Do Bacterial Vaginosis and Chlamydial Infection Affect Serum Cytokine Level?

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SUMMARY

Introduction Serbia is the country with extremely low birth rate and a relatively high percentage of preterm deliveries (8%). With this in mind, discovering new diagnostic methods that could be used for the prediction of preterm delivery is of great importance. In this study we tried to determine whether bacterial vaginosis and chlamydial infection could provoke preterm delivery by activation of systemic cytokine network.

Objective The aim of this study was to determine serum levels of proinflammatory cytokines (IL-1β, IL-8, IFN-γ, IL-6 and TNF-α) in pregnant women with symptoms of preterm delivery and to make correlation between these parameters and the presence of bacterial vaginosis or chlamydia infection.

Method In the serum of 35 pregnant women, which were divided in groups according to the presence or absence of bacterial vaginosis and chlamydial infection, commercial ELISA tests for proinflammatory cytokines were performed.

Results The serum level of IFN-γ was significantly increased in pregnant women having chlamydial infection, as well as the level of IL-1β in women with bacterial vaginosis. The levels of TNF-α, IL-6 and IL-8 were not significantly different between the investigated groups.

Conclusion The preliminary results obtained in this research point out the possibility that not only intrauterine or systemic infections, but also bacterial vaginosis and chlamydial infection can cause a partial activation of systemic cytokine network and contribute to the occurrence of preterm delivery.

Keywords: interleukines; preterm delivery; bacterial vaginosis; Chlamydia trachomatis

INTRODUCTION

Preterm delivery is one of the most significant factors of perinatal morbidity and mortality. Considering the fact that Serbia is thought to be the country with extremely low birth rate and a relatively great number of preterm deliveries (8%), it is of utmost importance to bring every pregnancy to its term, reaching a live and viable birth [1, 2]. The aetiology of preterm deliveries is very complex. Last data indicate that infection can be one of the causes of preterm delivery, especially at a low gestational age (<30 weeks) [3, 4].

Bacterial vaginosis (BV) is characterized by a disturbance of the normal vaginal flora, with a loss of H₂O₂-producing Lactobacillus spp. and an increase in the number of gram-variable coccobacilli (Gardnerella vaginalis), anaerobic organisms (Mobiluncus spp., Bacteroides spp., Fusobacterium spp., Prevotella spp., Peptostreptococcus spp.), and genital mycoplasmas (Mycoplasma hominis) [5]. These changes in the vaginal flora are associated with increase of the vaginal pH and changes in vaginal secretion. Conventional diagnostic methods for BV are methods of Amsel et al. [6] and Nugent et al. [7].

Chlamydia trachomatis (Chl) is a carrier of a sexually transmitted disease, which is often manifested by asymptomatic infection of the lower genital tract. It is assumed that this infection can influence the course and the result of pregnancy.

In the early phase of local immunological response to the infection, activated macrophages produce a large quantity of cytokines, which activate prostaglandine F₂-α and E₂ leading to the increase of contractility of myometrium and premature rupture of amniotic membranes [8].

OBJECTIVE

The aim of this pilot study, was to determine if there was a correlation between the serum levels of proinflammatory cytokines (IL-1β, IL-8, IFN-γ, IL-6 and TNF-α) in pregnant women with symptoms of preterm delivery and to make correlation between these parameters and the presence of bacterial vaginosis or chlamydial infection.
at a gestational age range from 24 to 35 weeks of gestation (GW) were enrolled into the study. The women were divided in groups according to the following criteria: 1) patients with BV and/or chlamydial infection (n=17, group BV/Chl) and patients without BV or chlamydial infection (n=18, group without BV/Chl), 2) patients with BV (n=13, group with BV) and patients without BV (n=22, group without BV), 3) pregnant women with Chl (n=8, group with Chl) and pregnant women without Chl (n=28, group without Chl).

Some other factors that could cause preterm delivery, such as general factors (diseases during pregnancy: cardiovascular diseases - preeclampsia, kidney diseases, urinary infection, diabetes mellitus), than local factors (uterine malformation, cervical insufficiency, uterine and adnexal tumours, Asherman syndrome, cervical conization, other genital infections) and obstetric risk factors (multiple pregnancy, polyhydramnion) were excluded in all patients [1]. Furthermore, the factors which could influence the level of interleukins in the serum, such as autoimmune diseases, hormonal disorders, special complications of hypersensitivity and infectious diseases were also excluded during the selection of patients [9].

