

SHORT PAPER

Maternal Serum and Cervicovaginal IL-6 in Patients with Symptoms of Preterm Labor

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ABSTRACT

Background: Preterm birth is a common problem in obstetrics. **Objective:** To measure maternal serum interleukin-6 in mothers with preterm uterine contractions and compare it with cervicovaginal interleukin-6 in the same women. **Methods:** In this cross-sectional study, we measured interleukin-6 in the sera and cervicovaginal fluids of 86 women with preterm uterine contractions. All participants had an intact membrane. Interleukin-6 was measured by using ELISA method. Statistical analysis was done using U-Mann Whitney, Chi-Square and Kendall's tests. **Results:** The mean and median (Quartile25, Quartile75) of interleukin-6 in cervicovaginal fluid were higher than maternal serum interleukin-6. There was a statistically significant difference in the median of interleukin-6 in sera and cervicovaginal fluid ($P < 0.0001$). There was no significant correlation between serum and cervicovaginal interleukin-6 ($r = 0.048$, $p = 0.548$). There was no significant correlation between serum and cervicovaginal interleukin-6 ($r = 0.048$, $p = 0.548$). **Conclusion:** We found no relationship between serum interleukin-6 and preterm labor and the maternal serum Interleukin-6 does not seem to be a suitable biomarker for predicting preterm delivery.

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Keywords: Cervicovaginal Fluid, Interleukin-6, Maternal Serum, Preterm Labor

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INTRODUCTION

Preterm birth (PTB) is defined when delivery happens at less than 37 completed weeks and more than 20 completed weeks of gestation (1). In the United State, the prevalence rate of preterm delivery was 11.7% in 2011 (2). It is mentioned that the prevalence of PTB is higher in developing countries (3). In different cities of Iran the prevalence rate of preterm delivery was between 5.6% in Qoum and 39.4% in Kerman in 2012.(4).

PTB has serious effects on mothers, children and health system, making it an important public health issue. In 2005, the cost of health care for PTB has been estimated to be \$26 billion US (5). PTB is the cause of 35% neonatal deaths worldwide (6), and the second most common reason of death after pneumonia in children under five years of age (7). Preterm delivery has complex and multifactorial etiology including race and ethnicity (8), mother's age (9), previous preterm birth (10), infection (11) and etc.

One of the most common causes of preterm labor is intrauterine infection. It is estimated that near 50% of all cases of preterm labor caused by intrauterine infection (1). Early uterine contractions that are caused by infection have two pathways: 1) Bacteria existing in the vagina and cervix extend upward to choriodecidual space, release toxins and these toxins activate fetal membranes and decidua to produce cytokines likes interleukin-6 (IL-6) and interleukin-8 (IL-8), and finally, these factors activate prostaglandins and consequently stimulate premature contractions (11, 12); 2) Some women may have chronic intrauterine infection even between pregnancies that decrease the activity of prostaglandin dehydrogenase and cause an increase in prostaglandins and stimulate preterm contractions (13,14).

The history of obstetrics, symptoms and epidemiological risk factors are neither sensitive nor specific for predicting preterm delivery (15). Many biochemical markers like IL-8, IL-6, fetal fibronectin, C-reactive protein (CRP), etc. are studied for a prediction of preterm labor (16,17).

IL-6 is one of the most important pro-inflammatory cytokines in innate immune system. Its gene is located on chromosome 7p21 (18), it is synthesized by macrophages, endothelial cells and T-cells and is increased in many systemic and chronic inflammatory diseases such as autoimmune diseases, cancer, infection, sepsis and hypertension (19). IL-6 has both local and systemic effects; It can be secreted in the local sites of infection, affect vascular endothelial cells, leukocytes and increase the local delivery of cells to fight infections. It can also be produced at inflammatory sites and enter the blood and bone marrow and increase the supply of cells that can be recruited to the sites of infection (20).

Increases of IL-6 in amniotic fluid and cervical secretions have been studied in several surveys (21-23). Although IL-6 increases in amniotic fluid (22) using amniotic fluid for detecting IL-6 is costly, invasive and requires trained staffs (16). Increases of cervicovaginal IL-6 have also been shown in many studies (23-25).

Sampling cervicovaginal secretions in women with preterm contractions can stimulate preterm delivery but collecting blood from a mother in this condition is less invasive. The increase of IL-6 level in sera of women with preterm delivery remains controversial (26,27). For example, in a case-control study, Shahshahan *et al.* compared 75 women in preterm uterine contractions and 75 women in term uterine contractions, found that serum IL-6 increases in preterm delivery and it was an appropriate marker for prediction of preterm delivery (26). However, Bahar *et al.* did not find any evidence of IL-6 increasing in maternal serum during preterm birth (27).

