

## Original Article

### The Value of Interleukin-8 and Interleukin-6 in Cervical Secretions as Predictors of Preterm Delivery

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#### ABSTRACT

**Background and Objective:** Preterm birth occurs in 8% to 11% of all pregnancies and is responsible for 75% to 80% of all neonatal deaths. Cytokines may be involved in the etiology of preterm birth through their stimulation of prostaglandin synthesis. Cytokines may be released into cervicovaginal fluid during the breakdown of the chorio-decidual adhesion or from an inflammatory reaction in the same area. The aim of this study was to determine whether the values of interleukin 8 and 6 in cervical secretions could predict spontaneous preterm birth in asymptomatic high-risk pregnant women.

**Materials and Methods:** Levels of interleukin 6 and 8 in cervical samples, collected from 100 pregnant women between 22 to 28 weeks of gestation were measured by ELISA test in Mostafa Khomeini and hazrat-zeinab university hospitals in Tehran, from December 2006 to July 2007. Gestational age at time of delivery was recorded. Receiver operator characteristic curve analysis was used.

**Results:** There were 4.8, and 4.4, -fold increase in cervical interleukin 8 and 6 concentrations in early preterm versus term delivery. A single interleukin 8 >751.25 pg/ml, and 1 interleukin 6 >157 pg/ml, was identified early preterm versus term delivery. Sensitivity, specificity, positive and negative predictive values of interleukin 8 in early preterm birth were 89%, 83%, 69%, 94% and for interleukin 6 as 89%, 78%, 63%, 88%, respectively.

**Conclusion:** Cervical interleukin 8 and 6 is a sensitive and specific predictor preterm delivery.

**Keywords:** Interleukin 6, Interleukin 8, Preterm Birth, Preterm Labor

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## Introduction

Preterm birth, which is defined as delivery before 37 weeks of gestation, is a common obstetric problem (1), and occurs in 8% to 11% of all pregnancies. This obstetric complication is responsible for 75% to 80% of all neonatal deaths (2-4). Infants born prematurely are at increased risk for long-term disability and death (5).

Cytokines has been shown that involve in the etiology of preterm birth. Some choriodecidual cells produce substantial amount of cytokines and their production can stimulate prostaglandin synthesis (6). Increased serum and amniotic fluid concentrations of several cytokines, including interleukin 6 (IL-6), have been reported in women with preterm labor (7-11).

The use of cervicovaginal fluid has been suggested as an alternative to amniotic fluid testing because this fluid is released through mechanical or inflammation-mediated damage to the membranes or placenta before birth (12). Cytokines may be entered into cervicovaginal fluid as result of divaricated of the chorio-decidual adhesion or from an inflammatory process. Inflammation without infection may cause preterm labor on its own, as intraamniotic infusion of interleukin-1 (IL- 1) causes uterine contractility in primates (13). Cytokine release due to intrauterine infections seems to play an important role in a considerable percentage of preterm deliveries.

In cervicovaginal fluid, significant associations between elevated cytokine levels and preterm birth have been described previously for IL-1, IL-6, IL-8, and IL-18 (14-20).

The purpose of this study was to evaluate the usefulness of measuring cervico-vaginal proinflammatory cytokines (IL-6 and IL-8) in asymptomatic pregnant women considered high risk for preterm delivery in the prediction of preterm delivery.

## Materials and Methods

### Study design, sample collection, and population.

A cohort study was undertaken with cervical

samples collected from 50 pregnant women who had at least one risk factor for preterm delivery and 50 pregnant women who were low risk for SPD as a control group. All women were followed at the two prenatal clinics of Mostafa Khomeini and hazrat-zeinab university hospitals in Tehran from December 2006 to July 2007.

The study was approved by the Ethics Committee of Tarbiat Modares University, Tehran, Iran. Written consent was obtained after detailed information was given to every patient selected for the study.

Risk factors were designed such as history of Spontaneous Preterm Birth SPB in previous pregnancies, a history of second trimester abortion, a history of incompetent cervix and uterine malformation. Criteria for exclusion from the study were fetal congenital anomalies, placenta previa, vaginal bleeding during sampling, pregnancy induced hypertension or preeclampsia, fetal growth restriction, fetal distress, preterm rupture of membranes, and multiple pregnancy.

Each subject was enrolled between 22 and 28 complete weeks of pregnancy. Demographic, obstetric and outcome data were collected for all women. Gestational age was based on the last menstruation if the last menstrual period and the earliest ultrasound biparietal diameter did not differ by more than 10 days. If not, the biparietal diameter was used to define gestational age.

Cervical secretions were sampled with separate Dacron polyester fiber swabs that were left in place for 30 seconds to achieve saturation (150G). After the initial examination at 22 and 28 weeks of gestation, patients were examined at 2-week intervals. The primary outcome was defined as a SPB (after preterm rupture of membranes or spontaneous labor) at <34 weeks of gestation.

