

## CLINICAL STUDY

IL-1 $\beta$ , IL-6 and IL-8 levels in gynecologic infectionsBEATRIZ BASSO<sup>1</sup>, FRANCISCO GIMÉNEZ<sup>2</sup>, & CARLOS LÓPEZ<sup>3</sup><sup>1</sup>Laboratorio de Microbiología, Cátedra de Clínica Pediátrica, <sup>2</sup>Cátedra de Obstetricia, and <sup>3</sup>Cátedra de Ginecología, Hospital Universitario de Maternidad y Neonatología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina

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**Abstract**

**Objective.** During pregnancy cytokines and inflammatory mediators stimulate the expression of prostaglandin, the levels of which determine the onset of labor. The aim of this work was to study interleukin IL-1 $\beta$ , IL-6 and IL-8 levels in the vaginal discharge, serum and urine of pregnant women with genitourinary infection before and after specific treatment. One hundred and fifty-one patients were studied during the second or third trimester of their pregnancy.

**Methods.** The selected patients were: healthy or control group ( $n = 52$ ), those with bacterial vaginosis ( $n = 47$ ), those with vaginitis ( $n = 37$ ), those with asymptomatic urinary infection ( $n = 15$ ) and post-treatment. The level of cytokines was assayed by ELISA test. The Mann–Whitney U-test was used for statistical analysis.

**Results.** The IL-1 $\beta$  levels in vaginal discharge were: control  $103.5 \pm 24.2$  pg/ml, bacterial vaginosis  $1030 \pm 59.5$ , vaginitis  $749.14 \pm 66.71$  ( $p < 0.0001$ ), post-treatment  $101.4 \pm 28.7$ . IL-6 values were similar in both control and infected groups, and there were no patients with chorioamnionitis. In vaginal discharge IL-6: control  $14.2 \pm 3.9$  pg/ml, bacterial vaginosis  $13.2 \pm 3.8$ , vaginitis  $13 \pm 4.2$ . IL-8 levels were: control  $1643 \pm 130.3$  pg/ml, bacterial vaginosis  $2612.7 \pm 257.7$ , vaginitis  $3437 \pm 460$  ( $p < 0.0001$ ), post-treatment  $1693 \pm 126.6$ . In urine the results were: control  $40.2 \pm 17$  pg/ml, asymptomatic urinary infection  $1200.7 \pm 375$  ( $p < 0.0001$ ). In patients with therapeutic success both IL-1 $\beta$  and IL-8 returned to normal levels.

**Conclusions.** Genitourinary infections induce a significant increase in IL-1 $\beta$  and IL-8 levels in vaginal secretions, and IL-8 in urine as well. Both cytokines could be useful as evolutive markers of infection.

**Keywords:** Cytokines, pregnant women, bacterial vaginosis, vaginitis, urinary infections

**Introduction**

During pregnancy, inflammatory mediators, such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) directly stimulate the expression of prostaglandin, the levels of which determine the onset of labor. These cytokines, together with IL-8, activate the secretion of IL-6 by deciduas and chorionic membrane cells encouraging the membranes to rupture. It has been demonstrated that during labor, cervical dilatation is associated with an increase in IL-1 $\beta$  and IL-8 concentrations in the lower uterine segment [1]. Meanwhile, it has been observed at experimental level that in normal conditions, the cervix fibroblast is responsible for IL-8 synthesis [2,3]. On the other hand, the cytokine production of immunocompetent cells such as macrophages, lymphocytes, natural killer, and other cells plays an

essential role in the course of infection, being responsible for the onset and maintenance of the immune response to the aggressor agent [4,5]. Moreover, innate immunity, especially the cytokines, plays an important role in the maintenance of pregnancy and in the regulation of the onset of labor, as well as in the cause of spontaneous abortion, either caused by infection or by other factors. It has also been demonstrated that vaginal infections during pregnancy produce complications that include pre-term onset of labor, premature labor, premature rupture of the membrane, chorioamnionitis and endometritis. Also associated with bacterial vaginosis are pelvic inflammatory disease, post-abortion inflammatory disease and mucopurulent cervicitis. Urinary infections are also common, with complications associated to preterm onset of labor, pyelonephritis, and sepsis [6]. These situations

can produce a rise in perinatal morbidity/mortality, with development of meningitis, respiratory distress syndrome, neonatal sepsis, or necrotizing enterocolitis and intraventricular hemorrhage [7].

It has been observed that pregnant women with genitourinary infections are more likely to have premature rupture of the membrane or pre-term labor [8]. As a consequence of this observation, the purpose of this work was to study the IL-1 $\beta$ , IL-6 and IL-8 levels in pregnant women with genitourinary infections, and to analyze their potential use as evolutive markers.