The purpose of this study was to determine the maternal serum IL-6 in mothers with preterm uterine contractions and compare it with cervicovaginal IL-6 in the same women.

MATERIALS AND METHODS

Study Design and Participants. This cross-sectional study, was conducted in Al-Zahra hospital from January until April 2015 in Tabriz (in the Northwest of Iran). The Tabriz University of Medical Sciences Review Board approved the original study protocol (93/10/10-5/4/9267) and the participants provided written informed consent at the time of study enrollment.

Pregnant women with the following criteria were eligible in the study: gestational age between 28^{0/7} and 36^{6/7} weeks, singleton pregnancy, having at least two contractions during 10 minutes that lasting 30-40 seconds and a fetal heart rate ranging 120-160 bpm (beats per minute).

Women with vaginal bleeding, received any antibiotics and corticosteroids in hospital, with cervical cerclage or any medical problems including high blood pressure, diabetes, having infectious disease during pregnancy and fetus malformations were excluded.

Eighty six consecutive women with preterm uterine contractions and intact membrane participated in this research. Participants were at gestational ages of 28^{0/7}–36^{6/7} weeks according to sonography that was performed in 7th to 11th gestational weeks of pregnancy. Preterm labor was defined as regular uterine contractions at a frequency of three in 10 minutes with cervical dilation and effacement more than one centimeter and 80% respectively. All participants had intact membranes at study enrollment, and this was confirmed by sterile speculum examination. Vaginal examination and contraction control was conducted by the researcher.

Sample size was determined at 86, considering a 95% confidence interval, 80% power, two sided test

based on a mean (SD) of IL-6 [29.7 (31.67)], and information was obtained from a study by Shahgheibi *et al.*(28).

Data Collection. In this survey, a questionnaire including sociodemographic and reproductive history was filled out by researcher. For collecting cervicovaginal fluid, first women lied in lithotomy position, then sterile speculum was used to see the cervix and a Dacron swab placed on the posterior fornix of the vagina for 10 seconds. The swab was inserted into a tube containing one milliliter normal saline and was shaken for five minutes. Two milliliters of blood was collected from all participants, too. Samples were referred to the laboratory in 30 minutes. Whole samples were centrifuged for 10 minutes with 6000×g Serum and cervicovaginal fluid were stored at -30°C until assayed. Before analysis, samples were thawed to 25°C and centrifuged at 1700×g (gravity) for five minutes.

Data Validity. For evaluation of sociodemographic validity, the questionnaire was given to 10 professors of Tabriz University of Medical Sciences and after collecting their ideas, needed changes was done and finally used for study.

IL-6 concentrations were measured by human enzyme-linked immunosorbent assay (eBioscience, Australia) ELISA kit. The assay was carried out by strictly following the instructions provided by the manufacturer, and all samples were measured in duplicate. The calculated inter-assay coefficients of variation (CV) for IL-6 immunoassays in our

laboratory were 5.2%. Calculated intra-assay CVs for IL-6 were 3.4%. The calculated detection limits (sensitivity) were 0.92 ng/ml.

Cytokine Assay. For IL-6 assessment, first 50 microliter samples and 50 microliter assay buffer were added to microwells that were coated with antibody and was incubated for 2 hours; after washing microwells, 100 microliter streptavidin-HRP was added to microwells and was incubated for 1 hours. Then microwells were washed and 100 microliter substrate solution reactive with HRP was added to microwells. The absorbances were measured at 450 nm using ELISA reader and resulting data were analyzed according to the standard curve.

Analysis. Data were analyzed through descriptive and inferential statistical tests such as mean (Standard Deviation) for normal data distribution and median (Quartile 25 to Quartile 75) for abnormal data distribution by using SPSS (Statistical Package for the Social Science) version 13 software. Inferential statistical tests were U-Mann Whitney and Kendall's. P-value less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The purpose of this study was to determine maternal serum concentration of IL-6 and its comparison with cervicovaginal fluid IL-6 in the same women to find a correlation between serum and cervicovaginal IL-6, as well as to help for choosing a less invasive method to predict women that are at high risk of preterm delivery. In our setting, for all women with symptoms of preterm delivery, recommended treatments include tocolytics (magnesium sulfate), steroids (betamethasone) and antibiotics (especially ampicillin). This strategy involve unnecessary treatment in a number of symptomatic women who eventually will not deliver pretermly. So, there is a need for valid and non-invasive tools to determine women at a high risk of preterm delivery and those at a low risk.