The pregnant women and their providers of obstetric care were blinded to the results of the interleukin 8 and 6 assays.

we categorized subjects into 1 of 3 strata: early SPB who delivered before 34 weeks of gestation; late SPB who delivered between 34 and 37 weeks of gestation ; and term delivery who delivered after

37 weeks of gestation. The control group consisted of 50 pregnant women with singleton gestations who had no risk factor.

**Immunoassay:** The swabs were then inserted into a polypropylene tube containing 1 ml of sample buffer (1% bovine serum albumin in Tris buffer with 5 mmol/L ethylenediaminetetraacetic acid, 5 mmol/L phenylmethylsulfonyl fluoride, and 0.5 trypsin-inhibitory unit aprotinin). Samples were stored at 4° C and transported to the Laboratory of Immunology, Shahed University, Tehran, within 48 hours. From this original sample, a 0.5 to 1.0 ml aliquot was stored at -20° C for up to 6 months for analysis. Concentrations of IL 6 and 8 in the antiprotease buffer were measured by immunoassay (Quantikine Human IL 6 and 8 Immunoassay, R&D System, Minneapolis). Results are reported in pg/ml. Clinicians were blinded to laboratory results, and technicians were blinded to clinical data.

**Data analysis:** Statistical analysis was performed with spss14 software. A Kruskal-Wallis test, Chi square test, Fisher Exact test and One-Way Anova

were performed as indicated. Receiver-operator characteristic (ROC) curve analysis was used to establish the cut-off values for cervical interleukin 8 and 6, which optimized the prediction of SPB. Sensitivity, specificity, positive and negative predictive values with their 95% confidence intervals were also calculated. A value of  $P < 0.05$  was considered significant.

## Results

Among 50 pregnant women considered at high risk for SPB, 9 (18%) delivered before 34 weeks' gestation. Fourteen (28%) delivered between 34 and before 37 weeks' gestation and 27 (54%) delivered at term and the entire control group delivered at term.

The maternal, obstetric, and neonatal characteristics of women who had early and late SPB in comparison with those delivered at term are shown in Table 1. Mean maternal age ( $\pm$ S.D.) for the study cohort was  $28.2 \pm 4.6$ , 54% had a history of preterm delivery, 40% a second trimester abortion, and 6% an incompetent cervix.

**Table 1:** Demographic and obstetric characteristics of patients with spontaneous preterm delivery vs. term delivery

	Spontaneous Preterm Delivery n=9 #	Late Spontaneous Preterm Delivery n=14 ~	Term Delivery n=27	Control Group Term Delivery n=50	Significance
Maternal age (yr)	28.55 $\pm$ 6.61	27.00 $\pm$ 4.27	28.74 $\pm$ 3.85	27.97 $\pm$ 3.85	NS*
Indication for enrollment					
History of previous preterm delivery (n, %)	8(88)	10(71)	18(66)	0	NS*
History of second trimester abortion (n, %)	3(33)	5(35)	12(44)	0	NS*
History of Incompetent cervix (n, %)	1(11)	1(%)	1(3)	0	NS*
Gestational age at delivery (weeks $\pm$ SD)	30.26 $\pm$ 1.94	35.40 $\pm$ .93	38.58 $\pm$ .70	39.00 $\pm$ .70	Significance
Birth weight (g $\pm$ mean $\pm$ SD)	1646.66 $\pm$ 376.36	2540.00 $\pm$ 332.61	3280.70 $\pm$ 286.49	3320.51 $\pm$ 320.25	Significance

\*Non-significant

# Birth before 34 weeks

~ Between 34 and before 37 weeks

Fig. 1 and 2 show interleukin 8 and 6 values obtained from cervical secretion in women delivered term or early and late preterm. The mean ( $\pm$ S.D.) interleukin 8 and 6 value in the early and late preterm group were significantly higher in comparison with the terms group. Receiver–operating characteristic (ROC) curve analysis was used to establish the optimal cut-off values the interleukin 8 and 6 levels of cervical secretions in order to predict preterm delivery (Fig. 3-4). An interleukin 8 value of 751.25 pg/ml in early preterm birth had a sensitivity of 89% (95%CI: 52%, 99%) and specificity of 83% with positive and negative predictive values of 69% and 94%. An interleukin 8 value of 296.6 pg/ml in late preterm birth had a sensitivity of 79% (95% CI:

