

**University of Medicine and Pharmacy of Craiova**

**PhD THESIS  
-ABSTRACT-**

**SEXUALLY TRANSMITTED INFECTIONS CAUSED BY  
CHLAMYDIA, UREAPLASMA AND MYCOPLASMA -  
IMPLICATION IN THE PRETERM BIRTH**

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**KEY WORDS:** premature birth, vaginal infection, interleukin 6, fetal fibronectine, length of the cervical channel.

## INTRODUCTION

The premature birth defined by the WHO in 1961 as being the birth under 37 weeks of gestational age, affects 7-13% of the pregnancies, fact which has led to the attempt of elaborating efficient prevention programs (1, 2).

Premature birth (PTB) is one of the important problems in obstetrics and modern medicine due to its special impact on the neonatal mortality and morbidity as well as for the complications developed by children born without congenital malformations and the long-term consequences on the health of the population.

The short term complications include: respiratory distress syndrome, bronchopulmonary dysplasia, fetal inflammatory response syndrome, intraventricular haemorrhage, retinopathy of prematurity, anemia of prematurity, necrotizing enterocolitis and other organic dysfunctions. The long term complications include: cerebral palsy and behavioral and psychological problems (3).

The modification of the normal vaginal flora with pro-inflammatory microorganisms leads to the initiation of a cascade of events with the risk of premature birth. The genital mycoplasmas (*Mycoplasma hominis* and *Ureaplasma* spp.) are suspected for the development of these pathological conditions (4). The vaginal infection is the single pathological process proved to be directly correlated with the premature birth (5). The ascending infection of the genital tract has a major impact both on the prematurity at birth but also on neonatal complications. So the production of inflammatory mediators such as interleukin 6 (IL-6) is stimulated, the major modulator of the response of the host towards the infection and/or tissue aggression (5). Even the functional polymorphism of the interleukin 6 gene is a risk factor for cerebral palsy (6).

The inflammation in fetal territory is connected to the debut of the labour in patients with ascending intrauterine infection, especially with *Chlamydia trachomatis* (Chl), *Mycoplasma hominis* or *Ureaplasma* spp. (My/Uu) (7).

## **OBJECTIVES**

The aim of the study was to investigate a possible relation between the premature birth and the presence of inflammation in the genital territory caused by the modification of the vaginal and cervical flora.

Another aim of the study was the evaluation of IL-6cv as a component of the immune response, in pregnant women with premature birth risk compared to a normal pregnancy. In this sense, I observed a relationship between the levels of interleukin 6 from the cervical-vaginal fluid (IL-6cv) and the presence of *Mycoplasma hominis*, *Ureaplasma spp.* and/or *Chlamydia trachomatis*, as well as their association in the case of women with premature birth risk. I also pointed a relationship between the levels of interleukin 6 in the cervical-vaginal fluid, the fetal fibronectin (fFN) and the length of the cervical channel (LCC), in women with premature birth risk and the superiority of testing IL-6cv at 24 hours, as an independent predictor of PTB.

Originality of the study was the determination of IL-6 in the cervical-vaginal fluid 24 hours after the sampling, which was not found in any other published study in the reference literature.

The novelty of this paper was to study the most important microorganisms that may be involved in premature birth and the correlation with IL-6cv growth, which was not found in literature.

I also proposed as screening tests the identification of *Chlamydia trachomatis* and genital mycoplasmas in pregnant women with a premature birth risk.

## **MATERIAL AND METHODS**

The observational clinical study was performed in Obstetrics and Gynaecology sections of the Clinical Emergency Hospital No.1 and the Municipal Hospital Filantropia Craiova in the period 17 November 2009/30 July 2012 and included a number of 262 women (of whom 106 were pregnant women with a gestational age between 22 and 34 weeks); samples

from the endocervix were collected in order to investigate the infection etiology and to determine the IL-6 cv.