In the present study, 86 women with symptoms of preterm delivery were enrolled and samples of both cervicovaginal fluid and blood were taken at the time that participants entered to hospital. Mean (SD) age and BMI of participants (Body Mass Index) before pregnancy were 27.8 (6.51) and 24.91 (4.22), respectively. Thirty two women (37.2%) were primigravida and 11 women (12.8%) were pregnant using ART (Assisted Reproductive Technology) method. None of participants used cigarette and alcohol.

The mean (SD) of serum and cervicovaginal IL-6 did not have normal distribution based on the Kolmogorov-Smirnov test (5.69 (7.12) and 45.94 (65.16), respectively) There was a statistically significant difference between median (Q_{25} - Q_{75}) of serum and cervicovaginal IL-6 in preterm labor using the U-Mann Whitney test [2.89 (4.45 to 4.72) and 20.90 (4.94 to 61.45) respectively, ($P < 0.0001$)].

The mean (SD) of gestational age in participants was 33.95 (2.47) and the normal range of serum IL-6 was from non-detectable to 12.7 ng/ml. The relation between gestational age and serum IL-6 levels was assessed by the Chi-Square test (Table 1).

There was no statistically significant correlation between serum IL-6 and gestational age ($p = 0.157$), [OR (Odds ratio): 2.00; CI (Confidence Interval) 95%: 0.23-17.12]. Kendall's test was performed to determine correlation coefficient between serum IL-6 and gestational age. There was no significant correlation between serum IL-6 and gestational age ($r = 0.071$, $p = 0.383$). The Figure shows scatter plot of these data.

This finding is in accordance with a study by Isik *et al.* in which 22 women with term labor were compared with 22 women in preterm labor; the authors found that there was

no correlation between serum IL-6 and preterm delivery (28). Moreover Bahar *et al.* found that there was no correlation between an increase of serum IL-6 and gestational age (26); while Shahshahan *et al.* found that serum IL-6 level increases in preterm delivery, but there is not a significant correlation between serum IL-6 and gestational age (25).

Table 1. Relation between serum and cervicovaginal IL-6 with gestational age in preterm labor (n=86).

	GA ^a ≤ 32 week	GA > 32 week	P ^b
Serum IL-6 ≤ 12.7 (ng/ml) N (%)	18 (100)	60 (87.9)	0.157
Serum IL-6 > 12.7 (ng/ml) N (%)	0 (0)	8 (12.1)	
Cervicovaginal IL-6 ≤ 35 (ng/ml) N (%)	17 (73.3)	45 (65.5)	0.773
Cervicovaginal IL-6 > 35 (ng/ml) N (%)	5 (26.7)	23 (34.5)	

^b Fisher exact test

^a Gestational Age

An increase of cervicovaginal IL-6 in preterm labor is consistent in previous studies in which cervical IL-6 increases in preterm labor (29).

Table 1 shows the correlation between cervicovaginal IL-6 and gestational age under 32 weeks and above 32 weeks in preterm labor. The cut-off point of 35 ng/ml were used based on Coelman *et al.* study (30).

