Ginekol Pol. 2013, 84, 368-372

Maternal serum interlukin-6 level in preterm labor

Interleukina 6 w surowicy krwi matki a poród przedwczesny

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Abstract

Objective: *The aim of this study was to measure interleukin-6 (IL-6) levels in maternal serum of women undergoing preterm labor without a clear infection.*

Materials and Methods: *Twenty two pregnant women with diagnosis of preterm labor who presented to the outpatient clinic of 19 Mayıs University Faculty of Medicine from July 2011 through December 2011 were enrolled in the study group. Twenty two healthy pregnant women who were at the same gestational age as the study group were selected as the control group.*

Results: *Gestational age in the study and control groups varied from 24 weeks and 4 days to 34 weeks and 6 days. In the study group, 11 patients (50%) underwent preterm birth. Pregnant women in preterm labor were compared to healthy pregnant women with regards to serum IL-6 levels. No significant difference was found in the IL-6 levels of maternal serum between the 2 groups.*

Conclusion: *In this study, we have shown that there is no increase in IL-6 levels in patients undergoing preterm labor without clinical or biochemical infection signs.*

Key words: **preterm labor** / **interleukin-6** / **preterm birth** /

Streszczenie

Cel: *Celem badania było określenie poziomu interleukiny 6 (IL-6) w surowicy krwi matki u kobiet, które przebyły poród przedwczesny bez oczywistej infekcji.*

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Otrzymano: **09.05.2012**
Zaakceptowano do druku: **10.04.2013**

Materiał i metoda: *Do badania włączono dwadzieścia dwie kobiety z diagnozą porodu przedwczesnego, które zgłosiły się do poradni przyszpitalnej przy Mayis University Faculty of Medicine w terminie od lipca 2011 do grudnia 2011. Grupę kontrolną stanowiły 22 zdrowe kobiety w takim samym wieku ciążowym jak grupa badana.*

Wyniki: *Wiek ciążowy w obu grupach mieścił się w granicach od 24 tyg+4 dni do 34 tyg+6 dni. W grupie badanej 11 kobiet (50%) przebyło poród przedwczesny. Kobiet ciężarne, które urodziły przedwcześnie były porównane z kobietami zdrowymi, pod względem poziomu IL-6 w surowicy. Nie znaleziono istotnych różnic w poziomie IL-6 w surowicy krwi pomiędzy obiema grupami.*

Wnioski: *Porodowi przedwczesnemu bez klinicznych i biochemicznych objawów infekcji nie towarzyszy wzrost poziomu IL-6.*

Słowa kluczowe: **poród przedwczesny** / **Interleukina 6** /

Introduction

Preterm labor is defined as labor starting after 20 weeks of gestation and before 37 weeks of gestation. Preterm birth complicates 12.7% of all pregnancies in the U.S. and is responsible for 75% of perinatal mortality [1, 2, 3, 4].

Labor involves cervical effacement and dilatation in conjunction with coordinated uterine contractions that are maintained until the fetus and placenta are delivered. Four principal mechanisms are emphasized with regards to the occurrence of preterm labor. The first highlights premature activation of normal physiological delivery mechanisms, while the second addresses excess uterine strain. The third and fourth mechanisms involve inflammation and decidual bleeding, respectively. The underlying cause of preterm labor is idiopathic in 50% of pregnancies [5]. At present, many tests are used for the diagnosis of preterm labor, since there are many causes in its etiology. Idiopathic cases of preterm labor are most common. However, excluding idiopathic cases, preterm labor can be related to various obstetric, medical, and anatomic disorders. Preterm labor is directly linked to 4 principal causes [5]:

1. İnduced delivery due to maternal or fetal indications or caesarean section before labor
2. Spontaneous idiopathic preterm labor without membrane rupture
3. Idiopathic preterm premature rupture of membrane (PPROM)
4. Twins or multi-fetal deliveries

