

# Assessment of IL-6 activity in cervico-vaginal mucus connected with cervical length concerning elderly pregnant women over 35 years of age at a risk of preterm delivery

## *Ocena aktywności interleukiny 6 w śluzie szyjkowo-pochwowym w zależności od długości szyjki macicy u kobiet powyżej 35. roku życia z ryzykiem porodu przedwczesnego*

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### Summary

Interleukin-6 is a pleiotropic cytokine with multidirectional influence on the cells of both innate and adaptive immune system. The main role of IL-6 is to initiate and regulate acute inflammatory response and adaptive immunity. It has a number of physiological effects. Overproduction of IL-6 causes a change from acute to chronic inflammation. Its higher concentration may initiate premature occurrence of preterm uterine contractions. Preterm delivery is the main reason for perinatal mortality and morbidity of neonates. Pro-inflammatory cytokines, mainly IL-1 and IL-6, which stimulate prostaglandin production are considered to be crucial reasons for preterm delivery.

**Key words:** interleukin-6, preterm delivery, assessment of uterine cervix.

### Streszczenie

Interleukina 6 jest cytokiną plejotropową oddziałującą wielokierunkowo na komórki odporności wrodzonej i nabytej. Najważniejszą rolą IL-6 jest inicjowanie i regulacja ostrej odpowiedzi zapalnej oraz ułatwienie rozwoju i ukierunkowanie odpowiedzi nabytej. Wykazuje wiele działań ogólnoustrojowych. Zwiększone wytwarzanie IL-6 powoduje przejście ostrej reakcji zapalnej w fazę przewlekłą. Jej wyższe stężenie może inicjować przedwczesne wystąpienie czynności skurczowej macicy. Poród przedwczesny stanowi główną przyczynę umieralności okołoporodowej oraz zachorowalności noworodków. Kluczową rolę w jego wyzwalaniu odgrywają prozapalne cytokiny, głównie IL-1, IL-6, które stymulują produkcję działających naskurczowo prostaglandyn.

**Słowa kluczowe:** interleukina 6, poród przedwczesny, ocena szyjki macicy.

### Introduction

Preterm delivery is recognised as the most important problem of contemporary obstetrics and it is the one of the most common reasons for perinatal mortality and morbidity. According to the definition, preterm delivery refers to the birth of a baby between 23<sup>rd</sup> and 37<sup>th</sup> week of gestation [2, 3, 6, 10]. The recent clinical and experimental examinations indicate that

infections and inflammations play the main role in preterm delivery. Intrauterine infection is known to be the most important reason for preterm delivery. It is mainly caused by the ascending infection originating from the endocervical canal and vagina. Researchers proposed four stages of development of this process. At the beginning, there is an excessive growth in the endocervical canal and vagina. It leads to the infection

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of decidua and then to chorionitis and the consequence of that is the infection of the amnion and the amniotic cavity as well as foetal vessels [7–9, 12]. The result of the aspiration of the infected amniotic fluid by the foetus is the congenital pneumonia. The presence of bacteria in its blood leads to bacteraemia and sepsis. In mechanisms that release the preterm uterine contractions the main role is played by bacterial metabolism's products, tissue macrophages, cytokines and prostaglandins [2, 9, 11–13]. Uterine contractions occur as a result of the increase in the level of intrauterine prostaglandins. Additionally, IL-6 stimulates activity of collagenases which may be found in the cervix and foetal membranes. The essential requirement of delivery at term and preterm delivery is the maturation of the cervix. It is connected with the inflammatory responses which suggest the participation of cytokines in this process [1, 9, 11, 12]. Prostaglandins stimulating contractions of uterine are released by proinflammatory cytokines, mainly IL-1 and IL-6. IL-6 is currently considered to be an early and sensitive, but not specific, indicator of inflammation. The value of a test in diagnosis and prediction of preterm delivery must take into account its diagnostic utility, clinical availability, possibility of rapid performance, short time to obtain the result and costs of performance. The activity of IL-6 was detected by Milenia QuickLine IL-6 tests. The results were read using a PicoScan device.