## Subjects and methods

### Patients

One hundred and fifty-one pregnant women in the second or third trimesters pregnancy were selected when they came to a control or medical checkup at the Hospital Obstetrics Department. All the patients gave a written consent to participate in the research protocol.

The patients were classified into the following groups by clinical examination and laboratory diagnosis: healthy pregnant women (control group, **C**)  $n = 52$ , pregnant women with bacterial vaginosis (**BV**)  $n = 47$ , pregnant women with vaginitis (**V**)  $n = 37$ , and pregnant women with asymptomatic urinary infection (**AUI**)  $n = 15$ .

### Treatment

Patients with infections were exposed to a 10-day treatment with metronidazol suppositories for bacterial vaginosis and parasitic vaginitis, and ketoconazol suppositories for mycosis. For urinary infections, the selected antibiotic depended on the antibiotic sensitivity test results (ampicillin, amoxicillin and first or second generation cephalosporins). The cytokine levels of these patients were also studied (post-treatment, **PT**).

### Urine cultures

Urine cultures were performed with colony count and, in the case of urinary infections, microorganism identification was carried out by API Test (a system to identify *Enterobactereaceae* Gram-negative rods and Gram-positive cocci that uses 21 standardized biochemical tests) and antibiotic sensitivity test (Mueller–Hinton agar).

### Vaginal discharge tests

Bacteriological and mycological studies: Gram-staining of vaginal discharge smears was performed for the diagnosis of bacterial vaginosis or vaginitis

(mycosis) in the pregnant women. Aerobic and anaerobic culture media were also inoculated.

Parasitological studies: Gram-staining and fresh examination between slide and cover-slide were made to observe the morphology and mobility of *Trichomona vaginalis*.

A second sample was taken from patients who returned to control status upon completion of the therapeutic treatment.

### Samples for cytokine determination

For serum, venous blood was drawn into sterile tubes, and centrifuged at 600 g for 10 min. The serum aliquots were frozen at  $-70^{\circ}\text{C}$ . Urine was obtained in a sterile way and aliquots were frozen at  $-70^{\circ}\text{C}$ . Vaginal discharge was drawn into sterile tubes with 2 ml sterile phosphate buffered saline (PBS) pH 7.2 and kept at  $-70^{\circ}\text{C}$  until use.

### Cytokine determination

The IL-1 $\beta$ , IL-6 and IL-8 determinations were carried out by enzyme-linked immunosorbent assays (ELISA), using ICN Biomedical Inc. kits, following the protocol instructions. Briefly, immunoplates coated with monoclonal antibodies to the studied cytokine were incubated with samples and standards. After washing, a biotin-conjugated polyclonal anti-serum to each cytokine was added; the plates were then were incubated and washed. Next, avidin-peroxidase was added and, following incubation and washing, the color reaction was developed with tetramethyl bencidine.  $\text{SO}_4\text{H}_2$  was added as a stopper and absorbencies were measured at 450 nm by means of an ELISA plate reader. Values were calculated from standard curves based on prepared dilutions of recombinant cytokines.

### Statistical analyses

The Mann–Whitney U-test was used to determine the statistical significance in cytokine levels comparing the control group with the other groups. A  $p$  value of  $< 0.05$  was considered to be significant. A ROC curve was use to determine the relationship between sensitivity (true positive rate) and specificity (true negative rate) of diagnostic tests [9].

## Results

In the microbiological tests of the studied pregnant women's vaginal secretions we obtained the following results.

In healthy patients, control (**C**) the bacterial flora was normal, with mainly the presence of *Lactobacillus sp.*, *Difteroides* and *Staphylococcus sp.*

In patients with bacterial vaginosis (**BV**) the microbial flora was represented mainly by the presence of *Gardnerella vaginalis* with 'clue cells', *Mobiluncus sp* and, in some patients, *Clostridium sp* and *Fusobacterium sp*.

In patients with vaginitis (**V**) the presence of yeast, *Candida albicans* was detected in some patients (anyone with pseudomycelia). In other patients *Trichomonas vaginalis* was observed.

The studies performed by urine culture revealed 15 patients with **AUI**, with more than 100 000 CFU/ml of urine. The isolated microorganisms were *Escherichia coli* (n=10), *Proteus mirabilis* (n=4) or *Streptococcus faecalis* (n=1).

