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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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Received: 5 November 2011 Accepted: 2 December 2011 Published: 16 December 2011

Abstract

Pelvic inflammatory disease (PID) occurs with the incidence of 100 - 200/ 100 000. The aim of this study was to determine whether there is a correlation between serum proinflammatory cytokines IL-1? and IFN-? 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 with the average age of 32 years. The results of this study reveal that women with bacterial vaginoses and PID level of IL- 1? in serum is increased, whereas in women with Chlamydial infection and PID serum level of IFN-? is increased. The study showed that in patients with PID, in whom there was no diagnosis of BV and infection with Chlamydia trachomatis, the levels of IL-1? and IFN-? are increased. The conclusion of this research points out to the importance of monitoring levels of cytokines in patients with homeostasis of vaginal flora disorders in the prevention of PID.

Index terms— Bacterial vaginosis, Chlamydia trachomatis, interleukins, pelvic minor infection

1 INTRODUCTION

acterial vaginosis (BV) is a disorder of normal vaginal flora, characterized by reduction of the number of lactobacilli (Lactobacillus H 2 O 2 spp) and an increase in the number of anaerobic microorganisms (Mobiluncus spp, Bacteroides spp, Fusobacterium spp, Prevotella spp, and Peptostreptococcus spp and Prophiromanas spp), gram-variable coccobacilli (Gardnerella vaginalis), and genital mycoplasmas (Micoplasma hominis) (Hillier et al., 1993). These changes in vaginal flora were associated with an increase in vaginal pH and changes in vaginal secretion. Chlamydia trachomatis (Chl) is the carrier of sexually transmitted diseases, which often manifest as asymptomatic infection 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-? and E2 (Pickering et al., 2006;JerantPati?, 2000). The spectrum of genital infections in women includes, beside the vaginal inflammation (colpitis or vaginitis) or vulva (vulvitis), a number of diseases, which beside their separate occurrence, they also occur in causal connection in various combinations. Inflammation of the cervix (cervicitis), inflammation of the mucous Author : MD, PhD. Gynecology and Obstetrics clinic, clinical centre Kragujevac , Vojislava Kalanovi?a 1A/3, 34000 kragujevkac, serbia ; Tel.: +381-64-616-8999 ; Fax: +381-34-370-151. E-mail : drloncar@sezampro.rs membrane of the uterus (endometritis), and inflammation of the oviducts and ovaries (salpingitis /adnexitis) are in fact very often inherent in both the etiology and in the clinical and therapeutic terms, and are referred to the term pelvic inflammatory disease (PID). PID occurs with an incidence of 100-200 /100 000 women, that is in the age of adolescence: one of 8 girls (Soper & Mead, 2005) . The aim of this study was to determine whether there is a correlation between serum pro-inflammatory cytokines IL-1? and IFN-? and the presence of bacterial vaginosis or Chlamydial infections in women with symptoms of inflammatory processes in the pelvis minor (pelvic inflammatory disease-PID).

II.

2 MATERIALS AND METHODS

The research was conducted, as a prospective study, 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 as a PID category, had to meet the following criteria: hypersensitivity and infectious diseases were also excluded in the selection of patients. A sample of vaginal secretion was taken from the vaginal side walls 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) form endocervical samples of all subjects. Sample preparation for determination of cytokines was performed as follows: 5 ml of blood was collected from the patient's cubital veins. Blood was placed into test tubes to separate the serum, and after half an hour, the sample was centrifuged for 30 minutes at 1000 rpm per minute. Furthermore, serum samples were immediately frozen and stored at -20 ° C until use. In the serum samples the levels of IL-1? and IFN-? were determined by ELISA kit (I & R systems, UK). Sensitivity of the test for IL-1? was 1.0 pg/L, and for IFN-? was 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.

3 III.

4 RESULTS

The average age of women who participated in this study was 32 years and ranged between 22 and 40. 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-1? in the PID group with BV (14.6%) (table 1) and highest for IFN-? in the PID group with BV (42.2%) (table 1). In 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 BV group and the group without BV. However, in the patients group, according to the second criterion (table 2), it can be seen that women with Chlamydial infection and PID (10 patients) had increased level of IFN-? 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 is obvious that the average value of IFN-? was significantly higher in the group with Chlamydial infection ($p < 0.010$). The table **??**. shows the levels of interleukins in the group of patients with PID in whom we have not found vaginal flora disorder, where we showed a significant increase in both types of parameters. **??** 2005) , although this increase is generally less than twofold compared to the control group. In addition, it was found that in in vitro conditions, vaginal discharge collected from women with BV strongly induces IFN-? secretion from immune cells **??**Zariffard et al., 2005) . Levels of IL-6 and TNF-? in vaginal secretion of patients with BV were not increased compared to controls. There is no much data on the level of interleukin in serum with women with BV in prediction of PID. In this study, we found increased levels of IL-1? in serum of women with bacterial vaginosis compared with the controls, which 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-? (table 2) and small amounts of IL-10, IL-12, IL-23 and TNF-? (Srivastava et al., 2008;Golden, 2003) . This is consistent with the results of our study, where the level of IFN-? in 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 systemic, partially immune response of the woman, which may cause further boost of the inflammatory reaction. Modulation of the immune response during inflammatory process may be an explanation of our contradictory results in the group of patients with PID, in which we have not demonstrated vaginal flora disorder (table **??**). Due to the fact that the PID pathophysiology is not yet known, the results of this study may contribute to its explanation. [1] [2] [3] [4]

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Figure 1: 1) present pelvic pain 2)

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IV. DISCUSSION

Many clinical studies have shown that with women with PID and bacterial infection, intrauterine endo and exotoxin are the cause of hyperproduction of pro-inflammatory IL (IL-1? and IFN-? (Curry et al., 2007; Basso et al., 2005; Hedges et al., 2006) . Cytokines can induce the synthesis of prostaglandins and metalloproteinases, whichmay increase inflammatory processes in

Figure 2: Table 1 .

1

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Figure 3: Table 1 :

Determination of 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). Results of this study demonstrate that in women with bacterial vaginosis and PID, level of IL-1? in serum is increased, whereas in women with Chlamydial infection and PID, serum level of IFN-? is increased. In addition, the study showed that in patients with PID, in whom there was no diagnosis of BV and Chlamydial trachomatis infection levels of IL-1? and IFN-? are also increased. The conclusion of this research points out to the importance of monitoring levels of cytokines in patients with homeostasis of vaginal flora disorders in the prevention of PID.

Competing interests: none declared

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