

Cytokines and cervical length: a pilot study of relationship to incidence of preterm birth

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Abstract

Objective: To test if levels of interleukins (IL) in a low risk obstetric population coupled with transvaginal cervical length help predict spontaneous pre-term birth.

Materials and methods: Prospective pilot study of 39 patients presenting for initial prenatal care. Vaginal swabs from each patient evaluated for IL-1A, IL-1B, IL-6, IL-8, IL-10 and IL-13 levels via ELISA kits. Mean cytokine levels compared between preterm/term birth groups with Student t-test. Cytokine levels tested to evaluate correlation with shorter cervical lengths using Pearson's correlation.

Results: Of the 39 patients enrolled, 8 (20.5%) delivered pre-term. Mean IL-1A, IL-4, IL-10 and IL-13 levels were not statistically different between pre-term and term births. However, IL-1B and IL-6 levels were significantly lower in preterm birth group (6.28 pg/ml \pm 12.7 and 3.96 pg/ml \pm 3.8) compared to term births (25.41 pg/ml \pm 41.6; $p=0.035$ and 35.7 pg/ml \pm 55.3; $p=0.003$). There was moderate correlation between IL-10 and cervical length ($r=0.54$; $p=0.002$). IL-1A, IL-1B, IL-6, IL-10 and IL-13 had no correlation with cervical length.

Conclusions: Unlike previous reports, IL-1B and IL-6 levels were lower in our preterm birth patients. IL-10 may provide a moderating effect on both inflammatory cytokines and affect cervical length by its anti-inflammatory action. Further study with larger numbers of patients is warranted.

Keywords: Cervical length, cytokines, preterm birth, interleukins, pearson's correlation

Introduction

Amniotic and chorionic phospholipase A₂ activity precipitates term labor. Numerous investigators have suggested that normal labor is triggered by activation of phospholipase A₂, which produces an increase in free arachidonic acid and increase in the synthesis of prostaglandins by hydrolyzing the phospholipids in the placental membranes [1].

Phospholipase A₂ activities are higher in microorganisms that cause urinary tract infections, bacteria vaginosis, and neonatal sepsis, in comparison to that of membrane phospholipase activities. There is a strong association between premature labor and intrauterine infection, urinary tract infection and early neonatal sepsis. The cytokines represent a family of immunologic products that have significant effects on reproduction. A few relevant inflammatory cytokines are interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-alpha). Some of the regulatory cytokines are IL-4, IL-10, and IL-13.

Further, the modulating cytokines of interleukin (IL)-4, IL-10, and IL-13 decrease the inflammatory response by IL-6, IL-8, and TNF-alpha [6]. Recent information by [6], on the receptor activity of IL-4, IL-10, and IL-13, notes that these anti-inflammatory

cytokines may be involved in the maternal response to infection. Specifically, the authors found that African-American women had a decrease in the production of IL-10 in the face of bacterial vaginosis which may partially explain African-American women's increased risk for preterm delivery as a result of infectious processes. Microorganisms may augment premature labor with phospholipase A₂ activity from endocervical and/or intrauterine contamination or infection, producing deacylation of arachidonic acid from amniotic phospholipids with increased concentrations of free arachidonic acid and increased prostaglandin synthesis, which triggers labor [1].

Age at gestation and cervical length measurement are also linked to preterm birth due to the shortening of the cervix space by the colonizing phospholipase producing bacteria and reduction in a normal vaginal flora that may correlate to the number of life sexual partners. Teenagers with limited sexual exposures may have less normal vaginal flora than an adult with numerous life sexual partners. All of the above factors may compound the occurrence of preterm birth. Rates of preterm birth have continued to increase despite intensive research efforts over the last several decades. A woman who has a

spontaneous preterm birth is at high risk for a subsequent preterm birth [8]. Studies have identified clinical, sonographic, and biochemical markers that help to identify the women at highest risk [4]. Determining cervical length, routine culturing of cervical microorganisms, and determination of cytokines could identify and potentially prevent preterm birth. Effective interventions to prevent preterm birth, however, remain elusive.