A swab sample of the vaginal secretion was taken from the lateral wall and used for diagnosis of BV by Amsel and Nugent methods [6, 7]. One step immunochromatographic test for selective identification of LPS antigen of the Chlamydia trachomatis species with a high degree of sensitivity (Biorapid Chlamydia AG Kit 20 Tests, BIOKIT S.A., Barcelona, Spain) was used for the detection of Chlamydia trachomatis in endocervical specimens of all pregnant women.

Serum samples preparation and immunoassays for cytokines: we collected 5 ml of blood from the cubital vein of the pregnant women. The blood was placed in a serum separator tube, and after half an hour the samples were centrifugated for 30 minutes at 1000 rpm. Next, the serum samples were immediately frozen and kept at -20 °C until the moment of use. In the serum samples the levels of IL-1β, IL-8, IFN-γ, IL-6 and TNF-α were determined. For IL-1α, IL-8, IFN-γ we used ELISA kits (R&D Systems, UK). The sensitivity of assays was 1.0 pg/mL for IL-1α, 1.5-7.5 pg/mL for IL-8, with the mean minimum detectable dose of 3.5 pg/mL and finally 8.0 pg/mL for IFN-γ. The ELISA kits (ImmunoTech, France) were used for the evaluation of IL-6 and TNFα. The assay sensitivity was 3.0 pg/mL for IL-6 and 5.0 pg/mL for TNF-α.

The results were statistically evaluated with non-parametric Mann Whitney test, p-values less than 0.05 were considered as statistically significant.

### RESULTS

The average age of women involved in this research was 26 years and varied between 20 and 35 years.

The presence of BV was found in 9 patients, chlamydial infection in 4 women, whereas 4 patients had both BV and chlamydial infection. Eighteen patients had neither BV nor Chl.

From the obtained results for cytokine levels in the serum, detectibility of methods, the average, minimal and maximal values, standard deviation and p-values were calculated. The values of calculated parameters are shown in Tables 1, 2 and 3.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Group with BV/Chl (n=17)</th>
<th>Group without BV/Chl (n=18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detectability X SD Min Max</td>
<td>Detectability X SD Min Max</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>70.6% 11.7 12.1 3.6 42.4</td>
<td>66.7% 13.8 22.6 3.6 86.1</td>
<td>0.86</td>
</tr>
<tr>
<td>IL-8</td>
<td>76.5% 23.3 33.9 3.9 126.0</td>
<td>50.0% 8.2 5.2 4.3 4.34 20.7</td>
<td>0.27</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>35.3% 35.1 41.6 8.7 117.0</td>
<td>27.8% 13.1 5.6 8.3 4.34 22.8</td>
<td>0.36</td>
</tr>
<tr>
<td>TNF-α</td>
<td>87.5% 62.0 50.0 7.5 189.0</td>
<td>94.1% 75.1 67.5 9.1 9.15 286.0</td>
<td>0.46</td>
</tr>
<tr>
<td>IL-1β</td>
<td>17.6% 1.5 0.2 1.3 1.7</td>
<td>44.4% 1.4 0.3 1.1 2.1</td>
<td>0.28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Group with Chl (n=8)</th>
<th>Group without Chl (n=27)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detectability X SD Min Max</td>
<td>Detectability X SD Min Max</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>75.0% 8.31 8.5 3.6 25</td>
<td>66.7% 14.2 19.9 3.6 84</td>
<td>0.46</td>
</tr>
<tr>
<td>IL-8</td>
<td>62.5% 15.9 12.4 4.3 35</td>
<td>63.0% 17.5 30.2 3.9 126</td>
<td>0.43</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>37.5% 59.7 50 23 117</td>
<td>29.3% 12.1 4.6 8.3 117</td>
<td>0.014</td>
</tr>
<tr>
<td>TNF-α</td>
<td>100% 75.7 63 18 189</td>
<td>88.5% 66.8 59.1 7.6 287</td>
<td>0.85</td>
</tr>
<tr>
<td>IL-1β</td>
<td>25.0% 1.51 0.21 1.3 1.7</td>
<td>33.3% 1.41 0.32 1.1 2.1</td>
<td>0.55</td>
</tr>
</tbody>
</table>
are significantly higher (about 10 times) in BV [15-21] and with BV and healthy flora, have reported that IL-1β levels tryptory cytokine levels in the genital tract fluid from women and preterm delivery.