The studied patients were divided into two groups:

- I. A control group which included 40 pregnant women with an average age of 26,8 years old (age interval 18-36 years old);
- II. A group of 66 pregnant women with an average age of 32,3 years old (age interval 18-40 years old) with detected premature birth significant risk factors: presence of bacterial vaginitis/ vaginosis, history of spontaneous abortion or history of premature birth.

The 66 pregnant women group was divided into two subgroups:

- a. First subgroup – with risk factors consisting of antecedents +/- bacterial vaginitis/ vaginosis (35 patients);
- b. Second subgroup - without risk factors consisting of antecedents, but presenting bacterial vaginitis/ vaginosis (31 patients).

Samples were collected from the endocervix for infection aetiology investigation (Chlamydia trachomatis, anaerobic germs, cultivation, identification, quantitative assessment and testing the susceptibility to antibiotics of Ureaplasma spp., Mycoplasma hominis, Streptococcus B, Escherichia coli, Candidosis) and to determine the level of IL-6cv.

Samples were also collected from the endocervix in order to determine the IL-6 and fFN in the cervical-vaginal fluid. The samples were preserved to 37°C in a thermostat, for 24 hours after the first determination of IL-6, in the same recipient each sample was transported in.

Each participating patient gave her informed consent regarding the non-invasive character and the purpose of this study.

The study was mainly focused on the group of pregnant women with a modified vagina flora in order to correlate the level of IL-6cv with the presence or association of some bacterial species in of genital infections.

To prove the superiority of IL-6cv testing after 24 hours from collecting the sample as an independent predictor of PTB, I studied a number of 64 patients out of which 36 pregnant

women with PTB risk were selected after a previous evaluation through the determination of IL-6 and fFN levels in the cervical-vaginal liquid, the vaginal secretion examination and trans-vaginal ultrasound for measuring the length of cervical channel in the single pregnancy.

Inclusion criteria:

- Monofetal pregnancy;
- Gestational age certified through ultrasound evaluation during the first 16 weeks with the measurement of cervical channel length (CLL) during weeks 7-13 and/or of biparietal diameter (DBP) and the length of the thigh-bone between 13- 16 weeks;
- Absence of fetal morphological abnormalities and of maternal genital organs abnormalities;
- Informed consent regarding the participation to the study.

To indicate the superiority of IL-6cv testing after 24 hours, as an independent predictor of PTB, I have chosen a group of 36 pregnant women with an average age of 30,6 years old (between 18-40 years old) with 2 or more significant risk factors for premature birth detected: vaginal bleeding during pregnancy; premature contractions; previous records of spontaneous miscarriage; length of the uterine cervix <20 mm; fFN positive test (>50 ng/ml); IL-6 levels in the cervical vaginal fluid >58 ng/ml; presence of My/Uu and/or Chlamydia in the urogenital tract.

The followed risk factors for spontaneous premature birth were: the presence of fetal fibronectin (>50ng/ml); the presence of My/Uu and/or Chlamydia in vaginal secretion; the growth of IL-6cv above the cut-off of 58 ng/ml; LCC under 20 mm.

Exclusion criteria:

- Multiple pregnancies or abnormal foetuses
- Interruption of the pregnancy before the 24th week for reasons which exclude the infectious pathology;
- Performing the genetic amniocentesis;

- Prophylactic cervical cerclage performed before the 14th week;
- Elective premature birth due to medical recommendation or obstetrical complications of any other cause than infectious.

In order to identify the germs I used:

- Test for the identification of fetal fibronectin - Adeza™ Fetal Fibronectin Kit;
- Test for the diagnosis of My/Uu from the urogenital tract (cultivation, identification, quantitative estimation, susceptibility testing to antibiotics) - Mycoplasma IST 2 / Mycoview. The antibiogram simultaneously provided results for the identification, quantitative estimation and susceptibility testing of My/Uu to 11 antibiotics.
- Test for fast diagnosis of Chlamydia from the cervical-vaginal liquid - The QuickVue Chlamydia / “Diaquick” Chlamydia test;
- Kits for the identification of Interleukin 6 from pathological products - Elecsys IL-6 test (ECLIA method– Roche) and Human IL-6 (ELISA method– Invitrogen).
- Transvaginal ultrasound transmitter of Medison Sonoace 8000 for the LCC measurement.
- The microbial associations were isolated through enriched and selective environments, the antibiogram being performed only for the aerobic bacterial species.

