational age at the time of sampling was 29.0 weeks. Mean time between sampling and delivery was 9.3 weeks (S.D. 4.7). Median cervicovaginal concentrations of IL-1α, IL-1β, IL-6 and IL-8 did not differ between preterm and term delivery group. Women with lower genital tract pathological flora and IL-1α concentration below 25th percentile presented significant risk of subsequent preterm delivery as compared with women with no low cytokines (OR = 10.7; 95% CI, 2.0–58.1). Women with more than one cytokine' low concentration (below 25th percentile) presented an increased risk of preterm delivery – OR = 11.8 (95% CI, 1.8–78.0).

Conclusions
The midgestation cytokines' measurement in cervicovaginal fluid of pregnant women could be useful for prediction of preterm delivery only among women with lower genital tract pathological flora.

Introduction

Intrauterine infection is one of the most important causes of spontaneous preterm delivery.[1,2] The main mechanism of this infection is ascending microbial invasion by lower genital tract organisms which could produce local inflammation, subclinical chorioamnionitis leading to preterm rupture of membranes and/or pretem labor and possibly preterm birth.[3]

Many bacteria involved in ascending infection produce phospholipases A_2 and C, proteinases and

endotoxins activating placental, decidual, amnion and fetal membrane cells.[4–6] This in turn may stimulate these cells to production of proinflammatory cytokines and prostaglandins which plays an important role in the initiation of parturition, especially in the cases related to chorioamnionitis.[7–10] Recent studies have suggested that proinflammatory cytokines could have an important role in the mechanism of preterm labor and delivery.

Interleukin-1 (IL-1) has a crucial role in cervical ripening through stimulating the production of collagenases and elastases.[11,12] Interleukin-8 (IL-8) strongly promotes the attraction of neutrophils and its degranulation. IL-1β and IL-8 can also induce hyaluronic acid production by human cervical fibroblasts.[12] Interleukin-6 (IL-6) activates acute phase response and stimulates immunoglobulin production.

Maternal inflammatory response to lower genital tract infection has been described as an important link between maternal infection and preterm delivery. The majority of investigations concerning the role of cytokines in the pathomechanisms of preterm delivery has employed amniotic fluid measurements.[13–15] Elevation of proinflammatory cytokines in maternal serum or in amniotic fluid during the infection and shortly before parturition has been extensively described.[15–18] There are also studies which indicates that the evaluation of cytokines concentration in cervicovaginal fluid could be of some value for the prediction of intrauterine infection and preterm birth especially in relatively short period before the delivery.[19–22] However, there are no sufficient data concerning cytokines characteristics in cervicovaginal fluid at several weeks before the delivery. We hypothesize that the highest risk of preterm delivery pertains to women who have impaired cytokines production in early pregnancy which could enhance an ascending microbial invasion of the upper genital tract thus leading to preterm birth.

The main aim of this study was to investigate the relationship between selected proinflammatory cytokines (IL-1α, IL-1β, IL-6 and IL-8) concentrations in cervicovaginal fluid, as measured in midgestation, and the risk of subsequent preterm delivery.

Material and methods

The study population comprised 120 women at 22–34 weeks' gestation recruited from the patients of the clinical hospital at the Department of Perinatology, Medical University of Lodz, Poland, between May 2001 and December 2002. Only the singleton pregnancies were qualified for inclusion in the survey. The exclusion criteria were as follows: antibiotic therapy within 4 weeks prior to the examination, multiple gestation, vaginal bleeding and serious maternal diseases. Of 120 women enrolled six women were lost to follow up and excluded from further analyses so final study group comprised 114 women.

This prospective cohort study was approved by the Biomedical Ethics Committee of the Medical University of Lodz, Poland (Decision No. RNN/215/00). Each participant provided a written informed consent to participate in the study.

A standard questionnaire covering medical, socioeconomic, demographic and constitutional aspects as well as tobacco smoking was administered to every subject and verified based on medical records. Routine ultrasound examination of fetal biometry was performed. The gestational age at the time of sampling was based on the time of last menstruation and verified by early ultrasound [crown-rump length (CRL)] of the fetus.

For the assessment of biocenosis in the lower genital tract, vaginal and cervical swabs were collected from the pregnant women under study. At first, bacteriological tests of cervical swabs were made to screen for *Mycoplasma hominis* and *Ureaplasma urealyticum*. For isolation and identification of genital mycoplasmas the commercially available Mycoplasma DUO kits (Sanofi Diagnostics, Pasteur) were used. Bacterial vaginosis (BV) was diagnosed by Gram stain of vaginal smear according to Spiegel's criteria.[23]

Cervicovaginal fluids were obtained by Dacron swabs from the posterior fornix. Then, Dacron swabs were placed in a glass probe containing 2 mL of phosphate-buffer saline solution and stored at −70°C. The samples were analyzed for the concentrations of selected cytokines by commercially available standard enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (Endogen; Woburn, MA, USA).

