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# Interleukin 6 in Maternal Serum as Marker of Bacterial Infection and Preterm Delivery

## ABSTRACT

**Introduction:** Preterm delivery remains a burning issue all over the world, especially in Serbia because of the rate of negative natural increase. IL-6 can stimulate the release of prostaglandins and cause premature contractions and premature labor.

**Aim of the Study:** The aim of this research is to examine the importance of infection in the occurrence of premature contractions and to examine whether the preterm labor is associated with increased concentrations of IL-6 in patients with intact fetal membranes.

**Patients and Methods:** We examined 83 pregnant women. The age range was between 15 and 43 years. The experimental group had 53 pregnant women and a control group was consisted of 30 pregnant women. All pregnant women had singleton pregnancies. The age of pregnancy in both groups was between the 21st and 35th week of gestation. The experimental group was divided into three groups according to the localization of infection: bacterial vaginosis, an infection of the cervix and urinary tract infections. The laboratory identification of IL-6 was performed as a double sandwich ELISA method. The reagents used for the identification of IL-6 were manufactured by Beckman-Coulter and were strictly intended for the research.

**Results:** There were 34 pregnant women or 64% with infection of the cervix, 26 pregnant women or 49% had a positive urine culture, while bacterial vaginosis was present in 47 pregnant women or 89%. A positive finding on all localization was found in 14 pregnant women or 26%. In the experimental group, IL-6 was detected in 37 pregnant women or 70%. The mean value of IL-6 proven in the experimental group was 20.6 pg/ml (SD=18.2, n=53).

**Conclusion:** This research demonstrated a direct link between a bacterial infection and preterm delivery. IL-6 can be used as a serological marker of bacterial infection and preterm delivery.

**Key words:** Interleukin-6, bacterial infections, premature birth, serologic tests

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## Introduction

Preterm birth is the end of pregnancy before the 37<sup>th</sup> week of gestation. Prematureness is an important single cause of neonatal morbidity, mortality and late consequences of survivors.<sup>1</sup> From an etiological point of view, one third of preterm births is caused by infection and premature rupture of the fetal membranes (PROM). One third is due to maternal and fetal factors and the last third, which according to some data goes up to 50%, is of unknown cause. There is evidence that an increase of IL-6 levels in amniotic fluid is associated with frequent spontaneous abortions, intrauterine fetal deaths and spontaneous premature births.<sup>1</sup> An increase of IL-6 levels is often the result of a subclinical infection of various genital tract localities. The infection is usually present for several weeks before the possibility of the onset of premature birth or unwanted complications.<sup>1</sup> Along with IL-6, the level of proinflammatory cytokines IL-1 and TNF- $\alpha$  in intrauterine infection and premature birth also increases in amniotic fluid.<sup>1,2</sup> The relationship between the two subpopulations of Th1 and Th2 cells (lymphocytes) affects the secretion of cytokines and the immune status of the pregnant woman.<sup>3-5</sup> Cellular immunity is mostly dependent on Th1 cells, while humoral immunity predominantly depends on Th2 cells.<sup>6</sup> At the beginning of pregnancy, the influence of Th1 cells is dominant. The balance exists in the second trimester, while the influence of Th2 cells is dominant before the end of pregnancy.<sup>3,7</sup> Th1 cells were involved in the pathogenesis of premature labor, preeclampsia and spontaneous abortion, while Th2 cells were predominantly due to successfully delivered pregnancy.<sup>8-10</sup> Proinflammatory cytokines were identified in amniotic fluid, maternal and fetal blood and vaginal fluid.

## Aim of the Study

The aim of this study is to demonstrate the correlation between the presence of infection (bacterial infections of the cervix, bacterial vaginosis and urinary infections) and increased level of interleukin 6 in the maternal serum. The second aim is to investigate whether the level of the measured values of interleukin 6 can be used in the detection of early intrauterine infection and premature birth, in pregnant women with intact fetal membranes.

