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ANALYSIS OF THE BACTERIAL VAGINOSIS PREDICTIVE SIGNIFICANCE IN THE DIAGNOSIS OF INFLAMMATORY PROCESSES IN FEMALE PELVIC MINOR

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Abstract - Pelvic inflammatory disease (PID) has an incidence of 100-200/100,000. The aim of this study was to determine whether there is a correlation between the serum proinflammatory cytokines IL-1β and IFN-γ and the presence of bacterial vaginosis (BV) or chlamydia infections (Chl) in women with symptoms of inflammatory processes in the pelvic minor. The study included fifty patients diagnosed with PID and an average age of 32 years. The results of this study revealed that the number of women with BV and PID presented increased IL-1β levels in the serum, whereas in women with chlamydial infections and PID serum the level of IFN-γ was increased. The study shows that in patients with PID, in whom there was no diagnosis of BV and infection with *Chlamydia trachomatis*, the levels of IL-1β and IFN-γ were increased. The conclusion of this research points to the importance of monitoring levels of cytokines in patients with homeostasis of vaginal flora disorders in the prevention of PID.

Key words: Bacterial vaginosis, *Chlamydia trachomatis*, interleukins, pelvic minor infection

INTRODUCTION

Bacterial vaginosis (BV) is a disorder of normal vaginal flora, characterized by the reduction of the number of lactobacilli (*Lactobacillus* H_2O_2 spp.) and an increase in the number of anaerobic microorganisms (*Mobiluncus* spp., *Bacteroides* spp., *Fusobacterium* spp., *Prevotella* spp., *Peptostreptococcus* spp. *and Prophiromanas* spp.), gram-variable coccobacilli (*Gardnerella vaginalis*), and genital mycoplasmas (*Mycoplasma hominis*) (Hillier et al., 1993). These changes in vaginal flora are associated with an increase in vaginal pH and changes in vaginal secretion. *Chlamydia trachomatis* (Chl) is the carrier of sexually transmitted diseases that often manifest as asymptomatic infections of the lower genital tract. In the early phase of the local immune response to infection, activated macrophages produce large amounts of cytokines, which activate prostaglandin F2-α and E2 (Pickering et al., 2006; JerantPatić, 2000). The spectrum of genital infections in women includes, beside vaginal inflammation (colpitis or vaginitis) or vulva (vulvitis), a number of diseases that beside their separate occurrence, also occur in causal connection in various combinations. Inflammation of the cervix (cervicitis), inflammation of the mucous membrane of the uterus (endometritis) and inflammation of the oviducts and ovaries (salpingitis/adnexitis) are in fact very often inherent in both etiology and clinical and therapeutic terms, and are referred to the term pelvic inflammatory disease (PID). PID has an incidence of 100-200/100,000 women (i.e. at adolescence 1 in 8 girls) (Soper and Mead, 2005). The aim of this study was to determine whether there is a correlation between serum levels of pro-inflammatory cytokines IL-1β and IFN-γ and the presence of bacterial vaginosis or chlamydial infections in women with symptoms

of inflammatory processes in the pelvis minor (pelvic inflammatory disease-PID).

MATERIALS AND METHODS

The research was conducted at the Department of Gynecology and Obstetrics, Clinical Center in Kragujevac. The protocol was approved by the Ethics Committee Institution of the Clinical Center in Kragujevac. The study included fifty women diagnosed with PID. The subjects were divided into groups according to the following criteria: 1) PID patients with bacterial vaginosis - BV (N = 18) and 2) PID patients with *Chlamydia trachomatis* infection – Chl (N = 10). The women that were classified in the PID category had to meet the following criteria: 1) present pelvic pain; 2) a positive bimanual gynecological finding; 3) elevated body temperature > 38.5° C, measured rectally; 4) a positive laboratory finding for the presence of infection as follows: number of leukocytes ≥ 10.0 $x10^9$ / L, neutrophilic granulocytes ≥ 75%, sedimentation ≥ 30 mm/h, C-reactive protein ≥ 30.0 mg/L, fibrinogen ≥ 6.0 g/L; 5) a positive ultrasound finding of pelvic, and 6) normal neutrophilic colposcopic examination and Papanicolaou test findings.