Preterm delivery was defined as birth before the completed 37th week of gestation. To evaluate the risk of preterm delivery associated with cytokines levels the odds ratios (OR) and its 95% confidence intervals (CI) were calculated. The crude and adjusted odds ratios for preterm delivery were computed.

Cervical incompetence treated by cervical cerclage and uterine contractions presented before inclusion to the study were considered as possible confounders. We considered lower genital tract pathological microflora as an effect modifier and estimated risk of preterm delivery in this subgroup. Median concentrations of cytokines were compared using least absolute value (LAV) regression model. Observed differences were considered statistically significant at standard level 0.05. Statistical analysis was carried out using STATA 8 software (Stata Corp. College Station, TX, USA).

Results

The characteristics of the examined population is presented in Table I. The mean gestational age at the time of cytokines measurement was 29.0 weeks (S.D. 4.0). The mean time between sampling and delivery was 9.3 weeks (S.D. 4.7). The mean maternal age of the study group was 27.3 years (S.D. 4.7). Twenty-one percent of the subjects were unmarried and 16.1% had primary education. In the study population, 60.5% of women were nulliparous and 15.8% were smoking during pregnancy.

According to Gram stain results BV was diagnosed among 31 women (27.2%). *Mycoplasma hominis* was diagnosed in 26 and *U. urealyticum* in 30 of pregnant women. Among 49 women pathological vaginal microflora defined as presence of *M. hominis* or *U. urealyticum* or BV was detected. Mean gestational age at the time of delivery was 38.3 weeks and mean newborns' birthweight was 3146 g. Of 114 analyzed women 15 (13.2%) delivered before 37th week of gestation.

Table I The Characteristic of Examined Population

Patients' characteristics	$N = 114$
Mean gestational age at entry to study (weeks)	29.0 (S.D. 4.0)
Mean maternal age (years)	27.3 (S.D. 4.7)
Mean maternal weight (kg)	56.5 (S.D. 8.0)
Parity:	
Nulliparous	69 (60.5%)
Multiparous	45 (39.5%)
Mean number of deliveries	1.54 (S.D. 0.96)
Smoking:	
Yes	18 (15.8%)
No	96 (84.2%)
Vaginal pH	5.14 (S.D. 0.63)

Table II The Risk Factors of Preterm Delivery in Examined Population

		Preterm delivery	
	$N = 114$	*n*	OR (95% CI)
Cervical cerclage			
No	104	11	1
Yes	10	4	5.6 (1.4–23.1)
Uterine contractions			
No	53	3	1
Yes	61	12	4.1 (1.1–15.4)
Bacterial vaginosis			
No	83	10	1
Yes	31	5	1.4 (0.4–4.5)
M. hominis			
No	88	8	1
Yes	26	7	3.7 (1.2–11.4)
U. urealyticum			
No	84	9	1
Yes	30	6	2.1 (0.7–6.5)

In univariate analysis the significant risk factors of preterm delivery were as follows: lower genital tract colonization by *M. hominis* (OR = 3.7; 95% CI 1.2–11.4), cervical incompetence treated by cerclage (OR = 5.6; 95% CI 1.4–23.1) and uterine contractions (OR = 4.1; 95% CI 1.1–15.4) (Table II).

Although women with preterm delivery had lower concentrations of analyzed cytokines no significant differences of median cervicovaginal concentrations, as measured during pregnancy, between women with subsequent preterm and term delivery was found (Table III). Median concentrations of IL-1α, IL-1β and IL-8 but no IL-6 were significantly higher among women with pathological vaginal microflora as compared with women with normal flora (Table IV).

Table III Median Cervicovaginal Concentrations of Analyzed Cytokines Among Women with Preterm and Term Delivery

Cytokines	Preterm delivery ($N = 15$) [median (inter quartile range)]	Term delivery ($N = 99$) [median (inter quartile range)]	*P*-value
IL-8 (pg/mL)	375 (94–713)	506 (198–830)	0.53
IL-6 (pg/mL)	6.0 (0.1–12.2)	6.4 (2–16.5)	0.92
IL-1α (pg/mL)	13.4 (2–75)	31.3 (7.6–107.4)	0.37
IL-1β (pg/mL)	2.3 (0.1–13.4)	11.4 (1–39.5)	0.22

Table IV Median Cervicovaginal Concentrations of Selected Cytokines Among Women with Lower Genital Tract Pathological Microflora and Normal Flora

