ORIGINAL ARTICLE

Maternal serum interleukin 6 and 8 and C-reactive protein in predicting the tocolytic therapy in preterm labor

Zahra Shahshahan, Leila Hashemi, Ousha Rasouli
Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: This study aimed to evaluate the relation between C-reactive protein (CRP), interleukin (IL) 6 and 8 with the response to tocolytic therapy. **Materials and Methods:** A total of 75 singleton pregnant women between 18 and 35 years old, and with symptoms of preterm labor were hospitalized in "Shahid Beheshti" hospital in Isfahan, Iran. Tocolysis in patients was performed first with infusion of 4 g of magnesium sulfate 20% and then 2 g per hour continued. Next, they were followed till delivery time to assess the response to the treatment. Baseline data and serum levels of IL-6 and IL-8 and CRP were all recorded. **Results:** A total of 16 patients with symptoms of preterm labor did not respond to the treatment and delivered prematurely and 59 women responded to tocolytic treatment and delivered at term. There was a significant relationship between serum IL-6, IL-8 and CRP levels with response to the treatment in cut-off > 45 for IL-6 [area under the curve [(AUC), 0.894; SE, 0.042; *P*-value < 0.0001, >171 for IL-8 (AUC, 0.864; SE, 0.059; *P*-value < 0.0001)] and >1.8 for CRP (AUC, 0.738; SE, 0.076; *P*-value = 0.001). Also, pairwise comparison of receiver operating characteristic curves between CRP, IL-6, and IL-8 showed that there were no significant differences between areas for IL-6 with IL-8 (*P*-value = 0.46); IL-6 with CRP (*P*-value = 0.086); and IL-8 with CRP (*P*-value = 0.18). **Conclusion:** Maternal serum concentrations of IL-6 and IL-8 and CRP can be used as appropriate biomarkers for predicting the response to tocolytic therapy in pregnant women and there were no significant differences between these markers in predicting tocolytic therapy.

Key words: Cytokines, interleukin, IL, preterm labor, tocolytic

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INTRODUCTION

One of the important indicators of hygienic cares in the national level is women's health and also the number of women at pregnancy age forms a large and effective amount of population in the society.[1]

Frequency of preterm birth which is defined as childbirth occurring in less than 259 days of gestation or 37 complete weeks since the 1st day of the women's last menstrual period is from 5% to 13% in countries with high income and the incidence is increasing[2,3] compared with term-birth infants in infants born prematurely; higher morbidity and mortality rates were reported.[4] Preterm birth is the leading cause of child deaths and perinatal morbidity and mortality worldwide, in almost all high-income and middle-income countries. The rate of annual neonatal deaths is reported to be 3.1 million in the world; while, preterm birth complications are estimated to be answerable for 35% of them.[5] Also, after pneumonia, preterm birth, is the second most common cause of death in children less than 5 years old.[6] Across the world, preterm birth is an important perinatal health problem and has high economic and social cost in terms of neonatal intensive care, for the families and health-care systems.[7,8]

Spontaneous preterm birth is known as the cause of around half of the preterm births, for which there are no known effective prevention measures.[2] C-reactive protein (CRP) is a sensitive inflammatory marker whose serum level increases and then remains constant during the infectious and inflammatory processes. On the contrary, CRP measurement is quick, noninvasive, and risk-free which can be a useful diagnostic test for evaluating and categorizing the risk levels and also anticipating the morbidity of both mother and fetus.[9,10] Also, cytokines have been explored as biomarkers of impending preterm labor, but there is paucity of data in the current literature concerning the association between maternal serum concentrations of IL-6, IL-8, and CRP and preterm delivery.[11] The presence of increased concentration of IL-6 and IL-8 in a variety of biological fluids including maternal and/or fetal blood, amniotic fluid, urine, cervical and/or vaginal secretions, and

Address for Correspondence : Dr. Zahra Shahshahan, Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: shahshahan@med.mui.ac.ir
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placental tissue is an independent risk factor for preterm labor.[12-15] Additionally, some studies show that in women with intrauterine infections, CRP levels of amniotic fluid are higher compared with the control group.[16,17]

Even though tocolytics have not been shown to improve neonatal outcomes, they are an important intervention in obstetrics to delay preterm delivery.[18,19] The aim of tocolysis is to delay preterm delivery long enough for antenatal corticosteroids to be administered or for the mother to be transported to a tertiary care center, thereby, reducing neonatal morbidity and mortality.[20,21] The use of tocolytic agents in contemporary obstetric practice should be customized and according to the available evidence basis for efficacy and fetomaternal safety, gestational age, the maternal condition, and potential side-effects of the drug.[22] Magnesium sulfate is one of the tocolytic agents, the administration of which to women at the risk of preterm birth helps protect the baby's brain, reduce rates of cerebral palsy and improve long-term neonatal health outcomes and in spite of maternal side effects, magnesium sulfate is commonly used for tocolysis.[23]