The IL-1 $\beta$  levels in vaginal secretion (see Figure 1) were: **C** 103.5  $\pm$  24.2 pg/ml, **BV** 1030  $\pm$  59.5 pg/ml, **V** 749.1  $\pm$  66.7 pg/ml (**BV** and **V** versus **C** p < 0.0001). The **PT** group levels fell to normal values (101.4  $\pm$  28.7 pg/ml). In urine and serum this cytokine could not be detected.

According to the ROC curve analysis with 200 pg/ml as the cut-off level, the determination of IL-1 $\beta$  had 100% sensitivity and 95% specificity for **BV** and **V**.

IL-6 levels in all studied materials (serum, vaginal discharge and urine) of infected patients were no different from those obtained in healthy pregnant women. For vaginal discharge the results were: **C** 14.2  $\pm$  3.9 pg/ml, **BV** 13.2  $\pm$  3.8 pg/ml, **V** 13  $\pm$  4.2 pg/ml. In urine and serum IL-6 was not detected either.

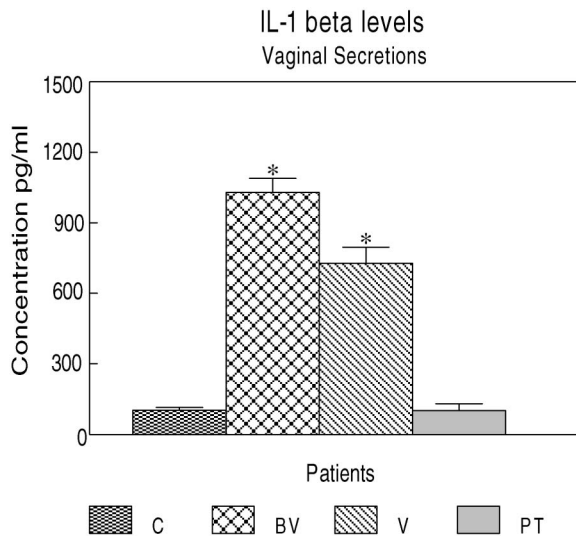


Figure 1. IL-1 $\beta$  levels in vaginal secretions of pregnant women with genitourinary infections. Bacterial vaginosis (**BV**), vaginitis (**V**) specific post-treatment (**PT**), and healthy women (**C**). The average  $\pm$  standard error is represented. The differences among the cytokine levels in each infected group with respect to the control group were evaluated by the Mann-Whitney U-test. \*p < 0.0001.

Figure 2 shows the IL-8 levels in vaginal secretions: **C** 1643  $\pm$  130.3 pg/ml, **BV** 2612.7  $\pm$  257.7 pg/ml, **V** 3437.6  $\pm$  420 pg/ml (p < 0.0001). The level of this cytokine in the **PT** group was 1693  $\pm$  126.6 pg/ml. As with the other studied cytokines, IL-8 could not be detected in serum.

In the patients with **AUI**, the IL-8 level was 1200.7  $\pm$  375 pg/ml and in the **C** group it was 40.2  $\pm$  17 pg/ml (p < 0.0001), see Figure 3.

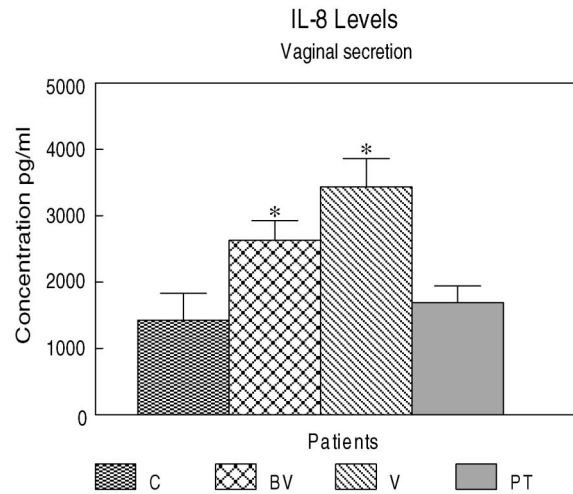


Figure 2. IL-8 levels in vaginal secretions of pregnant women with genitourinary infections. Bacterial vaginosis (**BV**), vaginitis (**V**) specific post-treatment (**PT**), and healthy women (**C**). The average  $\pm$  standard error is represented. The differences among the cytokine levels in each infected group in relation to the control group were evaluated by the Mann-Whitney U-test. \*p < 0.0001.