In addition, factors that may affect the level of interleukin in serum, such as autoimmune diseases, hormonal disorders, and particularly complications of hypersensitivity and infectious diseases, were also excluded in the selection of patients. A sample of vaginal secretion was taken from the vaginal sidewalls and was used for the diagnosis of BV by Amsel and Nugent methods (Amsel, 1983; Nugent, 1991). In one step, an immunochromatographic test was used for selective identification of LPS antigen for Chlamydia trachomatis (Biorapid Chlamidia AG kit for 20 tests, BIOKIT SA, Barcelona, Spain) from endocervical samples of all subjects. Sample preparation for the determination of cytokines was performed as follows: 5 ml of blood was collected from the patient's cubital veins. Blood was placed in test tubes to separate the serum, and after 30 min the sample was centrifuged for 30 min at 1000 rpm. The serum samples were immediately frozen and stored at -20° C until use. In the serum samples, the levels of IL-1β and IFN-γ were determined by ELISA (I & R systems, UK). The sensitivity of the test for IL-1β was 1.0 pg/L, and for IFN-γ, 8.0 pg/ml. The results were statistically analyzed using the nonparametric Mann-Whitney test; a p-value less than 0.05 was considered statistically significant.

RESULTS

The average age of the women who participated in this study was 32 and ranged between 22 and 40 years. The presence of BV was found in 18 patients with PID, chlamydial infection (Chl) in 10 women with PID, while 6 patients with PID had BV and chlamydial infection as well. Sixteen patients with inflammatory syndrome in the pelvis minor had neither BV nor chlamydial infection. The calculated values of parameters are shown in Tables 1, 2 and 3, depending on the criteria used to divide patients into groups. It can be seen that the lowest detectable value was found for IL-1β in the PID group with BV (14.6%) (Table 1) and the highest for IFN-γ in the PID group with BV (42.2%) (Table 1). In the patients with PID divided into two groups according to the first criterion (Table 1), there were no statistically significant differences between the levels of interleukins in the serum of women from the BV group and the group without BV. However, in the group according to the second criterion (Table 2), it can be seen that women with chlamydial infection and PID (10 patients) had an increased level of IFN-γ in relation to the group with BV ($p < 0.010$), while for other interleukins, there were no significant differences. On the other hand, when we compared the levels of interleukins obtained from the blood of PID patients with chlamydial infection (10 women) with the values of the PID patients without chlamydial infection (40 women), it was obvious that the average value of IFN-γ was significantly higher in the group with chlamydial infection ($p < 0.010$). Table 3 shows the levels of interleukins in the group of patients with PID in whom we did not find a vaginal flora disorder.

Table 1. Sensitivity and statistical analysis of cytokine results in the patient group with PID BV.

Cytokine	PID group with BV (N = 18)					PID group without BV (N=32)					P
	Detectability	Max	Min	X sr	SD	Detectability	Max	Min	X sr	SD	
IFN-□	42.2%	41.3	5.4	22.4	12.1	30.4%	80.9	10.0	30.3	36.0	0.993
IL-1β	14.6%	1.6	0.8	16.2	2.6	48.0%	2.4	1.5	1.6	0.41	0.092

Table 2. Sensitivity and statistical analysis of cytokine results in the patient group with PID Chl.

Cytokine	PID group with Chl (N = 10)					PID group without Chl (N=40)					P
	Detectability	Max	Min	X sr	SD	Detectability	Max	Min	X sr	SD	
IFN-□	32.2%	117.4	16.4	52.4	47.1	32.4%	22.0	9.1	14.0	5.0	0.010
IL- 1β	28.6%	2.6	1.8	1.62	0.42	43.8%	3.9	1.9	1.7	0.40	0.617

Table 3. Sensitivity and statistical analysis of cytokine results in the patient group with PID and negative finding of BV and Chl.