#### Statistical analysis

The description of categorial data (frequency, average, standard deviation) and differences between groups environments (Student test), the correlation analysis and the chi-test were performed with the following software program packages: SPSS v.14.0 for Windows, Epi Info v. 3.3.2 and Microsoft Excel. The data correlations and comparisons were considered statistically significant at  $p < 0,05$ .

## **RESULTS AND DISCUSSIONS**

The genital tract of pregnant woman suffers a series of inflammatory reactions which will initiate the labour both in premature birth and in spontaneous full term birth.

Recent observations show that during pregnancy, the inflammatory cells invade the chorion and amnios thus being a source of inflammation mediators.

### **Vaginal infection caused by Chlamydia, Mycoplasma and Ureaplasma with implication in premature birth**

In this study I found out that, there is a possibility that the stimuli produced by certain bacteria, (specially mycoplasmas) will create inflammatory reactions in the pregnant uterus by releasing some cytokines, thus initiating a cascade of events eventually finalised with PTB (8).

My/Uu and Chl are all bacteria involved in urogenital and respiratory tract infections, being differentiated by the “in vitro” way of cultivation: acellular specific environments for My/Uu, methods using cellular cultures for Chl, but the tetracycline, macrolids, lincosamid, streptogramins and fluoroquinolones act on both microorganisms.

The presence of My/Uu and Chl, but especially their association with subgroups 1 and 2 has shown significantly higher ( $p < 0,05$ ) IL-6cv levels as compared to the control group . Comparing the two subgroups we can observe that the average value of IL-was higher for the subgroup 1, which possibly indicates that the persons with a risk factor in their history develop a higher predisposition towards a premature birth. Thus the question whether the intrauterine infection might be considered a chronic process must be considered.

We noticed that the presence of the Mycoplasma hominis, Ureaplasma spp. and /or Chlamydia trachomatis in the group of women with premature birth risk significantly increases IL-6cv level, thus being incriminated in the pathogenesis of the premature birth.

There were identified other bacterial species incriminated in the development of the bacterial vaginosis but the IL-6cv values did not have any statistical significance compared to the control group, these results being similar to the data also found in the reference literature (9).

In the case of the studied group, it is worth mentioning the frequent bacterial colonisation with a B group streptococcus (35.9%) among the microbial agents identified in the vaginal infection, followed by Ureaplasma spp. (19,23%), Enterococcus (12,82%),



*Mycoplasma hominis*, *Chlamydia trachomatis* and *enterobacteriaceae*, each with 6,41%. The B group streptococcus (35,9%) is the cause of invasive infections in newborn through a vertical transmission from mother to foetus after the rupture of the membranes or the start of the labour (10, 11).

I evaluated the sensibility to antibiotics of the identified My/Uu. The studied bacterial strains have manifested an increased resistance to fluoroquinolone and azithromycin and maximum sensibility to streptogramins.

Fluoroquinolones act by blocking the topoisomerase IV and DNA-gyrase, needed in the whirling of bacterial chromosome. The resistance to fluoroquinolone was described for urogenital species of My/Uu through the emergence of mutations in the regions which codify the genes of DNA-gyrase and topoisomerase IV enzymes. (12). The multiple resistance to antibiotics was explained by the active efflux phenomenon on the level of the bacterial membrane through the efflux pumps (13). The resistance to macrolids was given to the mutations of the ribosomal proteins L4 and L22 of the subunit 50S and of the mutation in the position C2243N in ARNr 23S (14). Pereyre et al. issued the hypothesis that several mutations present both in the ARNr subunits and also at the level of the ribosomal proteins are necessary conditions for the emergence of the increased resistance to macrolids (15, 16).

