Interferon Gamma, Interleukin 8 and Interleukin 10 in Serum of Patients with the Cervical Infection and Symptoms of the Imminent Preterm Delivery

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SUMMARY

Introduction Preterm delivery (PTD), defined as a delivery between 24th and 37th completed week, increases the risk of neonatal morbidity and mortality. There is a growing body of evidence that the intrauterine infection as well as cervicovaginal bacterial infections and Chlamydia infections are possible causes of preterm delivery. Host response to cervicovaginal and/or intrauterine infections is possible with a release of various inflammatory mediators, many of which are cytokines.

Objective The aim of the study was to find out if cervical infection influenced the serum levels of interferon-γ (IFN-γ), interleukin 8 (IL-8) and interleukin 10 (IL-10) in patients with the clinical symptoms of the imminent preterm delivery.

Methods A total of 128 pregnant women (from 24 to 30 weeks of gestation) with the clinical symptoms of the imminent preterm delivery were divided in: study group – 85 patients with the cervical infection, and control group – 43 patients without the cervical infection. The level of cytokines in the serum was measured with commercial ELISA tests.

Results No significant difference could be found in serum levels of IFN-γ (p=0.632), IL-8 (p=0.712) and IL-10 (p=0.676) between these two investigated groups.

Conclusion The results of our study suggest that there is no significant difference in serum IFN-γ, IL-8 and IL-10 concentrations between pregnant women with the symptoms of the imminent preterm delivery who had and had no cervical infection.

Keywords: interferon gamma; interleukin 8; interleukin 10; preterm delivery; bacterial infection; Candida; Chlamydia trachomatis

INTRODUCTION

Preterm delivery (PTD), defined as delivery prior to 37 completed weeks of gestation, increases the risk of neonatal morbidity and mortality [1]. It is now well accepted that intrauterine infection causes a significant proportion of spontaneous PTDs, particularly earlier ones. Bacteria can ascend from the lower genital tract before or during pregnancy, infect the membranes and initiate an inflammatory response culminating in preterm labor (PTL) or preterm premature rupture of membranes (PPROM) [2]. Cytokines are a diverse group of soluble proteins that mediate inflammation and many other processes. These proteins exhibit pleiotropy and functional redundancy, up and down-regulating one another to result in complex networks involved in the establishment and maintenance of pregnancy [3] and complications such as miscarriage, preeclampsia and spontaneous PTD [2]. A long-standing paradigm classifies cytokines based on their T-cell lineage as Th1 or Th2. Normal pregnancy has been characterized as Th2-dominant state [4], although the timecourse of the expected shift to Th2 dominance in peripheral blood is not clear [5]. Host response to cervicovaginal and/or intrauterine infections is represented with a release of various inflammatory mediators, many of which are cytokines [6]. Pro-inflammatory cytokines, in the settings of genital infection, could have an important role in the chain of events that lead to the preterm delivery, switching the Th1/Th2 relationship to the Th1 side which can be devastating for the foetus [7, 8]. In 1992, Shimoya et al. [9] showed the presence of interleukin 8 (IL-8) in trophoblast cells and macrophages in first, as well as in the third trimester of pregnancy with the elevated expression of this cytokine during the course of chorioamnionitis. Interleukin-10 (IL-10) is a pleiotropic anti-inflammatory cytokine involved in limiting and terminating inflammatory responses, recently defined as a ‘governor’ of inflammation [10]. Activated Th2 cells are the major source of IL-10 production, and IL-10 has been classically considered a Th2 cytokine marker. During pregnancy, sources of IL-10 include cytrophoblasts, syncytiotrophoblasts, and chorion as well as decidual
mononuclear cells/macrophages and decidual natural killer (NK) cells [11, 12, 13].