## Objective

The aim of the study was to indicate the activity of IL-6 in cervico-vaginal mucus connected with cervical length assessed by ultrasound in pregnancies at a risk of preterm delivery and in normal physiological pregnancies. The study also refers to the correlation between the level of IL-6 and the time of preterm delivery occurrence.

## Material and methods

The study included 80 primiparous and multiparous women over 35 years of age between 24<sup>th</sup> and 36<sup>th</sup> week of gestation. Singleton pregnancies were analysed only. Patients were hospitalized in the First Department of Obstetrics and Gynaecology of the Medical Centre of Postgraduate Education in Warsaw between 2008

and 2010. The study group consisted of 50 women whose pregnancies were at risk of preterm delivery. Threatening preterm delivery was defined as uterine contractions lasting at least 60 seconds, occurring at a rate of 1 or more every 10 minutes and detected by CTG. These uterine contractions led to cervical shortening and dilatation. Cervix was measured by ultrasound. The control group consisted of 30 women with uncomplicated pregnancy between 24<sup>th</sup> and 36<sup>th</sup> week of gestation. The inclusion criteria during this study were as follows: (1) gestational age between 24<sup>th</sup> and 36<sup>th</sup>, (2) uterine contractions detected by CTG, (3) singleton pregnancy, (4) amniotic sac intact, (5) patients older than 35. The exclusion criteria included: (1) plural pregnancy, (2) presence of amniotic fluid leakage, PPRM, (3) cervical cerclage, cervical pessary, (4) abnormal bleeding, (5) foetal abnormalities, (6) patients younger than 35. The research material was collected during uterine contractions. The cervico-vaginal mucus was obtained from the posterior vaginal vault using sterile Rayon applicators. Afterwards, the cervical length was measured by means of ultrasonography. IL-6 activity was determined by Milenia QuickLine IL-6 tests. The results were read by means of a PicoScan device and they were statistically analysed then using non-parametrical test Mann-Wilcoxon-Withney.

## Results

With reference to the control group, the activity of IL-6 in cervico-vaginal mucus collected from the posterior vaginal vault was higher in the study group. The results were included into three groups. The first group constitutes the control group's results. The second comprised patients suffering from threatening preterm delivery with IL-6 activity between 50 and 100 pg/ml. The last group included patients whose IL-6 activity was over 100 pg/ml. All patients were examined using ultrasound to assess the cervical length after material collection. The activity of IL-6 was under 50 pg/ml in the control group. The mean cervical length measured from internal to external orifice was 38.4 mm. In all cases (30 women, 100%) the delivery occurred at term. The IL-6 activity between 50 and 100 pg/ml was found in 36 women (72%) of the study group. The mean cervical length in this group was 25.7 mm. Apart from tocolytic

Tab. I. Study results

|  | Control group         | Study group (I) 36 women | Study group (I) 36 women |
|--|-----------------------|--------------------------|--------------------------|
| IL-6 activity  | < 50 pg/ml (negative) | 50–100 pg/ml             | > 100 pg/ml              |
| mean cervical length (USG)   | 38.4 mm               | 25.7 mm ( $p < 0.05$ )   | 18.6 mm ( $p < 0.01$ )   |
| percentage of preterm delivery even with administration of tocolytic treatment | 0%                    | 14%                      | 28%                      |

treatment (beta-2-mimetics, Mg<sup>2+</sup>) for 14 days, 5 women delivered a foetus before the term. This constituted 14% of this group and 10% of the study group. The activity of IL-6 over 100 pg/ml was observed in 14 women, which constituted 28% of the study group. The mean cervical length in this group was 18.6 mm. Within 14 days in 4 cases (28%) the tocolytic therapy was finished with failure and preterm deliveries were observed.