## Patients and Methods

83 pregnant women participated in this research. In the experimental group, there were pregnant women (n=53) hospitalized due to the symptoms of premature labor in which during the hospitalization infection was found at least in one of the examined localizations: vaginal swab, cervical swab and urine culture test. The experimental group of pregnant women was divided according to the

localization of bacterial infection into three following groups:

1. Positive bacteriological findings of the cervical swab
2. Presence of bacterial vaginosis (Nugent's scoring system)
3. Positive urine culture

The control group consisted of healthy pregnant women (n=30) who were included in the research based on the identical initial criteria as pregnant women of the experimental group. They were hospitalized due to prenatal diagnosis indicated by geneticists, due to biochemical markers or data on the birth of children with genetic malformations in previous pregnancies. It had been confirmed that the karyotypes were normal.

Clinical treatment, microbiological and immunological examination of markers were carried out in hospitalized patients with proven infection in at least one of the examined localizations, then documented contractions of the uterus (at least three in ten minutes), but with intact fetal membranes and without progressive changes of the cervix. The gynecological and ultrasound examination (SonoAce X6, "Samsung Medison") was performed by the doctors at the Department of Pathology of Pregnancy, Clinic for Gynecology and Obstetrics Clinical Centre Vojvodina. The 5 ml of full vein blood was taken early in the morning without anticoagulants. Such blood was left to coagulate for 30 minutes, after which it centrifuged at 3000 rpm for ten minutes. The separated serum was frozen at -70<sup>o</sup>. It was kept up to the moment of the definitive laboratory processing of the examined series. The identification of interleukin 6 from the patient's serum was done by a double sandwich ELISA method, an open system apparatus. Reagents used to identify interleukin 6 were manufactured by Beckman & Coulter and were designed strictly for research. The values obtained for these parameters were determined according to the tables and values given by the producer. In the second phase of the study, obtained vaginal, cervical swabs and urine were processed to identify the present bacterial flora. The bacterial diagnosis was based on the microscopy and cultivation of the taken material by examining: Gram-positive, Gram-negative bacteria and fungi. Before sowing, a direct preparation was made and painted according to Gram stain procedure.

## Results

83 pregnant women were analyzed. There were 53 pregnant women in the experimental group who had an infection of at least one examined localization. Of these 53

patients, 34 or 64% were with cervical infection. Positive urine culture had 26 pregnant women or 49%, while 47 pregnant women or 89% had bacterial vaginosis. Two patients only had cervical infection, one patient had only positive urine culture, while 10 pregnant women only had bacterial vaginosis. However the most pregnant women had joint infections and infections present in more than one investigated localization. Three pregnant women had positive urine culture and a positive cervical swab. Eight patients had a positive urine culture and bacterial vaginosis. 15 pregnant women had bacterial vaginosis and positive bacteriological finding on the cervix. A total of 26 pregnant women had two positive findings of possible three different localities. 14 pregnant women or 26% had a positive finding in all localities.

The average age of all pregnant women was 28 years

(SD=6.2, n=83). For the experimental group, the average was 27 years (SD=6.1, n=53) while the control group was 29 years old (SD=6.2, n=30). The youngest pregnant woman in the experimental group was 15, while in the control group the youngest pregnant woman was 18 years old. The oldest pregnant woman in the experimental group was 43 years old, while the oldest pregnant woman in the control group was 40 years old. Between the groups, there was no statistically significant difference (p=0.247) in the age. (Table 1.)

The gestational age in both groups was between the 21 and 35 week.

The mean gestational age in the experimental group was 25 weeks (SD=3.9, n=53), while the mean age in the control group was 26 weeks of gestation (SD=5.1, n=30).

**Table 1. The average of the age of pregnant women and gestational age**

Groups	The average of the age of pregnant women						Gestational age					
	N	Average	SD	Min	Max	P t-test	N	Average	SD	Min	Max	P t-test
Experimental	53	27.4	6.1	15	43	0.247	53	25.2	3.9	21	35	0.270
Control	30	29.0	6.2	18	40		30	26.3	5.1	21	35	
Total	83	28.0	6.2	15	43		83	25.6	4.4	21	35	

The average gestational age for all pregnant women was 26 weeks (SD=4.4, n=83). (Table 1.)