Pro-inflammatory cytokine, interferon gamma (IFN-γ), along with the interleukin 12 is a major promoter of differentiation of naïve Th cells into Th1 cell subpopulation that can induce the creation of hostile environment for the foetus. IFN-γ plays important roles in diverse cellular processes, including activating innate and adaptive immune responses, inhibiting cell proliferation, and inducing apoptosis [14]. It is also crucial in the immune responses against pathogens and immunosurveillance of tumours [15]. Cytokines in plasma or serum from healthy, non-pregnant adults are predominantly pro-inflammatory (Th1 type). Gradually, across the duration of normal pregnancy, women invert this ratio and become type 2 (regulatory) cytokine dominant in the third trimester [16]. Women showing type 1-dominant cytokine profiles in the third trimester are considered to have threatened pregnancies [16]. There are multiple sources for plasma cytokines in pregnant women, including trophoblasts, maternal endothelial cells, and circulating leukocytes. By the third trimester, the circulating leukocytes have acquired an activated phenotype, with gains in expression of CD11B, CD14, CD64, and intracellular reactive oxygen species. This has provided the concept that pregnancy is a pro-inflammatory state. Normally in late pregnancy, MHC class II expression on leukocytes is decreased, and IFN-γ levels are low [16]. A cascade of events proceeding from the Th1 immune-dominance could either act through stimulation of the uterine contractions (through the activation of prostaglandins) or by softening the cervix and weakening the chorioamniotic membranes (through the action of metalloproteinases) thus leading to their rupture. Sialidases and prolidases are the enzymes produced by anaerobic bacteria which are likely involved in the pathogenesis of bacterial vaginosis. The action of these enzymes may potentially alter the immune signals.

Pro-inflammatory cytokines could be a good marker of the intrauterine infections, whereas anti-inflammatory cytokines could be very important for normal course of pregnancy [14].

OBJECTIVE

The aim of the study was to investigate if local cervical infection could influence the serum levels of IFN-γ, IL-8 and IL-10 in pregnant women with the clinical symptoms of the imminent preterm delivery.

METHODS

The study enrolled 128 pregnant women at a gestational age ranging from 24 to 30 weeks of gestation (WG), admitted at the Department of Gynecology and Obstetrics at the Clinical Center of Vojvodina (Novi Sad), with the clinical symptoms of the imminent preterm delivery (presence of contractions of the uterus, but without cervical ripening or an evident rupture of membranes). Gestational age was based on last menstrual period and was confirmed by an early first trimester ultrasonography. The protocol was approved by the Institutional Ethics Board of the Faculty of Medicine, University of Novi Sad and Clinical Centre of Vojvodina (Novi Sad). Each patient was provided with the informed consent. Investigated pregnant women were divided in two groups according to the following criteria: 1) patients with the cervical bacterial infection (CB) or Candida infection (CI) or Chlamydial infection (Chl) (n=85, study group) and patients without CB, CI and Chl (n=43, control group).

Some other factors that can cause preterm delivery, such as general factors (mother diseases: cardiovascular diseases, preeclampsia, kidney diseases, urinary infections, diabetes mellitus; local factors: uterine malformation, cervical insufficiency, uterine and adnexal tumours, Asherman's syndrome, cervical conisation, other genital infections) and obstetric risk factors (multiple pregnancy, polyhydramnion) were excluded in all patients [17]. Furthermore, the factors which can 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.

Microbiological diagnosis was performed with direct cervical swab preparation coloured by methylene blue and by Gram stain. Cultivation of the material taken with cervical swab was performed by standard bacteriological techniques (isolation and identification), while Candida was cultivated on Sabouraud agar plates. Presence of Chlamydial infection was detected by Chromatographic immunoassay kit (BIOKIT S.A. LIICA d’ AMUNT, Barcelona, Spain) and by direct fluorescent microscopy (DIF test). All patients with positive cervical swab were treated with antibiotics according to drug susceptibility test (mostly from cephalosporin or penicillin groups), after the samples were collected from the patients.

Serum samples preparation and immunoassay for IFN-γ, IL-8 and IL-10: Venous blood was collected from all patients during the first 24 hours after the first symptoms of preterm delivery. Serum was obtained by centrifugation at 4000g for 15 minutes and stored at -20° C before use. Measurements of cytokines in the serum were performed by ELISA test (R&D Systems). Absorbance was measured in duplicates with a microplate reader (Beckman Coulter). The final concentration was expressed in pg/ml. Sensitivity of method was 8.0 pg/ml for IFN-γ, 3.5 pg/ml for IL-8, and 3.9 pg/ml for IL-10.