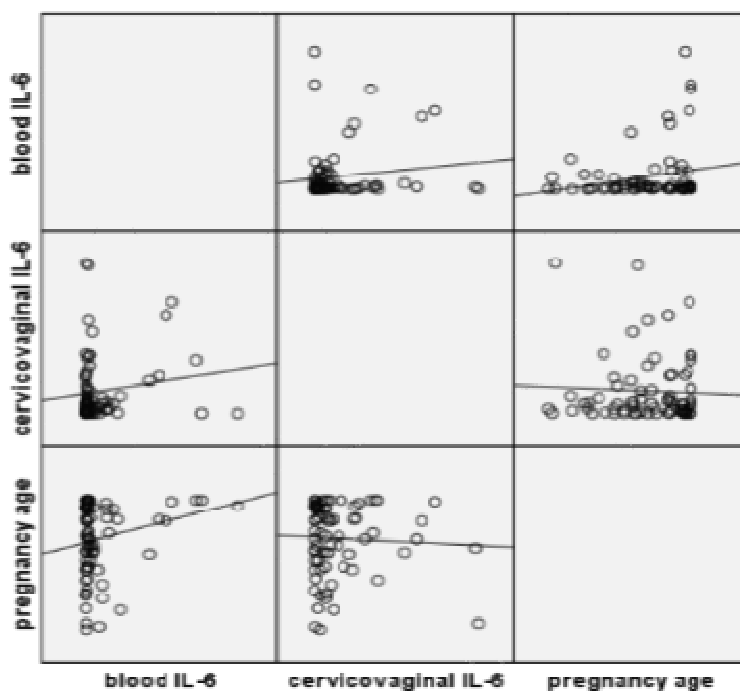


Figure 1. Scatter plot of Serum IL-6, Cervicovaginal IL-6 and pregnancy age.

There was no statistically significant correlation between cervicovaginal IL-6 and gestational age ($p=0.773$), [OR: 1.44; CI 95%: 0.41-5.13]. The Figure shows scatter plots of cervicovaginal IL-6 and gestational age. IL-6 in cervicovaginal fluid does not correlate with gestational age (correlation coefficient=0.014, $p=0.864$).

Coleman *et al.* used a cut-off of 35 ng/ml (the sensitivity of 60% and specificity of 77%) and stated that IL-6 increases in cervicovaginal fluid in preterm labor, but they did not find a significant relationship between cervicovaginal IL-6 and gestational age (30). Woodworth *et al.* collected cervicovaginal fluid of 660 pregnant women and found that cervicovaginal IL-6 increased in preterm labor and could be used for predicting preterm deliveries (21). In a study conducted by Hadzi-Lega *et al.*, the authors analyzed cervicovaginal IL-6 in 58 women and concluded that IL-6 test with a cut-off of 1305 pg/ml, (sensitivity of 69.4% and specificity of 68.2%) is a good biomarker to determine women at a high risk of preterm delivery (31). Lashay *et al.* used a cut-off of 100 pg/ml and found that there is no significant correlation between cervical IL-6 and delivery under 32 weeks ($p=0.11$) (32). The sensitivity of 50-100% and specificity of 67-87% have been reported in studies and such differences are due to the cut-off point (33).

The correlation between serum and cervicovaginal IL-6 levels was determined by Kendall's test. There was no statistically significant correlation between serum and cervicovaginal IL-6 in preterm labor (correlation coefficient=0.048, $p=0.548$).

To determine correlation between several pregnancy outcomes and IL-6, Kendall's test was performed (Table 2).

Table 2. Correlation between serum and cervicovaginal IL-6 with Several pregnancy outcomes (n=86).

Variables	Serum IL-6		Cervicovaginal IL-6	
	r	p ^b	r	p ^b
Cervical dilation (centimeter)	0.179	0.045	0.311	0.0001
Interval between starting contractions and delivery (day)	-0.166	0.041	-0.202	0.013
Gestational age in delivery (week)	-0.061	0.456	-0.108	0.185
Mother WBC ^{aa} before pregnancy ($\times 10^3/\text{mm}^3$)	0.058	0.553	-0.067	0.433
Baby weight (gram)	-0.002	0.977	-0.081	0.320
Apgar in first minute	0.023	0.804	-0.031	0.732
Apgar in fifth minute	-0.059	0.534	-0.078	0.410
Admission to NICU ^{aaa}	-0.006	0.953	0.105	0.277

^b Kendall's test

^{aa} White Blood Cell

^{aaa} Neonatal Intensive Care Unite

We found that cervical dilation had a significant correlation with serum and cervicovaginal IL-6 ($p=0.045$ and $p=0.0001$, respectively). Roghaei *et al.* using a cut-off of 18.1 pg/ml for cervicovaginal IL-6, found that the sensitivity and specificity of cervicovaginal IL-6 in predicting preterm delivery was 83.3% and 39.5%, respectively; although they did not find a significant correlation between cervical dilation and cervicovaginal IL-6 ($p=0.21$), they mentioned that the combination of these two characteristics had more sensitivity and specificity (50% and 65%, respectively) for predicting of preterm delivery (35).

The limitation of this study was the relatively small sample size for measuring cytokine with high variance. Also, we did not have direct evidence of previous intrauterine infection and had to trust the parents on reports, which was another limitation of this research.

In summary, although, IL-6 in cervicovaginal fluid increases in preterm labor, there is no significant correlation between serum and cervicovaginal IL-6. Therefore, maternal serum IL-6 is not a suitable biomarker for predicting preterm delivery.

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