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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 chlamydial 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 H_2O_2-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., Prophyromanas spp. and 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 macrofages produce a large quantity of citokynes, which activate prostaglandine F_2-α and E_2 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) and the presence of bacterial vaginosis or chlamydial infection in pregnant women with symptoms of preterm delivery.

METHODS

The research was devised as a prospective study which was conducted at the Clinic for Obstetrics and Gynaecology of the Clinical Centre of Vojvodina. The protocol was approved by the Institutional Ethical Board of the Faculty of Medicine, University of Novi Sad and Clinical Centre of Vojvodina, Novi Sad. Thirty-five pregnant women with symptoms of preterm delivery

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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 ^{0}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

Table 1. Detectability and statistical analysis results of cytokines in two groups of patients

Cytokine	Group with BV/Chl (n=17)					Group without BV/Chl (n=18)					p
	Detectability	$\bar{X}$	SD	Min	Max	Detectability	$\bar{X}$	SD	Min	Max	
IL-6	70.6%	11.7	12.1	3.6	42.4	66.7%	13.8	22.6	3.61	86.1	0.86
IL-8	76.5%	23.3	33.9	3.9	126.0	50.0%	8.2	5.2	4.34	20.7	0.27
IFN-γ	35.3%	35.1	41.6	8.7	117.0	27.8%	13.1	5.6	8.34	22.8	0.36
TNF-α	87.5%	62.0	50.0	7.5	189.0	94.1%	75.1	67.5	9.15	286.0	0.46
IL-1β	17.6%	1.5	0.2	1.3	1.7	44.4%	1.4	0.3	1.10	2.1	0.28

BV – bacterial vaginosis; Chl – chlamydial infection; n – number of patients; X – average value; SD – standard deviation; Min – minimal value; Max – maximal value

Table 2. Detectability and statistical analysis results of cytokines in two groups of patients

Cytokine	Group with BV (n=13)					Group without BV (n= 22)					p
	Detectability	$\bar{X}$	SD	Min	Max	Detectability	$\bar{X}$	SD	Min	Max	
IL-6	61.5%	12.6	13.4	3.6	42.4	72.7%	12.8	20.0	3.6	84.6	0.76
IL-8	76.9%	24.9	38.2	3.9	126	54.5%	10.6	9.2	4.3	35.5	0.49
IFN-γ	38.5%	18.8	12.8	8.7	39.4	27.3%	30.4	42.6	8.3	117	1.00
TNF-α	83.3%	58.4	53.4	7.6	189	95.2%	74.5	62.5	9.2	287	0.27
IL-1β	15.4%	1.65	0.0	1.6	1.6	40.9%	1.38	0.31	1.1	2.1	0.097

Table 3. Detectability and statistical analysis results of cytokines in two groups of patients

Cytokine	Group with Chl (n=8)					Group without Chl (n= 27)					p
	Detectability	$\bar{X}$	SD	Min.	Max.	Detectability	$\bar{X}$	SD	Min.	Max.	
IL-6	75.0%	8.31	8.5	3.6	25	66.7%	14.2	19.9	3.6	84	0.46
IL-8	62.5%	15.9	12.4	4.3	35	63.0%	17.5	30.2	3.9	126	0.43
IFN-γ	37.5%	59.7	50	23	117	29.3%	12.1	4.6	8.3	22.8	0.014
TNF-α	100%	75.7	63	18	189	88.5%	66.8	5.91	7.6	287	0.85
IL-1β	25.0%	1.51	0.21	1.3	1.7	33.3%	1.41	0.32	1.1	2.1	0.55

3 depending on the criteria that were used for the division of patients into groups. It can be seen that the lowest detectability was found for IL-1β in the group with BV (15.4%) (Table 2) and the highest for TNF-α in the group with Chl (100%) (Table 3). When patients were divided in two groups according to the first criteria (Table 1), there was no any statistically significant difference between interleukins levels in the serum of pregnant women from the group with BV/Chl and group without BV/Chl. However, when patients were divided in groups according to the second criteria (Table 2), it could be seen that pregnant women with BV (13 patients) had increased level of IL-1β in comparison to the group without BV ($p<0.097$), whereas for other interleukins there were no significant differences. On the other hand, when we compared the interleukins levels obtained from the blood of patients with Chlamydia trachomatis (8 women) to the levels of patients without Chl (27 women), it could be noted that the average value of IFN-γ was significantly higher in the group with Chl ($p<0.014$), whereas the levels of other interleukins were approximately the same in both groups (Table 3).