The incidence of preterm labor could be prevented by effective diagnosis and preventive factors, because of the importance of neonatal mortality and the fact that no effective treatment is currently available.[20,21] Also, to the best of our knowledge, there are no published studies reporting the role of CRP, IL-6, and IL-8 in the prediction of response to tocolytic treatment in women with the diagnosis of preterm labor. Therefore, the present study was designed to hypothesize: To determine if maternal serum concentrations of CRP, IL-6, and IL-8 have any values in the prediction of response to tocolytic treatment in these women.

MATERIALS AND METHODS

The present study was conducted on 75 singleton pregnant women with the diagnosis of new-onset preterm labor, who had referred to "Shahid Beheshti" hospital in Isfahan, Iran, from May, 2012 to October 2012. Diagnosis of new-onset preterm labor was defined as painful contractions at least every 10 min, documented, despite hydration, for 1 h on an external tocodynamometer or as regular labor detected by cardiotocography resulting in a cervical change. All of the cases did not have any clinical signs of infection or of any other maternal or fetal complications. Pregnant women's age range of between 18 and 35 years old with singleton pregnancy and gestational age of 24-34 complete weeks were eligible if they had less than five parturitions, no history of type 1 or 2 diabetes mellitus, hypertension, cardiovascular disease, and infectious diseases. Also, women with contraindication through using tocolytic drugs, fetus or amniotic fluid anomaly, uterian or cervical abnormality, emerging some undesirable conditions during parturition such as preeclampsia and abruption were excluded from the study. This study was approved by Institutional Review Board at the Isfahan University of Medical Sciences and written informed consent, was obtained from all participants after they were explained about and informed of the purposes of the study.

Tocolysis in eligible patients was performed with the use of magnesium sulfate (manufactured by Iran Pasteur Institute, Tehran, Iran) as follow: first with infusion of 4 g magnesium sulfate 20% and then 2 g per hour continued.[23] After that, they were followed till delivery time to assess the response to the treatment. Maternal serum that was collected at the first visit after diagnosis of preterm labor was analyzed to determine the levels of CRP, IL-6, and Il-8 using immunoassay method.

All the statistical analysis was done by SPSS-20 (SPSS IBM, New York, USA). Data were reported using number (%) for categorical variables and mean ± standard deviation for continuous variables. Categorical variables were compared using chi-square test and continuous variables were compared using independent sample t-test. For serum IL-6, IL-8, and CRP, a receiver operating characteristic (ROC) curve analysis was used to establish the cut-off values that optimized the response to tocolytic therapy. Sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV), and likelihood ratio (LR) were then calculated. In addition, area under the curve (AUC) were evaluated for all study tests. *P*-values less than 0.05 were considered to indicate statistical significance.

RESULTS

Of studied patients after tocolytic treatment, 59 women delivered at term, but 16 women despite tocolytic therapy delivered prematurely. The mean age for the samples was 27.1 ± 4.2 years old. Table 1 shows the differences between patients in regard to the results of tocolytic therapy for age, gestational age, history of miscarriage, gravidity, serum IL-6, IL-8, and CRP levels; there were no significant differences for age, gestational age, history of miscarriage, and gravidity. But in women with unsuccessful tocolytic treatment, levels of CRP, IL-6, and IL-8 were significantly higher than in women with successful treatment.

The results of ROC, describing the values of the serum IL-6 and IL-8 levels in predicting the response to tocolytic therapy in women with symptoms of preterm labor, are shown in Figure 1. There was a significant relationship between serum IL-6, IL-8, and CRP levels with response to the treatment in cut-off >45 pg/mL for IL-6 (AUC, 0.894; SE,

0.042; *P*-value < 0.0001), >171 pg/mL for IL-8 (AUC, 0.864; SE, 0.059; *P*-value < 0.0001), and cut-off >1.8 mg/L for CRP (AUC, 0.738; SE, 0.076; *P* value = 0.001). Also, the sensitivity, specificity, PPV, NPV, and LR for IL-6, IL-8, and CRP levels to predict response to tocolytic therapy in preterm women are shown in Table 2.

Pairwise comparison of ROC curves between CRP, IL-6, and IL-8 are shown in Table 3. As shown, there were no significant differences between areas in IL-6 with IL-8 (*P* value = 0.46); IL-6 with CRP (*P* value = 0.086); and IL-8 with CRP (*P* value = 0.18).