Dexamethasone therapy (four intramuscular doses of 6 mg given 12 hours apart) [18, 19] was administered in all patients for foetal lung maturation. In addition, the patients received tocolytic therapy (atosiban: amp. 0.9 ml iv. in bolus; after 2 amp. of 5 ml in 90 ml 0.9% NaCl in duration of 3 hours with the infusion pump; after 2 amp. of 5 ml in 90 ml 0.9% NaCl in duration of 24 hours with the infusion pump) and sedatives from benzodiazepine group (2 times tbl. 5 mg or amp. 10 mg). All the aforementioned therapy was introduced 30 min after the maternal blood collection and there was no significant difference in that interval between the two groups of patients.

doi: 10.2298/SARH1310623B
Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) for Windows. The results were statistically evaluated with the nonparametric Mann-Whitney test, and p-values less than 0.05 were considered statistically significant.

RESULTS

Maternal demographic characteristics (age of patients, gestational age, history of pregnancies and deliveries) in study group (SG) and control group (CG) were presented in Table 1. The isolated microbiological strains from cervical swabs of patients from study group were shown in Table 2. The following microbes from study group have been identified: Chlamydia trachomatis in 27, Candida sp. in 18, Escherichia coli in 12, both Enterococcus and Staphylococcus sp. in 8, Klebsiella pneumoniae in 7 and Streptococcus agalactiae in 5 pregnant women.

Serum IFN-γ levels in patients from study and control groups were summarized in Table 3. IFN-γ was detected in 32% of study cases and in 29% of the controls. The mean value of serum IFN-γ level in patients from the study group (pregnant women with the cervical infection) was 5.13±17.45 pg/ml. In the control group, the mean IFN-γ value was 3.23±5.48 pg/ml. The mean value of serum IFN-γ level in study group was not significantly different from that in the control group (p=0.632).

Serum levels of IL-8 in patients from study and control groups were illustrated in Table 4. IL-8 was detected in study group in 53% of cases and in 41% of patients from the controls. The mean value of serum IL-8 level in study group was 17.4±11.88 pg/ml. In the control group, the mean value of serum IL-8 level was 12.2±7.48 pg/ml. The mean value of serum IL-8 level in study group was not significantly different from that in the control group (p=0.712).

Serum levels of IL-10 in patients from study and control groups were demonstrated in Table 5. IL-10 was detected in study group in 61.4% of cases and in 65.7% of patients from the controls. The mean value of serum IL-10 level in patients from the study group (pregnant women with the cervical infection) was 19.4±14.9 pg/ml. In the control group, the mean value of serum IL-10 level was 21.2±9.58 pg/ml. The mean value of serum IL-10 level in study group was not significantly different from that in the control group (p=0.676).

Pregnancy outcome in the investigated groups of patients was shown in Table 6. In the study group, there were 30 (35.3%) preterm and 52 (61.1%) term deliveries, while in the control group, 17 women (39.5%) and 24 (55.8%) had preterm and in term deliveries, respectively. There were also 3 deaths of infants (3.6%) recorded in the study group and 2 (4.7%) in the controls. There was no statistically significant difference in distribution of pregnancy outcomes between two investigated groups of patients (p=0.875).

DISCUSSION

Cytokine pathways in pregnancy have been extensively investigated, with most studies examining the cytokines in
IFN-γ is defined as a pro-inflammatory cytokine belonging to Th1 immunological profile. It is a major promoter of differentiation of naive T cells into Th1 cell population. Almost all Th1 cells can produce IFN-γ, which predominantly activates antigen-presenting cells, macrophages, neutrophils and NK cells and stimulates production of other interleukins such as IL-1, IL-6, IL-8, IL-18 and tumour necrosis factor α (TNF-α). In normal pregnancy at term, IFN-γ is not detectable in amniotic fluid and is found at low concentrations in plasma and in significant amount in placenta, amnion, and chorio decidua [22]. Wilke et al. [23] reported that women delivering preterm had lower plasma IFN-γ between weeks 20 and 25 of gestation than women delivering at term, and that these levels tended to rise rather than decline between mid-gestation and birth. Not all studies find cytokine differences between these groups of women, or use common assay techniques. Even in women with the active malarial infection within their placentas, IFN-γ levels could not be correlated with preterm delivery [24]. In the paper of Piccinni [25], the concentrations of IFN-γ produced by decidual T cells of women with the unexplained recurrent abortions (URA) and normal pregnancy did not differ. No increased production of IFN-γ by decidual T cells was found in URA, as could be expected because of the potential role of Th1-type cytokines on allograft rejection, bearing in mind the paradigm of the foetus as the semi-allograft. Therefore, it might be appropriately concluded that there is a bias against type 1 cytokine expression and function in pregnancy. Type 2 cytokines may not be essential to pregnancy per se, but they may provide a bias away from type 1 cytokines. Surely Th1 cytokines, depending on their time of expression, the stage of gestation and their relative concentrations, could have a positive influence on pregnancy.