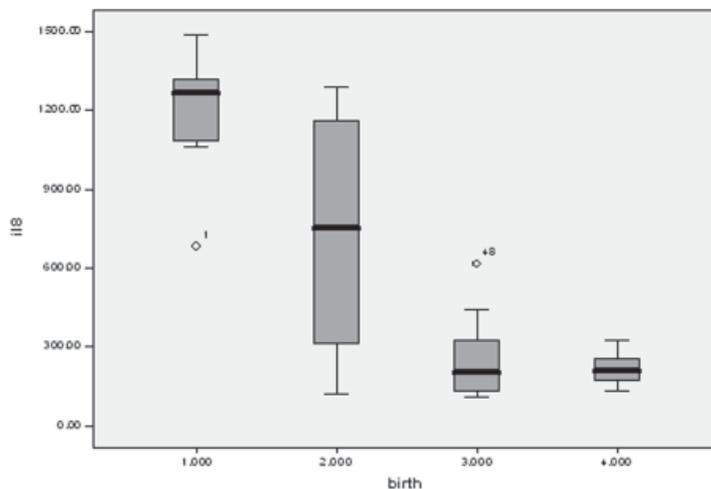
48%, 99%) and specificity of 53% with positive and negative predictive values of 42% and 85%. An interleukin 6 value of 157.00 pg/ml in early preterm birth had a sensitivity of 89% (95%CI: 52%, 99%) and specificity of 78% with positive and negative predictive values of 63% and 88%. A interleukin8 value of 123.65 pg/ml in late preterm birth had a sensitivity of 79% (95% CI: 48%, 99%) and specificity of 62% with positive and negative predictive values of 47% and 87%. Table 2 presents the mean $\pm$  SD values of interleukin 8 and 6 in early and late preterm delivery. Table 3 presents the sensitivity, specificity, positive and negative predictive values of interleukin 8 and 6 cut-off that were obtained at early and late preterm delivery.

**Table 2 :** Values of interleukin 8 and 6 in early and late preterm delivery and term delivery

	Early preterm birth	Late preterm birth	Term birth	Control /term birth	Significance
Interleukin8(pg/ml)	1192.30 $\pm$ 228.05	697.27 $\pm$ 435.49	254.84 $\pm$ 128.21	211.45 $\pm$ 107.25	0.0001
Interleukin6(pg/m)	388.15 $\pm$ 143.36	222.70 $\pm$ 140.70	88.15 $\pm$ 31.50	66.19 $\pm$ 52	0.0001

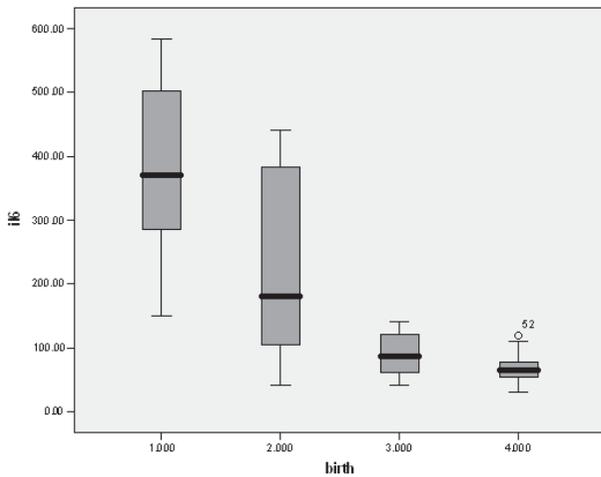
**Table 3:** Cut-off point, Sensitivity, specificity, positive and negative predictive Values of cervical interleukin 8 and 6 at early and late preterm for prediction of Spontaneous preterm birth

	Cut-off point	Sensitivity (%)	Specificity (%)	Positive PV (%)	Negative PV (%)
<b>Interleukin8</b>					
Early preterm birth	751.25pg/ml	89	83	69	94
Late preterm birth	296.6pg/ml	79	53	42	85
<b>Interleukin6</b>					
Early preterm birth	157. pg/ml	89	78	63	88
Late preterm birth	123.6pg/ml	79	62	47	87



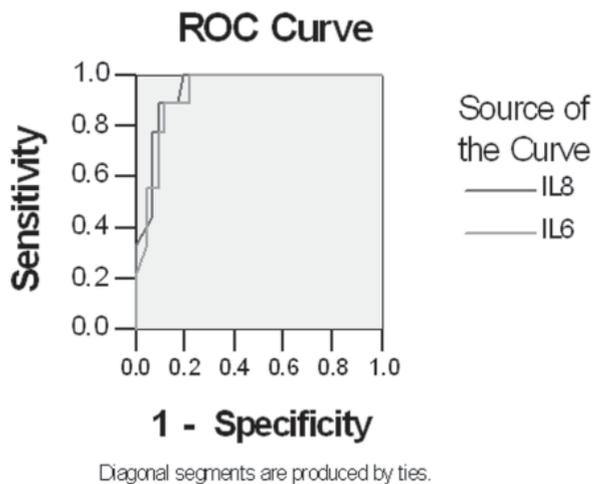
**Fig.1:** Cervical IL 8 values in the groups with early and late spontaneous preterm birth vs. a group with delivery at term ( $P<0.000$ )