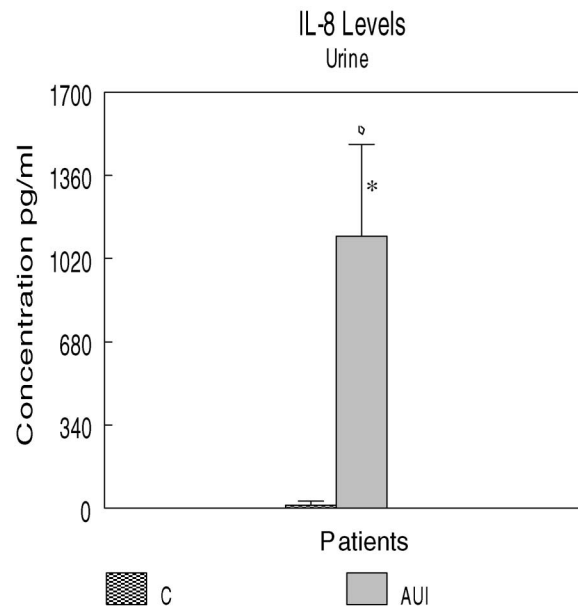


Figure 3. IL-8 levels in urine of pregnant women with urinary asymptomatic infection (**UAI**) and specific post-treatment (**PT**), and healthy women (**C**). The average  $\pm$  standard error is represented. The differences among cytokine levels in the infected group with respect to the control group were evaluated by the Mann-Whitney U-test. \*p < 0.0001.

According to the ROC curve, taking 1795 pg/ml as the cut-off level, IL-8 had 97% sensitivity and 86% specificity in **BV** and **V**. Meanwhile, in patients with urinary infection, IL-8 determination had 100% sensitivity and specificity, taking microbiological studies as gold standard.

## Discussion

In the past, important advances have been achieved in understanding the role that cytokines, cytokine receptors and soluble mediators play in different tumor, infectious or inflammatory pathologies, since they are, in many cases, involved in the pathogenesis of such diseases [10–14]. In the case of pregnant women, some cytokines can trigger situations that can induce preterm labor. At the same time, some of them can provide useful information for pathogenesis studies [12,15–17].

On the other hand, it has been demonstrated that the levels of pro-inflammatory cytokines, such as TNF- $\alpha$  and its soluble receptors R-55 and R-75 in amniotic fluid, are different in term and preterm pregnant women, even in absence of an infectious process. Maymon et al. [13] have shown that in preterm delivery, the TNF- $\alpha$  membrane concentration is eight times higher than in normal delivery. In an infectious process, these soluble mediators can play a double role. Indeed, the local synthesis turns beneficial for the host, allowing the elimination of the microorganisms responsible for the infection, while the high production with high systemic levels can induce toxic effects in the organism [15,16,18]. In this respect, in the present work it was observed that IL-1 $\beta$  and IL-8 levels increase in the urogenital tract, otherwise they could not be detected at systemic level.

It has been reported that the presence of bacteria or bacterial products can generate an inflammatory process induced mainly by cytokines [4,5]. Several studies have also revealed an inflammatory response in amniotic fluid infections with elevation of IL-6 levels [10,11,15,18]. The results obtained in the present work did not reveal an increase in this cytokine, even in patients with genitourinary infection, probably because none of the patients presented chorioamnionitis, which is known to be a risk factor for premature delivery [19].

This study showed that in pregnant patients with genitourinary infection there is a significant increase of at least two cytokines, IL-1 $\beta$  and IL-8 in vaginal secretions, and IL-8 in urine, as well. However, the patterns that they presented were different. In fact, IL-1 $\beta$ , a cytokine that mainly stimulates the acute phase response, was highly increased in bacterial vaginosis, whereas higher IL-8 levels were observed in vaginitis. This could be due to the fact that IL-8 is

a chemokine responsible for attracting a great number of leukocytes, which is typical in vaginitis. Moreover, higher IL-8 levels were observed in the urine of patients with urinary infection than in pyuria generally presented.

It is interesting to note that in the population included in this work an association in absence of preterm delivery and lack of detection of IL-1 $\beta$ , IL-6 and IL-8 in the serum of both infected and non-infected women was observed. These results will encourage future studies aimed at understanding the possible pathogenic significance of the systemic increase of these cytokines in the onset of labor, the premature rupture of the membranes and in preterm delivery.

On the other hand, antibiotic treatments revealed a fast return of both cytokines to normal levels, earlier than the normalization of microbiological studies. It is worth mentioning that in the group of studied patients, early treatment might have avoided the risk of preterm rupture of the membranes and premature onset of labor.

Finally, the results obtained in the present work suggest that IL-1 $\beta$  and IL-8 could be considered potentially eligible for use as infection predictive markers in the clinical context and in the evaluation of therapeutic success in genitourinary infections. Furthermore, the biological material (vaginal discharge and urine) used in this work is easy to obtain and non-invasive for the mother or the fetus, which could be an advantage over the use of amniotic fluid.

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