The following bacteria of the cervical swab were identified as *Streptococcus agalactiae*, *Enterococcus*, *Staphylococcus aureus*, *Staphylococcus species*, *Citrobacter*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Positive bacteriological findings of the cervix were present in 28 pregnant women. Six pregnant women had *Candida albicans*. 34 pregnant women of the experimental group had a positive finding of the cervix. *Streptococcus agalactiae* and *Enterococcus* were present in eight pregnant women, *Staphylococcus aureus* in two, *Staphylococcus species* in three, *Citrobacter* in one, *Escherichia coli* in four, *Klebsiella pneumoniae* in, *Proteus mirabilis* in one, while *Candida albicans* was found in six pregnant women.

27 patients of this group did not have positive urine culture. According to the positive findings of urine culture, the following bacteria were identified: *Streptococcus agalactiae*, *Enterococcus*, *Staphylococcus species*, *Escherichia coli* and *Klebsiella pneumoniae*. A positive finding of urine culture was present in 26 pregnant

women, *Streptococcus agalactiae* in six, *Enterococcus* in four, *Staphylococcus species* in two, while *Escherichia coli* was present in 13 pregnant women. *Klebsiella pneumoniae* was found in one pregnant woman.

The average value for IL-6 in both groups was 16.7 pg/ml (SD=16.5, n=83). The lowest measured value was 3.61 pg/ml in both groups. The highest measured value for IL-6 of the experimental group was 84.6 pg/ml, while the maximum value for IL-6 of the control group was 15.0 pg/ml (Table 2.). Determination of 3 pg/ml means that the measurement method used was able to measure only the values of IL-6 which were greater than the detection threshold of 3 pg/ml.

Parameters which were measured had a large range of values and the distribution of the measured values did not have a bell curve arrangement. The standard deviation (SD) values in this study were extremely high. This can be explained by a small sample within the available resources for this research. According to the distribution shown in the analysis, the Mann-Whitney test method was used. Statistical analysis found that IL-6 values in the experimental group were significantly higher than

values in the control group ( $p=0.001$ ).

**Table 2. The average values of IL-6**

Groups	N	The value of IL-6				P Mann Whitney
		Average	SD	Min	Max	
Experi- mental	53	20.6	18.2	3.61	84.6	0.001
Control	30	7.33	3.72	3.61	15.0	
Total	83	16.7	16.5	3.61	84.6	

### Discussion

The most important risk factors for a preterm delivery are multiple pregnancies, previous premature births, bleeding after the first trimester of pregnancy and low Body Mass Index (BMI).<sup>10</sup> Results of Mijovic et al. showed that bacterial vaginal infection was statistically more commonly diagnosed in third trimester in women who gave birth in a preterm (66.7%), compared to women who gave birth in a term (29.9%).<sup>11</sup> Some studies indicate an increased risk of spontaneous preterm labor before the 34th week of gestation in pregnant women in which serum reduced values of IL-18 and elevated levels of IL-12 are found.<sup>12-14</sup>

Increased level of IL-6 in serum is associated with preterm delivery in Caucasians but not in the black population.<sup>15</sup> Large studies which have examined the vaginal flora indicate that bacterial vaginosis is more present in black pregnant women, even in the same degree of health care.<sup>16,17</sup>

Pregnant women over 35 years of age in the first trimester of pregnancy have an increased levels of IL-6 and TNF- $\alpha$  in serum, compared to women under 35 years of age.<sup>17,18</sup> IL-6 and IFN- $\gamma$  have not been associated with all preterm deliveries before 37th weeks of gestation but are associated with premature birth and pregnant women under the 35th week of gestation who have proven horioamnionitis.<sup>8</sup> It has been shown there are no significant differences of serum levels of interleukins in pregnant women who have repeated premature births.<sup>19</sup>

The data of several comparative studies of the level of circulating cytokines in the first and second trimester of pregnancy show contradictory results. Some studies have shown and demonstrated the increased levels of