Cytokine	PID group (N = 16)					PID group without BV - Chl (N=34)					P
	Detectability	Max	Min	X sr	SD	Detectability	Max	Min	X sr	SD	
IFN-□	37.2%	135.4	26.4	62.4	58.1	29.4%	29.0	8.1	14.0	5.0	0.012
IL- 1β	16.3%	1.55	0.75	15.8	2.65	46.1%	2.38	1.34	1.65	0.61	0.091

DISCUSSION

Many clinical studies have shown that in women with PID and bacterial infection, intrauterine endo- and exotoxins are the cause of a hyperproduction of pro-inflammatory IL (IL-1β and IFN-γ (Curry et al., 2007; Basso et al., 2005; Hedges et al., 2006) . Cytokines can induce the synthesis of prostaglandins and metalloproteinases which can increase inflammatory processes in the pelvic minor. Studies have been published showing that the level of pro-inflammatory cytokines IL-1β in vaginal secretions of women with PID and BV (Table 1) compared to the healthy population, is significantly higher (about 10 times) than the control group (Cauci et al., 2002; AlvarezOlmos et al., 2004; Imseis et al., 1997; SturmRamirez et al., 2000; Spandorfer et al., 2001), and that these levels decrease after treatment of BV with metronidazole (Yudin et al., 2000). In addition, several studies have confirmed that the level of IL-8 in vaginal secretion in women with BV is elevated (Yudin et al., 2000; Zariffard et al., 2005), although this increase is generally less than twofold compared to the control group. It was also found that in *in vitro* conditions the vaginal discharge collected from women with BV strongly induces IFN-γ secretion from immune cells (Zariffard et al., 2005). The levels of IL-6 and TNF-α in the vaginal secretion of patients with BV were not increased compared to controls. There is not much data on the level of interleukin in serum with women with BV in the prediction of PID. In this study, we found increased levels of IL-1β in sera of women with bacterial vaginosis compared to healthy controls. This finding is consistent with recent results obtained for the levels of interleukins in vaginal secretions of women with BV and PID (Wennerholm et al., 1999; Gupta et al., 2009; Ondondo et al., 2009). In addition, in previous studies it was reported that cells infected with *Chlamydia trachomatis* produce high levels of IFN-γ (Table 2) and small amounts of IL-10, IL-12, IL-23 and TNF-α (Srivastava et al., 2008; Golden, 2003). This is consistent with the results of our study, where the level of IFN-γ in the serum of women with chlamydial infection and PID is significantly higher than in the control group. The results of our study indicate that bacterial vaginosis and chlamydial infections can cause a systemic, partially immune response that can further boost the inflammatory reaction. Modulation of the immune response during inflammatory process could explain our contradictory results in

the group of patients with PID, in which we did not find a vaginal flora disorder (Table 3). Because PID pathophysiology is not yet known, the results of this study could contribute to its clarification. Determination of the levels of interleukins in women with PID in the presence of vaginal flora disorders is still based on a small number of cases for the standardization of methods and possibilities of using interleukin as a marker of this pathological condition, which requires further investigation in resolving the problem (Ness, 2004). The results of this study demonstrate that in women with bacterial vaginosis and PID, the level of IL-1β in serum is increased, whereas in women with chlamydial infection and PID, the serum level of IFN-γ is increased. In addition, the study showed that in patients with PID, for whom there was no diagnosis of BV and *Chlamydial trachomatis* infection, the levels of IL-1β and IFN-γ are also increased. The conclusion of this research highlights the importance of monitoring the levels of cytokines in the prevention of PID, in patients with vaginal flora disorders.

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