DISCUSSION

Preterm birth is a major obstetric and neonatal challenge, and very preterm birth imposes a considerable burden on limited health care resources and is a main cause of mortality and morbidity for newborns. The most important causes of newborns' death, in Iran, were prematurity (63.24%), constituting nearly two-third of neonatal mortalities and that changes in perinatal management have been associated with a significant increase and better outcome of these infants, over the last 2 decades. One of the most important challenges in modern maternity care is the prevention of preterm birth.[3,24]

In the present study, serum IL-6, IL-8, and CRP levels in pregnant women with signs of preterm in predicting the response to tocolytic therapy were assessed. We observed that elevated serum IL-6 and IL-8 and CRP levels in preterm women with successful tocolytic therapy were markedly higher than in preterm women with unsuccessful tocolytic therapy. We found that cut-off values of IL-6 >45 pg/mL, IL-8 >171.5 pg/mL, and CRP >1.8 mg/L with a sensitivity

Table 1: Comparison of baseline characteristics, and the concentration of IL-6 and IL-8 between cases, in regard to response to tocolytic therapy

	Response to tocolytic therapy		
	Positive (*n* = 59)	Negative (*n* = 16)	*P* value
Age (year)	26.4±4.8	27.5±4.3	0.41*
Gestational age (week)	30.1±2.2	30.7±3.5	0.4*
History of miscarriage	14 (23.7)	3 (18.7)	0.9†
Gravida			
Pregnancy	25 (42.4)	5 (31.3)	0.12†
Pregnancy	21 (35.6)	10 (62.5)	
or more Pregnancy	13 (22)	1 (6.3)	
Interleukin-6 (pg/mL)	20.57±30.9	66.64±35.8	<0.0001*
Interleukin-8 (pg/mL)	69.54±83.8	187.96±109.8	<0.0001*
C-reactive protein (mg/L)	3.81±6.4	10.74±12.4	0.045*

Data are mean ± SD and number (%); *P* values are calculated by *Independent sample *t*-test and †Chi-square test; SD = Standard deviation

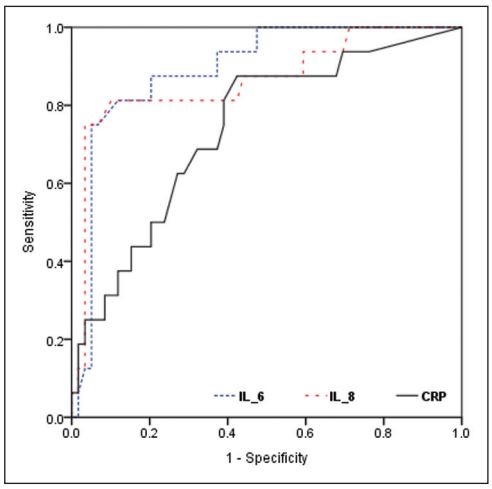

Figure 1: Receiver operating characteristic curves for maternal serum interleukin-6 (area under the curve, 0.894; SE, 0.055; *P* value <0.0001), interleukin-8 (Area under the curve, 0.864; SE, 0.061; *P* value <0.0001) and C-reactive protein (area under the curve, 0.738; SE, 0.076; *P* value = 0.001) levels for predicting the response to treatment in women with symptoms of preterm labor

Table 2: The prognostic value of evaluation of maternal serum IL-6 and IL-8 in preterm women for prediction of response to tocolytic therapy

	Sensitivity [95% CI]	Specificity [95% CI]	+PV [95% CI]	–PV [95% CI]	+LR [95% CI]	–LR [95% CI]
IL-6 (>45)	75 [47.6-92.6]	94.9 [85.8-98.9]	80 [51.9-95.4]	93.3 [83.8-98]	14.7 [11-19.7]	0.26 [0.07-1.1]
IL-8 (>171.5)	75 [47.6-92.6]	96.6 [88.3-99.5]	85.7 [57.2-98]	93.4 [84-98]	22.1 [16.6-29.5]	0.26 [0.5-1.3]
C-reactive protein (>1.8)	87.5 [61.6-98.1]	57.6 [44.1-70.4]	35.9 [21.2-52.8]	94.4 [81.3-99.2]	2.6 [1.6-2.8]	0.2 [0.06-0.8]

CI = Confidence interval; IL = Interleukin; LR = Likelihood ratios; PV = Predictive value

Table 3: Pairwise comparison of receiver operating characteristic curves for maternal serum Interleukin-6 and 8 and C-reactive protein after tocolytic therapy in preterm women