Interleukin-6 (IL-6) is a protein which is encoded by the IL-6 gene [6]. IL-6 functions as both a pro-inflammatory and an anti-inflammatory cytokine. It is released by T cells and macrophages to stimulate the immune response during infection or following tissue damage, particularly burns, which leads to inflammation. The role of IL-6 as an anti-inflammatory cytokine is mediated by inhibitory effects on tumor necrosis factor (TNF)-α and IL-1 and activation of IL-10. It has been proposed that IL-6 plays a direct role in preterm labor [7]. Although the causes of preterm birth are multifactorial, inflammatory cytokines may have roles in infection, PPROM, and smoking, which are factors known to be associated with preterm birth [8, 9].

It is known that IL-6 activates white cells to invade the uterus, increases the expression of oxytocin receptors, and increases prostaglandins by activating the hypothalamic-pituitary-adrenal (HPA) axis [5].

Materials and Methods

1. Study Method and Patient Details

Twenty two pregnant women with diagnosis of preterm labor between 24 and 34 weeks of gestation who presented to the outpatient clinic or were hospitalized in the inpatient clinic of the Gynecology and Obstetrics Department of Ondokuz Mayıs University Faculty of Medicine were enrolled in the study group. Twenty two healthy pregnant women who presented to the outpatient clinic at the same gestational age were selected as the control group.

Education status was not taken into consideration. Pregnant women in the study and control groups were not different in terms of smoking status, alcohol consumption, or illicit drug addiction and did not suffer from cervical or uterine anomalies, heart disease, asthma, diabetes, liver disease, kidney disease, or connective tissue disease.

Diagnosis of preterm delivery was based on the occurrence of 2 uterine contractions in 30 minutes, in association with cervical change or significant effacement and dilatation and/or occurrence of symptoms such as pelvic compression, menstruation-like cramps, and lower back pain. Presence of contractions was identified in all pregnant women via manual follow-up or non-stress test (NST).

In the study and control groups, week of gestation was calculated according to last menstrual period in patients who clearly knew the dates of their last menstrual cycle and had regular menstrual cycles. Week of gestation was calculated with regards to the measurement of crown-rump length in patients who could not remember or were unclear about their last menstrual period. All pregnant women with final diagnosis of preterm labor were admitted for inpatient treatment in our clinic.

Pregnant women with PPROM with>4 cm cervical effacement, pre-eclampsia, eclampsia, ablatio placenta, placenta previa, chorioamnionitis, intrauterine growth retardation, intrauterine exitus, or Rh immunization were not included in this study. In addition, those with multiple fetuses, diabetes mellitus, hyperthyroidism, significant heart disease, prominent uterine myoma, significant cervical or uterine anomalies, human immune deficiency virus (HIV) or other significant infection, evident urinary infection or confirmed urinary tract infection, leucorrhea or other vaginal infection or vaginal drainage, or uterine sensitivity were excluded. Other exclusion criteria included fetal tachycardia (> 160 beats per minute), maternal tachycardia (> 100 beats per

minute), maternal leukocytosis (leukocyte count > 15,000 mm^3), intra-amniotic infection indicated by amniotic fluid culture, maternal body temperature exceeding 37.8°C, and patients aged below 17 years or above 45 years. Ethical approval was received from the ethics committee of Ondokuz Mayıs University and all participants gave informed signed consent.

Pelvic examination was performed using a sterile speculum (Simpson speculum) for all pregnant women in the study and control groups, and vaginal and cervical status was evaluated. A study information form was issued to all pregnant women and patient details (age, body weight, gravidity, parity, abortion, live children, examination findings, current medications, Bishop's score, vital findings, and laboratory examinations) were recorded.

2. Maternal Serum

Maternal serum samples were obtained from 7–8 mL of whole blood drawn with a 10 mL syringe via puncture of the antecubital veins at room temperature (24°C) in a sitting position and irrespective of fasting status. Whole blood samples were centrifuged at 5000 rpm for 5 minutes at 4°C within 1 hour of collection in order to separate serum after blood clotting. Serum samples were stored at -30°C to allow study once an adequate number of patients were enrolled.