1 birth before 34weeks, 2 between 34 and before 37 weeks, 3 term delivery, 4control group/ term delivery



**Fig. 2:** Cervical IL 6 values in the group with early and late spontaneous preterm birth vs. a group with delivery at term ( $P<0.000$ )

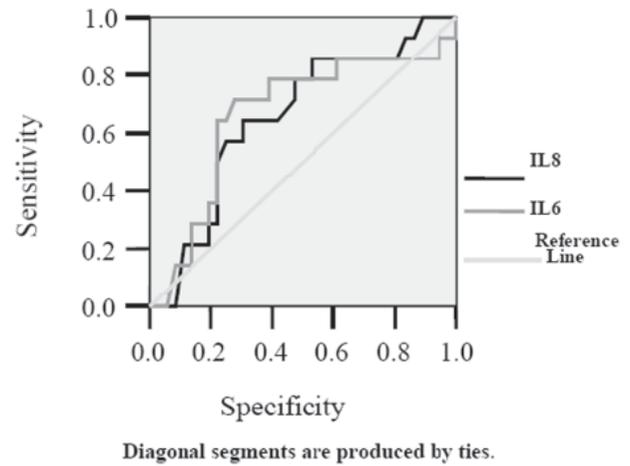
1 birth before 34weeks, 2. between 34 and before 37 weeks, 3.term delivery, 4.control group/ term delivery



**Fig. 3:** ROC curve for interleukin 8 and 6 for early spontaneous preterm birth

### Discussion

In the present study, the diagnostic value of interleukin 8 and 6 in cervical secretions to predict SPB were evaluated. The results indicate that cervical interleukin 8 and 6 measured between 22 and 28 weeks were significantly higher in patients delivered early and late preterm than in those delivered at term. Cervical interleukin 8 levels  $>751.25/\text{ml}$  and  $>296.60$  were associated with subsequent early and late preterm deliveries in about 89% and 79% of cases.



**Fig. 4:** ROC curve for interleukin 8&6 for late spontaneous preterm birth

Conversely, 83% and 53% of patients who delivered at term had cervical interleukin 8  $\leq 751.25$  pg/ml and  $\leq 296.60$ pg/ml. Cervical interleukin 6 levels  $>157.00$ pg/ml and  $>123.65$  were associated with subsequent early and late preterm deliveries in about 89% and 79%of cases. Conversely, 78% and 62% of patients who delivered at term had cervical interleukin6  $\leq 157.00$  pg/ml and  $\leq 123.65.60$  pg / ml. cervical interleukin 8 and 6 proved a better predictor of SPB than demographic and obstetric risk factors.

Human cervical cells produce large amounts of interleukin 8. IL8 may have its influence locally and not diffuse far into the amniotic fluid in cases of preterm labor (21). IL8 therefore may play a role in neutrophil-mediated cervical ripening which is supported by the cervical changes observed in the current study in those women with increase IL8.

IL8 is a potent chemo attractant for polymorph nuclear leucocytes (22).The collagenases that bring about cervical ripening are neutrophil-derived and IL8 in choriodecidual cells could be the controlling factor in neutrophil attraction. An increase of IL8 from cervical secretion is associated with cervical ripening and preterm labor. Cervical interleukin 8 levels obtained in 28 weeks' gestation were significantly higher in women with SPB compared with women who delivered at term (19).

There is a sufficiently high level of evidence in the literature to suggest that an increased level

of interleukin-6 in the amniotic fluid is related to preterm birth (23). It has been demonstrated that the decidua plays an important role in the inflammatory response to infection by production of cytokines (24). It indicates that chemical and vaginal IL-6 concentrations due to local inflammatory response, measured at 24 weeks of gestation were significantly higher in patients who delivered pre-term (25).

The cut-off value of cervical interleukin 6, was >250 pg/ml predict preterm labor with a sensitivity of 50%, specificity of 85.0% and positive predictive values of 47% in 24- 36 weeks' gestations (6). Elevated cervical IL6 levels could be a marker for cervicitis or local pathogens and thus an indirect indicator of infection associated preterm delivery.

Another possible explanation for the association between cervical IL6 and preterm delivery is that elevated cervical IL6 levels reflect a maternal genital tract hyper immune response. Perhaps such patients mount a more exuberant cytokine response to local bacteria, promoting preterm delivery before the developing of clinical chorioamnionitis. Alternatively, the presence of elevated cervical IL6 levels may reflect endocrine or paracrine events, unrelated to infection and/or inflammation but associated with subsequent preterm delivery.

## Conclusion

The results of this study suggest that a single cervical interleukin 8 and 6 measurement in high-risk pregnant women between 22 and 28 weeks' gestation might be used to predict SPB.

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