	IL_6 ~ IL_8	IL_6 ~ C-reactive protein	IL_8 ~ C-reactive protein
Difference between areas	0.0297	0.156	0.126
Standard error	0.0403	0.0906	0.0947
95% confidence interval	-0.0494 to 0.109	-0.0219 to 0.333	-0.0596 to 0.312
z statistic	0.735	1.718	1.331
P-value	0.462	0.086	0.183

CRP = C-reactive protein; IL = Interleukin

of 75, 75, and 87, respectively, were the best values in the prognosis of successful tocolytic therapy in preterm women. Using enzyme-linked immunosorbent assay techniques, CRP, IL-6, and IL-8 can be measured in serum, which gives the opportunity to detect these cytokines easily in routine practice. And, the evaluation of their impact on preterm treatment results can be useful. Our results showed that serum concentrations of CRP, IL-6, and IL-8 are good markers to the prediction of the response to tocolytic therapy in preterm women before 34 weeks. Whereas, CRP with LR +2.6, LR –0.2 sensitivity of 87.5%, IL-6 with LR +14.7, LR –0.26, sensitivity of 75%, and IL-8 with LR +22.1, LR –0.26, sensitivity of 75%, can be used as biomarkers for the response to tocolytic therapy in preterm labor women. Gravidity in women with unsuccessful tocolytic therapy was lower than in other women but was not statistically significant and there was no association between gravidity, and CRP, IL-6, and IL-8 levels. Also, the results of pairwise comparison of ROC curves showed that there were no significant differences between CRP, IL-6, and IL-8 to predict the response to tocolytic therapy in these women.

The association between maternal serum concentrations of IL-6 and IL-8 with early preterm delivery has been explored by a few studies on women at increased risk of preterm labor. In a nested case-control study, authors reported that for preterm birth before 35 weeks, maternal serum concentration of IL-6 was not a significant marker.[25] Another study suggested that elevated maternal serum concentration of IL-6 is a risk factor for early preterm delivery before 32 weeks.[26] Vogel *et al*.,[13] found that before 35 weeks, high maternal serum concentration of IL-6 was associated with an increased risk of spontaneous preterm birth. Also, Sorokin *et al*.,[26] in a large, multicenter, prospective trial revealed that elevated maternal serum concentrations of IL-6 and CRP were associated with preterm birth <32 weeks of gestation.

Data on the prediction of the response to tocolytic therapy in preterm labor women are limited in few studies; Kandil *et al*.,[27] evaluated abdominal electromyography in predicting the response to tocolysis in 40 pregnant women in preterm labor. They showed that abdominal electromyography had a specificity of 90%, PPV and NPV of 67% and 95%, and concluded that abdominal electromyography may predict the response to tocolysis in preterm labor. Ozden *et al*.,'s study[28] was performed prospectively on 204 pregnant women to determine the predictive value of transvaginal cervical measurements for evaluation of the response to tocolytic therapy. Authors found that the sensitivity, specificity, PPV, and NPV of the cervical length for prediction of preterm delivery were 80.9%, 72.2%, 87.2%, and 61.9%, respectively. In the present study, serum concentrations of IL-6, IL-8, and CRP can be used as suitable biomarkers for predicting the response to tocolytic therapy. However, these studies are different in methods and markers but show the possible way to predict the response to tocolysis in preterm labor.

Due to deficiency of researches carried out on the evaluation of the probable role of cytokines in the prediction of response to tocolytic therapy in preterm women and importance of prevention of preterm labor, the results of the present study can be noted as a basis for more other researches to find possible relations. Also, the main limitation of our study may be its small sample size in prediction of response to tocolytic therapy; whereas, 16 patients did not respond to the treatment compared to 59 patients responding to the treatment. Moreover, it is shown that increased cytokine levels are independent on inflammatory factors such as blood loss, anemia, and heavy infection and are a function of labor. IL-8 increases early during labor, possibly as a function of tissue stretching and membrane rupture. In addition, IL-6 showed the highest and most prolonged increase. IL-6 increase is also related to the strength of contractions and duration of labor. Therefore, the other limitation of our study is that we did not collect data on the strength of contractions and duration of labor that can affect IL-6 and IL-8 levels in pregnant women. We believe that these biomarkers must be assessed in further studies with a large sample size.

In conclusion, the findings of the present study demonstrated that the assessment of maternal serum concentrations of IL-6, IL-8, and CRP has good sensitivity and specificity and that they can be considered as suitable biomarkers for predicting the response to tocolytic therapy in pregnant women; however, this observation needs further studies to assess biologic markers as predictors, to a greater extent.

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