Methods and materials

We conducted a prospective pilot study of 50 obstetric patients. Our inclusion criteria consisted of: gestational age <34 weeks, singleton gestation, urinalysis and urine culture collected via clean catch, maternal age range 14-45 years, patient receiving prenatal care at CAMC Women's Medicine Center. Patients were excluded for the following reasons: women in labor or with uterine contractions, known fetal anomaly, cervical cerclage, multiple gestations, prior history of excisional cervical biopsy *eg.*, cold knife cone, loop electrosurgical excision procedure, laser conization, mullerian anomaly, patients with two or more D&C secondary to spontaneous or induced abortion.

For study consented patients, the following were performed in addition to standard prenatal care at the initial prenatal visit: transvaginal ultrasonograph cervical length measurements between 12 0/7 and 31 6/7 weeks of gestation, cervical cultures for standard microbiological analysis (including bacteria vaginosis, gonorrhea and chlamydia), as well as the collection of additional vaginal fluid for cytokines (IL-1A, IL-1B, IL-4, IL-6, IL-10 and IL-13) and both serum and vaginal phospholipase A₂ (PLA₂) samples for analysis.

Vaginal swab samples were placed individually into 250mL Tris and frozen while awaiting analysis. Peripheral venous blood samples were collected into BD Vacutainer ACD tubes by trained personnel using standard phlebotomy techniques. Buffy coat (white blood cells) were isolated from the blood sample via centrifugation at room temperature for 10 minutes at 3300 x g, no brake. Red blood cells were lysed by conventional hypotonic lysis with the resulting white cell pellet resuspended in 1 mL 1x PBS.

Vaginal swab samples were briefly vortexed upon thawing. One mL of vaginal sample solution sample was removed for sonication in parallel to harvested white blood cells from peripheral blood sample. Sonication was performed using Sonicator® Ultrasonic Processor XL by Misonix® Incorporated (Farmingdale, NY), 30% for 90 seconds, repeated for a total of two times per sample.

Protein concentration samples were determined using commercially available Pierce BCA Protein Assay Kit according to product insert with endpoint sample reading at 562 nm absorbance on BioTek® Instruments, Inc, µQuant Spectrophotometer Reader with KCJunior™ Software (Winooski, VT). sPLA₂ levels were determined in both vaginal swab and peripheral blood samples using commercially available Assay Designs Correlate-Enzyme Assay secretory Phospholipase A₂ kit (Ann Arbor, MI) according to kit instructions. Prepared

samples were read at 405nm on BioTek® Instruments, Inc, µQuant Spectrophotometer Reader with KCJunior™ Software (Winooski, VT). Samples were analyzed in duplicate and are expressed as the mean value.

Cytokine levels were determined from collected vaginal swab samples by thawing and briefly vortexing sample. Human IL-4, IL-6, IL-10, IL-13 levels were determined using commercially available Invitrogen Ultrasensitive Immunoassay kits. Human IL-1A was tested using commercially available Invitrogen Immunoassay Kit and human IL-1B was analyzed using commercially available Amersham Interleukin-1 Beta BioTrak ELISA System. All testing was done according to product insert instructions with IL-4, -6, -10, and -13 using samples diluted 1:100 in kit included standard diluents buffer and IL-1A and IL-1B using non-diluted samples. Samples were read at 450 nm on BioTek® Instruments, Inc, µQuant Spectrophotometer Reader with KCJunior™ Software (Winooski, VT). Samples were analyzed in duplicate and are expressed as the mean value.

Descriptive statistics and univariate analysis were used as appropriate for continuous or categorical variables. Continuous variables were presented as means and standard deviations and were compared by using the Student t-test. Categorical variables were reported as percentages and were compared using chi-square and if necessary, Fisher's exact test. Logistic regression analysis were used to control for potential confounders. An alpha of 0.05 was used as the determination of statistical significance.