rupture of amniotic membranes that can lead to PPROM uterine contractions, softening and dilatation of the cervix, prostaglandines and metaloproteinases, which can provoke [12, 13, 14]. Further these cytokines can induce synthesis of [15-21].

flamatory IL in amniotic fluid (IL-1β, TNFα, IL-6 and IL-8) [15-21]. ADH of interleukines in the serum of pregnant women is changing during pregnancy and that certain interleukines have different roles [12, 13, 14]. This is in accordance with the results obtained for the levels of interleukines in the genital secretion of pregnant women with BV [15-21].

Besides, in previous papers it was reported that cells from the Chlamydia trachomatis infected site release a high level of IFN-γ and small amounts of IL-10, IL-12, IL-23 and TNF-α [25, 26, 27]. This is in accordance with the results of our study, where significantly increased level of IFN-γ was found in the serum of pregnant women with chlamydial infection compared with the control group.

The results of this pilot study point out that bacterial vaginosis and chlamydial infection can partially provoke systemic immune response of pregnant woman, which can further cause cervix dilatation, contraction of uterus, preterm premature rupture of membranes and finally lead to preterm delivery. Considering the fact that pathophysiology of preterm delivery is still not well-known, the results of this study can contribute to its explanation. Because investigations of serum interleukines levels in pregnant women with preterm contractions and symptoms of preterm delivery in the presence of disturbed vaginal flora or intrauterine infection are still based on a small number of cases for standardization of methods and possible use of interleukines as markers of pathological conditions in pregnancy, further investigations are needed.

CONCLUSION

Results of this study suggest that in pregnant women with bacterial vaginosis the level of IL-1β in the serum is increased, while in pregnant women with chlamydial infection the serum level of IFN-γ is significantly increased. Besides, it has been found that bacterial vaginosis and genital chlamydial infection do not affect serum concentrations of IL-8, IL-6 and TNF-α. Results of this and similar studies point out the significance of monitoring of cytokines levels in patients with disturbed vaginal flora or chlamydial infection.

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КРАТАК САДРЖАЈ
Увод Србија је земља с екстремно ниском степеном природног прираштаја и релативно великом бројем преранових порођаја (8%). Због тога је проналасљење дијагностичких метода које би се могле користити за предвиђање прераног порођаја изузетно значајно. У овом раду покушали смо да откривемо да ли бактеријска вагиноза и инфекција хламидијом, преко активирања системске мреже цитокина, могу бити узрок прераног порођаја.

Циљ рада Циљ рада је био да се одреде нивои проинфламаторних цитокина (IL-1β, IL-8, IFN-γ, IL-6 и TNF-a) у серуму трудацке са симптомами прераног порођаја, а затим направи корелација ових параметара с присуством бактеријске вагинозе или инфекције хламидијом.

Методе рада Усеруму 35 испитаница, које су се налазиле у групе према постојању бактеријске вагинозе и инфекције хламидијом, ниво проинфламаторних цитокина су одређени комерцијалним ELISA тестовима.

Резултати Утврђено је статистички значајно повећање нивоа IFN-γ код трудаца с инфекцијом хламидијом, као и повећање нивоа IL-1β код жена са бактеријском вагинозом. Није било статистички значајних разлика у нивоима TNF-a, IL-6 и IL-8 између испитаних група.

Закључак Прелиминарни резултати доказују могућност да не само интруатерина или системска инфекција, већ и бактеријска вагиноза и инфекција хламидијом могу довести до делимичне активације системске мреже цитокина и допринети прераном порођају.

Кључне речи: интерлеукин; преранов порођај; бактеријска вагиноза; Chlamydia trachomatis

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