Cytokines	Lower genital tract pathological flora ($N = 49$) [median (inter quartile range)]	Lower genital tract normal flora ($N = 65$) [median (inter quartile range)]	*P*-value
IL-8 (pg/mL)	592 (298–829)	302 (91–710)	0.001
IL-6 (pg/mL)	9.1 (1.5–27.1)	5.1 (0.7–11.6)	0.12
IL-1α (pg/mL)	48.3 (8.2–136.8)	18.1 (3.5–56.8)	0.009
IL-1β (pg/mL)	21.5 (1.9–69.1)	6.2 (0.2–16)	0.003

Because of wide range and lack of normal distribution of cytokine concentrations we dichotomized the concentrations into <25th percentile and ≥25th percentile. The 25th percentile for evaluated cytokines as measured in group of women delivering at term were as follows: for IL-1α, 8 pg/mL; IL-1β, 0.8 pg/mL; IL-6, 2 pg/mL and for IL-8, 198 pg/mL.

Women who delivered before 37th week of gestation tended to have more frequently all the measured cytokines below 25th percentile (data not shown). As 10 of 15 (66.7%) preterm deliveries occurred in the group of women with midgestation pathological microflora, we made an analysis to determine an association between cervicovaginal cytokine' concentrations and subsequent preterm delivery restricted to the subgroup of women with lower genital tract pathological microflora (*M. hominis* or *U. urealyticum* or BV – $N = 49$).

Women with lower genital tract pathological microflora and IL-1α concentration below 25th percentile presented significant risk of subsequent preterm delivery in univariate and multivariate analysis ($OR_{adj} = 10.7$; 95% CI 2.0–58.1) (Table V). Similar tendency was noticed for low concentration of IL-6, IL-1β and IL-8.

Significantly higher risk of delivering premature baby was observed among women with lower genital tract pathological flora and low concentrations (below 25th percentile) of more than one cytokine ($OR_{adj} = 11.8$; 95% CI 1.8–78.0) (Table VI).

Table V Preterm Delivery in Relation to Cervicovaginal Cytokines Concentrations Among Women with Lower Genital Tract Pathological Microflora during Pregnancy

		Preterm delivery		
	$N = 49$	*n*	Crude OR (95% CI)	Adjusted[a] OR (95% CI)
IL-8				
>25%	39	7	1	1
<25%	10	3	1.9 (0.4–9.5)	2.7 (0.5–14.5)
IL-6				
>25%	37	7	1	1
<25%	12	3	1.4 (0.3–6.7)	2.2 (0.4–12.0)
IL-1α				
>25%	38	4	1	1
<25%	11	6	10.2 (2.1–49.3)	10.7 (2.0–58.1)
IL-1β				
>25%	38	6	1	1
<25%	11	4	3.0 (0.7–13.7)	4.9 (0.9–27.3)

[a]Odds ratios adjusted for cervical cerclage and uterine contractions.

Table VI The Risk of Preterm Delivery According to Number of Low Cervicovaginal Cytokines Concentrations (<25%) Among Women with Lower Genital Tract Pathological Microflora

		Preterm delivery		
Number of cytokines <25%	$N = 49$	*n*	Crude OR (95% CI)	Adjusted[a] OR (95% CI)
None	26	3	1	1
One	10	1	0.9 (0.1–9.3)	0.9 (0.1–12.8)
Two or more	13	6	6.6 (1.3–33.3)	11.8 (1.8–78.0)

[a]Odds ratios adjusted for cervical cerclage and uterine contractions.
Formula for risk prediction logit $P = -3.759$ [$-$ 0.063 × (one IL < 25%)] + [2.46 × (two IL < 25%)] + [1.85 × (cervical cerclage)] + [1.75 × (uterine contractions)].

Discussion

This study aimed at assessing the relationship between selected proinflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8) as measured in the cervicovaginal fluid at midgestation and the risk of subsequent preterm delivery. The mean interval between cytokines measurement and delivery was 9.3 weeks in our study group. This relatively long interval allowed for excluding the possible influence of uterine contraction activity and labor on the cytokines concentrations. We did not find any significant differences in median cytokines concentrations, as measured in midgestation, between the women with subsequent preterm and term deliveries.

The outcome of our study indicate that pregnant women with low cervicovaginal concentrations of proinflammatory cytokines (below 25th percentile) who at the same time have a pathological vaginal microflora presents an increased risk for delivering before the 37th week of gestation. The highest risk of preterm delivery was observed among women with low concentrations of IL-1α and IL-1β with OR = 10.7 and 4.9, respectively. Lower but still elevated risk was found for women with genital tract infection and low levels of IL-6 (OR = 2.2) and IL-8 (OR = 2.7). The highest risk of preterm delivery (OR = 11.8) was noted among the women for whom the concentrations of more than one of the cytokines studied were below the 25th percentile.