Results

Findings were analyzed in 39 patients (11 of the 50 did not have birth data available). Of the 39 women who met the inclusion criteria, several patient demographic characteristics were considered. (See **Table 1**) Of these patients enrolled, 8 (20.5%) delivered pre-term. Infection was associated with spontaneous pre-term birth, infection rate at 75% (6/8) versus 33% (10/31) for term birth ($p=0.045$). No patients had shortened cervixes <30 mm, while 9.68% had measurements <35mm. Mean IL-1A, IL-4, IL-10 and IL-13 levels were not statistically different between pre-term and term births as indicated in **Figure 1**. However, IL-1B and IL-6 levels were significantly lower in preterm birth group ($6.28 \text{ pq/ml} \pm 12.7$ and $3.96 \text{ pg/ml} \pm 3.8$) compared to term births ($25.41 \text{ pq/ml} \pm 41.6$; $p=0.035$ and $35.7 \text{ pq/ml} \pm 55.3$; $p=0.003$). There was moderate correlation between IL-10 and cervical length ($r=0.54$; $p=0.002$). IL-1A, IL-1B, IL-6, IL-4 and IL-13 had no correlation with cervical length. Cytokine concentrations were not associated with infection. (See **Table 2**).

Discussion

This pilot study is the first to attempt to prospectively evaluate the production of cytokines with relationship to cervical length and preterm birth. Unlike [5], we did not find elevated levels of IL-6 consistent with the finding of occult intramniotic infection as a precursor to preterm labor. Our findings of lower IL-B and

Table 1. Patient characteristics.

Patient Characteristics	Total (n=39) N (%) or mean (SD)	SPB (n=8) N (%) or mean (SD)	No SPB (n=31) N (%) or mean (SD)	p value
Maternal age (years)	24.0 (6.3)	25.1 (5.5)	23.7 (6.5)	0.576
Teen (<20 years)	8 (20.5%)	0 (0.0%)	8 (25.8%)	0.128
Adult (20-41 years)	31 (79.5%)	8 (100.0%)	23 (74.2%)	-
Ethnicity	-	-	-	0.110
European American	36 (92.3%)	7 (87.5%)	29 (93.5%)	-
African American	2 (5.1%)	0 (0.0%)	2 (6.5%)	-
Asian American	1 (2.6%)	1 (12.5%)	0 (0.0%)	-
Nulliparity	19 (48.7%)	5 (62.5%)	14 (45.2%)	0.317
Gestational Age (weeks)	10.8 (4.8)	10.6 (5.3)	10.8 (4.8)	0.912
Hx preterm birth	5 (12.8%)	2 (25.0%)	3 (9.7%)	0.268
Adequate Prenatal Care	10 (25.6%)	2 (25.0%)	8 (25.8%)	0.671
Tobacco Use	23 (59.0%)	5 (62.5%)	18 (58.1%)	0.575
Drug Use (All TCH, 1 Opiate, 1 Cocaine)	8 (20.5%)	3 (38%)	5 (16%)	0.323
Infection	16 (41.0%)	6 (75%)	10 (33%)	0.045
HTN	3 (7.7%)	1 (12.5%)	2 (6.5%)	0.508
DM	5 (12.8%)	2 (25%)	3 (9.7%)	0.268
-	-	n=7	n=24	-
Cervical Length	4.2 (0.9)	3.8 (0.5)	4.3 (1.0)	0.214