3. IL-6 Immunoassay

The IL-6 levels of maternal serum samples were determined via solid phase enzyme amplified sensitivity immunoassay using a commercial kit (DioSource Europa SA, Nivelles, Belgium) in accordance with the procedure specified by the manufacturer. Enzymatic reaction was evaluated with an automated micro-plate reader. Results were expressed as pg/mL. The lower cut-off value of the IL-6 kit was 2.0 pg/mL. The inter-assay coefficient of variation (CV) was 4.4% and the intra-assay CV was 4.2%. Samples with high values were diluted and repeated.

4. Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 15.0 software. Data were expressed as mean and median. Inter-group comparisons were made with Student's t-test, Mann-Whitney test, Fisher's exact test, and Chi-square test. A P value below 0.05 was considered statistically significant.

Results

The age distribution of the study group ranged between 17 years and 40 years (median: 27.5 years), while the age distribution of the controls ranged between 22 years and 45 years (median: 30.5 years). In the study group, gestation age ranged from 24 weeks and 4 days to 34 weeks and 6 days. For the control group, gestational age was between 26 weeks and 5 days and 34 weeks and 6 days. There was a statistically significant difference between the study and control groups in terms of age and body weight, while there was no difference in terms of gravidity and gestational week. (Table I).

Parenteral fluid therapy was started in all 22 pregnant women in the study group. Of these 22 women, 13 (59%) were started on pharmacologic tocolytic therapy. Twelve of the 13 women (92%) had tocolysis with nifedipine, while 1 (8%) had tocolysis with ritodrine.

Of the 22 pregnant women with diagnosis of preterm labor in the study group, 11 (50%) underwent preterm delivery. Of these 11 women, 8 (72.2%) had caesarean section and 3 (27.8%) had spontaneous vaginal delivery. For the 8 pregnant women who underwent caesarean section, delivery was completed within 7 days in 5 women (62.5%), while it exceeded 7 days (8 days, 9 days, and 28 days) in 3 women (37.5%).

Considering the 3 pregnant women who had spontaneous vaginal delivery, 1 (33.4%) delivered within 7 days, while the delivery period exceeded 7 days (18 days and 28 days) in 2 women (66.6%). One pregnant woman in the study group had diagnosis of chronic hypertension without attacks and significant proteinuria.

As compared to the control group, the maternal serum levels of IL-6 in the preterm labor group were elevated. The mean maternal serum level of IL-6 was 47.0 ± 15.4 pg/mL in the preterm labor group and 23.6 ±1.6 pg/mL in the control group. Although maternal serum IL-6 levels were found to be higher in patients with preterm labor when compared to controls this difference was statistically insignificant (P > 0.05). (Table II).

Table I. Comparison of study and control groups in terms of maternal particularities.

	Study Group N=22	Control Group N=22	P (significance)
	Mean±SD (Median)	Mean±SD (Median)	
Age (years)	26.7±1.27 (27.5)	30.7±1.21 (30.5)	0.026*
Body weight (kg)	66.2±2.21 (68.5)	73.3±2.44 (70.5)	0.037*
Gestational week	32.5±0.61 (33.7)	31.6±0.53 (32.3)	0.095
History of preterm delivery	1 (%4.5)	0 (%0)	1
History of habitual abortus	2 (%9.1)		0.488

*p<0.05 is significant

Table II. Comparison of study and control groups in terms of maternal serum IL–6 and white blood cell count.

	Study Group N=22	Control Group N=22	P (significance)
	Mean±SD (Median)	Mean±SD (Median)	
Interleukin–6 (pg/mL)	47.0±15.4 (23.1)	23.6±1.6 (22.1)	**0.605**
White blood cell count (mm^3)	11937,2±769.81 (10675.0)	9526.3±493.2 (9950.0)	**0.024**

*p<0.05 is significant

Table III. Comparison of maternal serum IL–6 levels of patients in study group in terms of preterm delivery in one week.