## Discussion

There are many papers which underline the connection between proinflammatory cytokines and preterm delivery. The result of our study confirms that IL-6 activity measured in cervico-vaginal mucus is significantly higher in the case of preterm delivery [3, 4, 7, 9]. Actually, it is extremely difficult to find any publications about the assessment of IL-6 activity in cervico-vaginal mucus in pregnant women over 35 years old. The 1990s brought several publications about the connection between the high level of IL-6 in cervico-vaginal mucus and preterm delivery. Lackwood et al. reported the activity of IL-6 in cervico-vaginal mucus of 161 pregnant women in 24<sup>th</sup> to 36<sup>th</sup> week of gestation [2]. They proved that IL-6 activity above 250 pg/ml was connected with a higher rate of preterm labours. Lange et al. analysed activity of IL-6 in 31 pregnant women. In all cases, IL-6 activity under 20 pg/ml was combined with the delivery at term [4]. In the recent publications Brik et al. [1, 6, 12] described the connection between IL-6 activity, cervical length examined using USG and preterm delivery. It was shown that frequency of preterm labour was significantly higher if the IL-6 activity was over 210 pg/ml and cervical length was less than 30 mm. In 35% of cases, the delivery occurred before 37<sup>th</sup> week of gestation and in 5% of cases – before 32<sup>nd</sup> week of gestation. In 12% of cases the cervical length was estimated to be under 15 mm and in 62% of cases – under 30 mm. Taking into consideration the fact that

there is an obvious correlation between preterm delivery and IL-6 activity, it is necessary to create a simple test which allows to assess IL-6 activity in a quick, accurate and reliable way [1, 3, 5, 8]. Without doubt, cervico-vaginal mucus can be treated as such material. The test satisfying the above mentioned requirements is a non-invasive Milenia test used in our study.

## References

1. Brik M, Pilar A, Perales-Puchalt A, et al. Cervical interleukin-6 as a predictive test for preterm delivery in symptomatic women: preliminary results Original Research Article, *European Journal of Obstetrics & Gynecology and Reproductive Biology*, In Press, Corrected Proof, Available online 4 December 2010.
2. Lockwood C. The diagnosis of preterm labor and the prediction of preterm delivery. *Clin Obstet Gynecol* 1995; 38: 675-87.
3. Lockwood C, Ghidini A, Wein R, et al. Increased interleukin-6 concentrations in cervical secretions are associated with preterm delivery. *Am J Obstet Gynecol* 1994; 171: 1097-102.
4. Lange M, Chen F, Wessel J, et al. Elevation of interleukin-6 levels in cervical secretions as a predictor of preterm delivery. *Acta Obstet Gynecol Scand* 2003; 82: 326-9.
5. Romero R, Mazor M, Brandt F. IL1 and IL1beta in preterm and term human parturition *Am J Reprod Immunol* 1992; 22: 117-23.
6. Torbe A. Maternal plasma procalcitonin concentrations in pregnancy complicated by preterm premature rupture of membranes. *Mediators Inflamm* 2007; 35782.
7. Romero R, Erez O, Espinoza J. Intrauterine infection, preterm labor, and cytokines. *J Soc Gynecol Investig* 2005; 12: 463-5.
8. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in preterm newborn. *Pediatr Res* 1997; 42: 1-8.
9. Menon R, Fortunato S. Fetal membrane inflammatory cytokines: a switching mechanism between the preterm premature rupture of the membranes and preterm labor pathways. *J Perinat Med* 2004; 32: 391-9.
10. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *The Lancet* 2008; 371: 75-84.
11. Burrus DR, Ernest JM, Veille J-C. Fetal fibronectin, interleukin-6, and C-reactive protein are useful in establishing prognostic subcategories of idiopathic preterm labor Original Research Article. *Am J Obstet Gynecol* 1995; 173: 1258-62.
12. Blank E, Hirsch JRG, Challis R, et al. Cytokine Signaling, Inflammation, Innate Immunity and preterm Labour – A Workshop Report Original Research Article *Placenta* 2008; 29 (Suppl 1): 102-4.
13. Steinborn A, Von Gall C, Hildenbrand R, et al. Identification of placental cytokine-producing cells in term and preterm labor Original Research Article *Obstetrics & Gynecology* 1998; 91: 329-35.