My/Uu present a natural resistance to several antibiotics, like the betalactamines and glycopeptides. The acquired resistance of the My/Uu and Chl to macrolids and fluoroquinolones is mainly caused by the abusive or incorrect use, which leads to therapeutic failure.

### **The value of interleukin 6 in preterm labor**

In the case of pregnant women with premature birth record or spontaneous miscarriage and the ones with a modification of the vaginal flora, IL-6 levels and the presence of the fetal fibronectin in the cervical vaginal fluid after the 24th week of pregnancy were determined, also providing prenatal assistance and supervising these high risk pregnancies until the birth.

In order to determine the IL-6 in the cervical vaginal fluid I proposed as a cut-off level the value of 58 ng/L, level above which the probability of a premature birth is significantly higher from a statistical point of view. In the case of these patients, a high specificity of testing was obtained when the length of the cervix was under 20 mm and the fibronectin test was positive, data also found in the referenced literature (17).

At the same time, due to the high sensitivity of the group with high risk factors for PTB (95%), the determination of IL-6cv at 24 hours from sampling can be used as a screening test.

The obtained results are according to the ones in the referenced literature. After a close observance of the LCC and of IL-6cv in the case of patients with a premature birth, Kurkinen et al. showed that only the determined IL-6 in the cervical vaginal fluid has a tendency to grow (18).

In the presence of bacterial vaginosis, the increase of IL-6cv was correlated with the growth of fFN, test used in order to assess the premature labour.

At present, the tissues responsible for the increased production of IL-6 are not fully known. This cytokin plays an important role in the defense mechanism of the host, being involved in recruiting the lymphocytes T in the inflammatory place, by increasing the adhesion between the endothelium and the lymphocyte (19).

The IL-6 level grows significantly after 24 hours from the sampling of the cervical vaginal fluid, before the clinical symptoms of labour are present and fFN becomes positive, due to the release of proinflammatory cytokines from the cells on the surface of the vaginal epithelium.

For the group of studied patients, the value of IL-6cv at 24 hours was the strongest predictor of the premature birth with an Odds Ratio of 21.86, CI95% 5.63-84.9.

Therefore I have observed that the IL-6 provides a connection to the mechanisms between the inflammatory stimulus and the delivery one, being a key cytokin in this process.

In the case of these pregnant women, we have to mention the frequent colonisation with *Ureaplasma* spp. (35.9%) identified among the microbial agents in the vaginal secretion,

followed by the B group streptococcus. We still do not know why the Ureaplasma invades the amniotic cavity only in the case of certain women. Several studies suggested that the Ureaplasma species such as U.parvum and U. urealyticum are different from the point of view of pathogenicity (20, 21, 22).

The IL-6cv values were significantly higher in pregnant women with a vaginal infection with Chlamydia, My/Uu ( $p < 0,05$ ) as compared to the control group. The high level of IL-6cv in the presence of the Mycoplasma, Ureaplasma, Chlamydia species and especially their association increases the risk of premature birth (23).

## **CONCLUSIONS**

Bacterial vaginosis/ vaginitis represents important etiopathogenic factors of the premature birth and the modern approach has to include the investigation and a quick treatment of the pathological condition within the risk population.

In the presence of some bacterial stimuli induced by Chl, My/Uu, there is a direct influence of the IL-6 production in the cells of the vaginal epithelium, a key cytokine of acute phase which acts as a major modulator of the response of the organism to infection.

The consequences of the infection with these microorganisms in the case of a pregnant woman should amplify the focus of obstetricians and neonatologists on this problem for the benefit of the mother and the conception product.

Interleukin 6 plays a very important role in the maturation process of the cervix, thus being considered a biomarker for the estimation of the premature birth.

IL-6cv and fFN are non-invasive tests which should be frequently used in the medical practice in order to avoid the immediate and late neonatal complications.

The diagnosis methods of genital infections (Chl, My/Uu) incriminated in the pathogenesis of premature birth might bring real benefits through a more adequate selection of the patients which are going to benefit from the antimicrobial treatment.

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