	Preterm labor group delivered in one week after diagnosis of preterm labor (n=6)	Preterm labor group not delivered in one week after diagnosis of preterm labor (n=16)	P (significance)
	Mean±SD	Mean±SD	
İnterlökin-6 (pg/mL)	24.1±17.4 (17.1)	57.7±22.1 (23.1)	0.185
White blood cell count (mm^3)	11568.57±1421 (10480.0)	12109.3±945 (11700.0)	0.582

*p<0.05 is significant

Interestingly, comparison of maternal serum IL-6 levels in patients who delivered within 7 days and those who did not showed that, although statistically insignificant, patients who delivered within 7 days after the diagnosis of preterm labor had lower serum levels of IL-6 ($P > 0.05$). (Table III).

Discussion

Preterm delivery is still an important obstetric problem in both developed and developing countries. Preterm deliveries are a significant cause of neonatal mortality and morbidity. Despite efforts to prevent preterm delivery, difficulties in understanding the underlying pathology, insufficient diagnostic methods, and non-efficient treatment methods have led to an increase in preterm delivery rates over the last 2 decades in many developed countries [10]. In this study, serum IL-6 was studied and its relationship with preterm labor was examined.

While some have found significant association between many pro-inflammatory cytokines, including IL-6, and spontaneous preterm labor [11, 12, 13, 14, 15], others have found no such association [16, 17]. In a randomized, controlled and multi-center study involving 475 patients, it was determined that in patients with diagnosis of preterm labor in gestational weeks 24–32, elevated IL-6 and C reactive protein (CRP) levels causes increased risk for preterm labor and neonatal intra-ventricular hemorrhage. Moreover, for pregnant women complicated with preterm labor or PPROM, a consistent relationship was determined between elevated maternal serum IL-6, CRP, and matrix metalloproteinase (MMP)-9 levels [1].

In an infection-induced mouse model of preterm delivery, acute administration of recombinant IL-6 did not result in significant shortening of gestation and the authors concluded that IL-6 has a limited biological role in preterm labor [18]. In contrast to this study, by using IL-6-deficient animals, it was shown that IL-6 deficiency results in delayed normal timing of delivery [19]. In the present study, although maternal serum levels of IL-6 were found to be higher in patients with preterm labor, there was no statistically significant difference in serum IL-6 levels between the study and control groups. This finding may indicate that significant increases in serum IL-6 levels occur secondary to overt infection, since patients with clinical or biochemical infection signs were not included in the current study. Studies conducted on this subject in the literature state that IL-6 largely increases secondary to infection [9].

It has also been stated that women with maternal signs of infection have significantly higher IL-6 levels than women with no signs of infection [20]. In a systemic review on the association between inflammatory cytokines and the risk of spontaneous preterm birth in asymptomatic women, the authors concluded that IL-6 in cervicovaginal fluid and IL-6 and CRP in amniotic fluid but not in plasma were strongly associated with spontaneous preterm birth, suggesting that inflammation at the maternal-fetal interface, rather than systemic inflammation, may be important in the occurrence of preterm birth [21].

Conclusion

In conclusion, in patients with preterm labor not associated with infection, no significant relationship was identified between

IL-6 and preterm labor. Infection and/or inflammation are the only known pathological processes in the development of preterm labor and birth [22]. An increase in pro-inflammatory cytokines during fetal infection and fetal HPA activation are 2 important mechanisms in the etiology of preterm birth [9].

Further studies may be beneficial in order to determine whether inflammatory cytokines increase following primary inflammation and/or clinically overt infection. Better understanding of the role of inflammatory cytokines in preterm birth may lead to future development of treatment strategies to prevent the inflammatory activation involved in preterm birth.

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