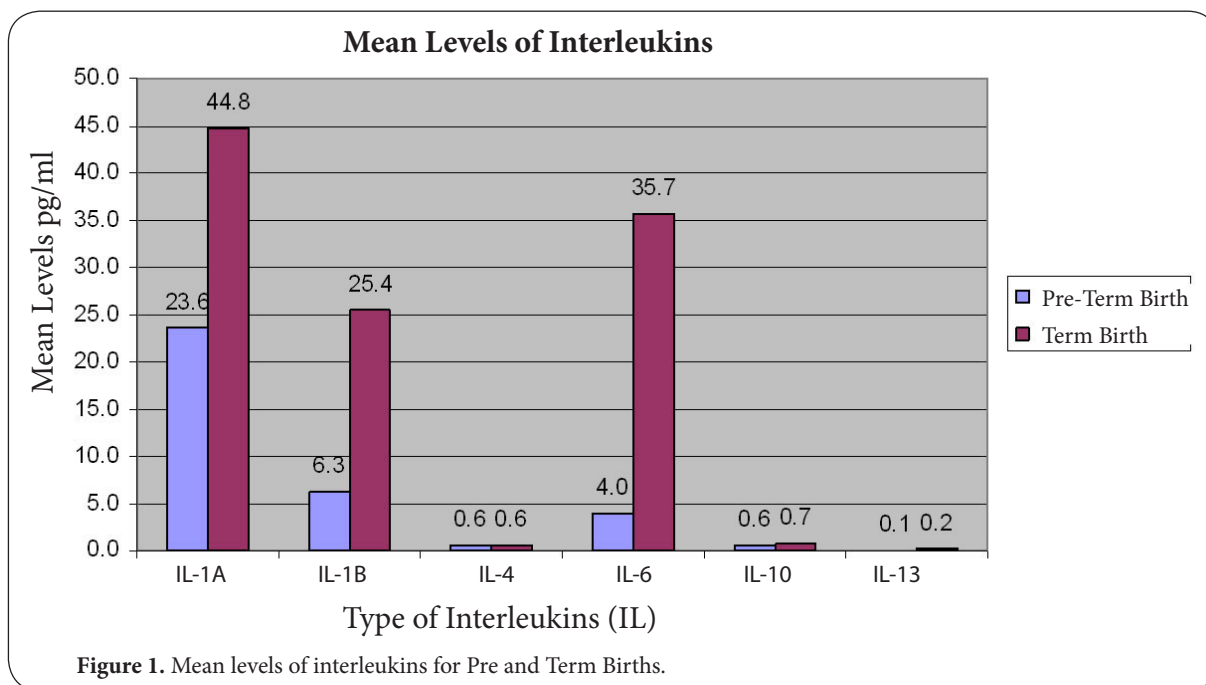


Figure 1. Mean levels of interleukins for Pre and Term Births.

IL-6 levels in the preterm birth patients are consistent with [3], who found that low levels of IL-A, IL-B, and IL-6 all were related to preterm birth [3]. The findings of [7] also found

lower levels of IL-B and IL-6 in patients who delivered prior to 34 weeks [7]. We also did not note any correlation with the inflammatory cytokines of IL-1A, IL-1B, and IL-13 with preterm

Table 2. Interleukin Levels by Pre and Term Births.

Variables	SPB (n=8) N (%) or mean (SD)	No SPB (n=31) N (%) or mean (SD)	P value
IL-1A	23.6 (34.3)	44.8	0.373
IL-1B	6.28 (12.7)	25.41 (41.6)	0.035
IL-4	0.62 (0.8)	0.59 (0.8)	0.943
IL-6	3.96 (3.8)	35.70 (55.3)	0.003
IL-10	0.62 (0.8)	0.67 (1.3)	0.918
IL-13	0.10 (0.1)	0.16 (0.2)	0.167

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birth. We did find a moderate correlation between IL-10 and cervical length ($r=0.54$; $p=0.002$). This is entirely consistent the concept of IL-10 as a mediator of inflammatory response. IL-1A, IL-1B, IL-6, IL-4 and IL-13 had no correlation with cervical length in our study. This is in contrast to the study by [2] which found a significant correlation with IL-6 and cervical length as a predictor of preterm birth [2].

Competing interests

The author declare no competing interests.

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