Our findings are supported by the data reported by Simham et al.[24] who investigated the possible relationship between cervicovaginal concentration of IL-1β and IL-8 in women at 8–20 weeks' gestation who had subsequently developed chorioamnionitis. They found that women with the lowest quartile of cervical concentrations of two or three cytokines (IL-1β, IL-6 and IL-8) early in pregnancy were significantly more likely subsequently to experience clinical chorioamnionitis than women with no low concentrations of proinflammatory cytokines (OR = 5.1). The condition underlying the observed increase in the risk both for chorioamnionitis and preterm delivery among women with low cytokine levels in early pregnancy may be the susceptibility to genitourinary tract infections, because of decreased cytokine concentrations, and the resulting development of the ascending infection. The findings of our study provided evidence for this probable pathomechanism: the increased risk for preterm delivery was found only in the group of women with lower genital tract infection who had low cervicovaginal concentrations of proinflammatory cytokines. The latter condition may also imply a lower reactivity of the maternal immune system that should normally result in diminishing the growth of pathological bacteria in the genitourinary tract. The more of the cytokines were found to have low concentration levels, the higher was the risk for preterm delivery. These finding may indicate that the probability of pregnancy complications increases with a growing extent of immunological disorders. It is worth noting, however, that immunological responses are partially genetically determined and hence may vary across populations.[25] The proinflammatory cytokines production could be modified to some extent by maternal genotype. According to Genc et al.[22] *IL- 1RN**2 carriage (allele 2 of the IL-1 receptor antagonist gene) is associated with a blunted proinflammatory interleukin-1β response to abnormal vaginal flora and this property could decrease susceptibility to infection-related preterm birth.

Probably not only early or midgestation immune hyporesponsiveness but also hyperresponsiveness could be related to subsequent intrauterine infections and preterm delivery. The influence of intrauterine infection on the concentration of cytokines and prostaglandins in amniotic fluid has been the subject to extensive investigations.[13,14,17] Some authors have reported increased concentrations of IL-1β, IL-1α, IL-10, TNF-alpha and IL-6 in pregnant women with intrauterine infection and with threatened preterm labor.[6,14] The increase concentrations of some proinflammatory cytokines in amniotic fluid was found to be a sensitive indicator of intrauterine infection and preterm delivery.[17] As reported by Putz et al.[17], high amniotic concentrations of IL-1β and IL-6 can be noted both in the case of intrauterine infection and preterm uterine contractions. The results of this study confirms an association between IL-1α, IL-1β and IL-8 but no IL-6 with pathological lower genital tract microflora.

In view of the invasive character of amniotic fluid aspiration attempts were made to determine cytokines concentrations in the cervicovaginal secretion. The studies by Jun et al.[26] performed on pregnant women with preterm rupture of membranes revealed a strong correlation between IL-6 concentrations measured in amniotic and in cervicovaginal fluids ($P < 0.001$). This finding has been confirmed by the results reported by Rizzo et al.[21]. Further, in the studies by Inglis et al.[27], the cervicovaginal levels of IL-6 and TNF-α were found to correlate with fetal fibronectin concentration.

Paternoster et al.[28], in their prospective studies on IL-6 concentration in cervicovaginal fluid in 24 weeks' gestation, reported significantly higher concentrations in women delivering before the 37th week of pregnancy as compared with women who delivered at term (608 pg/mL versus 58.9 pg/mL). This finding, however, was not confirmed for IL-8. Wenstrom et al.[18] found that amniotic concentration of IL-6 determined in II trimester of pregnancy reached significantly higher values in the women who subsequently had preterm delivery, which may imply a subclinical infection several weeks prior to the delivery. As reported by Lockwood et al.[29] the

amniotic and cervicovaginal concentrations of IL-6 between 24 and 36 week of pregnancy correlated positively with the risk of preterm delivery. These findings were not confirmed by Wennerholm et al.[30] who postulated that the correlation between preterm delivery and increased concentration of proinflammatory cytokines referred only to IL-8. It should be stressed, however, that these examinations concerned preterm delivery in multiple pregnancies.

Our findings indicates that midgestation maternal immune hyporesponsiveness, as represented by low cervicovaginal concentrations of various proinflammatory cytokine, constitute an increased risk for subsequent preterm delivery among women with lower genital tract pathological microflora. Probably, pregnant women with no adequate (hypo-and hyper) immune response are at risk for subsequent infection-related preterm birth. As in some populations abnormal lower genital tract flora during pregnancy is a common phenomenon[31] this results could also help at early identifying the women who are really at risk of subsequent preterm delivery.

Conclusions

1 The midgestation cytokines' measurement in cervicovaginal fluid of pregnant women could be useful for non-invasive prediction of preterm delivery only among women with lower genital tract pathological flora

2 Low concentrations of proinflammatory cytokines could creates a permissive environment for ascending infection and leads to subsequent preterm delivery.

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