IL-6 during pregnancy while others find no significantly increased level of IL-6 until the onset of delivery.<sup>16,20</sup> Many studies have attempted to identify a biomarker, a combination of multiple or clinical symptoms in order to facilitate the detection of pregnant women with an accompanying preterm delivery. If we want to overcome the above mentioned health problem successfully, it is necessary to analyze the existing approaches and methods in this field and to develop a wider multidisciplinary approach of research.<sup>21</sup>

Although studies show elevated values of different cytokines, the analysis of these results, which shows that the immune response in pregnancy is extremely complex, implies the interconnection of several different factors, which requires caution.<sup>22,23</sup> The premature opening of cervix without infection is basically a type of inflammatory reaction as well as cervical enlargement at the time of term delivery.<sup>24,25</sup> The differences that exist in different studies may be due to differences in a sample size. Comparison of different studies or level of cytokines may be limited by using different immunoassays, different population structure, laboratory methods and plasma or serum use.<sup>23</sup> Recent studies have similar results as the conducted research. Marconi et al. conducted a study in which they also demonstrated the correlation of preterm delivery with elevated values of IL-6.<sup>26</sup>

The influence of infection or other immune stimulants for cytokine levels is documented. There are still many unknown facts about how cytokine levels vary in pregnancy and depend on other maternal and fetal factors.

### Conclusion

This research demonstrated a direct link between bacterial infection and preterm delivery. IL-6 can be used as a serological marker of bacterial infection and preterm delivery.

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## Interleukin 6 u maternalnom serumu kao marker bakterijske infekcije i prijevremenog porođaja

### SAŽETAK

**Uvod:** Prijevremeni porođaj je aktuelni problem u cijelom svijetu, posebno u R. Srbiji zbog negativnog prirodnog priraštaja. IL-6 može stimulirati oslobađanje prostaglandina, te samim tim uzrokovati prijevremene kontrakcije i prijevremeni porođaj.

**Cilj rada:** Vidjeti značaj infekcije u nastanku prijevremenih kontrakcija, te ispitati da li je prijevremeni porođaj udružen sa povećanom koncentracijom IL-6 kod trudnica sa intaktnim plodovim ovojcima.

**Ispitanici i metode:** Istraživanje je izvedeno na 83 trudnice starosti između 15 i 43 godine, gde je 53 trudnice činilo eksperimentalnu grupu, a 30 zdravih trudnica, kontrolnu grupu. Sve trudnice su nosile jednoplodnu trudnoću, starosti između 21. i 35. nedelje gestacije. Eksperimentalna grupa je podijeljena u tri grupe prema lokalizaciji infekcije: bakterijska vaginoza, infekcija grlića materice i urinarna infekcija. Laboratorijska identifikacija IL-6 rađena je dvostrukom sendvič ELISA metodom, aparatom otvorenog sistema. Reagensi korišteni za identifikaciju IL-6, proizvedeni su od strane Beckman-Coulter, namijenjeni striktno za istraživanje.

**Rezultati:** Od 53 trudnice u eksperimentalnoj grupi bilo je 34 trudnice, odnosno 64%, sa infekcijom grlića materice, pozitivnu urino-kulturu imalo je 26 trudnica, odnosno 49%, dok je bakterijsku vaginozu imalo 47 trudnica, odnosno 89%. Pozitivan nalaz infekcije, na svim lokalizacijama, nađen je kod 14 trudnica, odnosno 26%. U eksperimentalnoj grupi IL-6 je dokazan kod 37 trudnica, odnosno 70%. Srednja vrijednost dokazanog IL-6 u eksperimentalnoj grupi iznosila je 20,6 pg/ml (SD=18.2, n=53).

**Zaključak:** Ovim istraživanjem dokazana je direktna povezanost bakterijske infekcije i prijevremenog porođaja. IL-6 se može koristiti kao serološki marker bakterijske infekcije i prijevremenog porođaja.

**Ključne riječi:** Interleukin-6, bakterijske infekcije, prijevremeni porođaj, serološki testovi