DISSCUSION

Peter Medawar first posed the riddle of the foetus-as-allograft nearly 60 years ago explaining the normal course of pregnancy by maternal-foetal interface, antigenic inertness of the foetus and maternal immune tolerance of foreign tissue [10]. The trophoblastic tissue and embryonic membranes, which are in close connection with the uterus, are also the allograft [10]. The fact that only the balance between Th2 and Th1 immunological response is necessary for normal pregnancy is too simplified. It is known that the concentration of interleukines in the maternal serum is changing during pregnancy and that certain interleukines have different roles in different stages of pregnancy (implantation and maturation period and later development of pregnancy and preparing for delivery) [11]. Different maternal factors (diseases – preeclampsia, kidney disorders, infections of urinary tract, diabetes mellitus, infectious diseases, tumours of uterus and adnex, Asherman syndrome, cervical conization) influence the level of interleukines in the serum.

It is assumed that cervical and vaginal bacterial infections are responsible for over 30% of all cases of preterm premature rupture of foetal membranes (PPROM) and preterm delivery [1]. Many clinical studies have confirmed that in pregnant women with intrauterine infections bacterial endo- and egzotoxins cause hyperproduction of proinflamatory IL in amniotic fluid (IL-1β, TNFα, IL-6 and IL-8) [12, 13, 14]. Further these cytokines can induce synthesis of prostaglandines and metaloproteinases, which can provoke uterine contractions, softening and dilatation of the cervix, rupture of amniotic membranes that can lead to PPROM and preterm delivery.

A number of studies that have compared proinflammatory cytokine levels in the genital tract fluid from women with BV and healthy flora, have reported that IL-1 β levels are significantly higher (about 10 times) in BV [15-21] and that it decreases after a successful treatment of BV with metronidazole [22]. Also, several studies found higher levels of IL-8 in the genital secretion of women with BV [15, 16, 21], although there was generally less than a twofold increase over controls. In addition, it was found that in vitro genital mucosal fluid collected from women with BV strongly induced secretion of TNF-α by immune cells [23]. However, the levels of IL-6 and TNF-α in vaginal secretion were not found to be increased in BV when compared with controls [16, 19, 24]. There is no much data about the level of interleukins in the serum of pregnant women with BV. In the present study, we found increased level of IL-1β in the serum of pregnant women with bacterial vaginosis compared with the control group, which is in accordance with the recent 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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Да ли бактеријска вагиноза и инфекција хламидијом утичу на ниво цитокина у серуму?

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КРАТАК САДРЖАЈ

Увод Србија је земља с екстремно ниском стопом природног прираштаја и релативно великим бројем превремених порођаја (8%). Због тога је проналажење дијагностичких метода које би се могле користити за предвиђање превременог порођаја изузетно значајно. У овом раду покушали смо да откријемо да ли бактеријска вагиноза и инфекција хламидијом, преко активирања системске мреже цитокина, могу бити узроци превременог порођаја.

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

Методе рада У серуму 35 испитаница, које су сврстане у групе према постојању бактеријске вагинозе и инфекције хламидијом, нивои проинфламаторних цитокина су одређени комерцијалним *ELISA* тестовима.

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

Закључак Прелиминарни резултати добијени овим истраживањем указују на могућност да не само интраутерина или системска инфекција, већ и бактеријска вагиноза и инфекција хламидијом могу довести до делимичне активације системске мреже цитокина и допринети превременом порођају.

Кључне речи: интерлеукини; превремени порођај; бактеријска вагиноза; *Chlamydia trachomatis*

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