The status of pro-inflammatory cytokines in the maternal serum or in the amniotic fluid during infection and shortly before parturition has also been extensively described [26]. In the study of Alvarez de la Rosa et al. [27], the authors noted that the concentration of the maternal serum interleukins 1, 6 and 8 in women 24–37 weeks who were in preterm labor, was elevated but not significantly in comparison to those who were not in labor. Torbe et al. [21] found in their study that only the concentrations of IL-1β were significantly higher in the serum of mothers with preterm delivery in conjunction with the early onset of infection in the newborns. IL-10 is believed to be a key cytokine for the maintenance of pregnancy. Production of this cytokine is significantly reduced in the placenta at term without labor compared with that in the first and second trimester tissues, suggesting that downregulation of IL-10 is a physiological event that favours an inflammatory state around the time of the onset of labor [28]. Cytokine and their receptor polymorphisms may explain some of the risk variations between women. High secretory genotypes of IFN-γ and IL-10 have been associated with an increased risk of the recurrent early pregnancy loss [29].

One can assume that the serum measurement of IFN-γ, IL-8 and IL-10 concentrations in women with the cervical infections and preterm delivery is of limited significance. Local alterations in cytokine profiles tend to remain relatively compartmentalised: indeed, although cervical mucus levels of some cytokines, like IL-8, are significantly higher in patients prior to miscarriage, these alterations are not always reflected in the circulation. [30] On the other hand, induction of Th1 immune response (IFN-γ being one of its major representatives), could be driven by multiple factors and cervicovaginal infection is only one of it. Once a chain of events for preterm delivery has been initiated, there are no major differences in cytokines concentrations proposed to have a role in it, regardless of the cause that triggered the process of preterm delivery.

CONCLUSION

The results of our study suggest that there is no significant difference in serum IFN-γ, IL-8 and IL-10 concentrations between pregnant women with the symptoms of the imminent preterm delivery who had and had no cervical infection. The conclusion may be that the physiologic cytokine network in circulation during pregnancy is irrespective of the presence of the cervicovaginal infections. Possible explanation for that fact could be the compartmentalisation of the immune response during the timecourse of the genital infection. Second explanation for our findings could be the fact that the induction of Th1 immune response (IFN-γ being one of the major representatives), which creates the hostile environment for the foetus, could be driven by multiple factors. Once a chain of events for preterm delivery has been initiated, there are no major differences in responsible cytokines concentrations, regardless of the cause triggering the process of preterm delivery.

The reported studies on measurement of cytokines in maternal serum in preterm labour or in the presence of the infection are still rare and have involved a small number of patients, so further studies are needed with standardized methods and in larger sample sizes.

ACKNOWLEDGEMENTS

The work was supported by the grant of the Regional Secretariat for Science and Technological Development of Vojvodina (Grant No. 114-451-00592/2005-01).
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Интерферон гама, интерлеукин 8 и интерлеукин 10 у серуму жена са инфекцијом грлића материце и претећим превременом порођајем

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КРАТАК САДРЖАЈ
Увод Превремени порођај се дефинише као порођај пре навршене 37. недеље трудноће. Појава превременог порођаја значајно повећава морбидитет и морtalитет новорођенчега. Истраживања у свету послећих година указују на значај интраутерине, цервициналне бактеријске и хламдијалне инфекције у настанку превременог порођаја. Одговор домаћина на интраутерину, односно цервико-вагиналну инфекцију праћен је ослобађањем различитих инфламаторних медијатора, од којих неки припадају групи цитокина.
Циљ рада Циљ истрживања је био да се утврди да ли инфекција грлића материце утиче на ниво интерферона гама (IFN-γ), интерлеукина 8 (IL-8) и интерлеукина 10 (IL-10) у серуму жена с клиничком сличом претећег превременог порођаја.
Методе рада У испитивању је укључено 128 трудница (24–30. недеље трудноће) с клиничком сличом претећег превременог порођаја. Испитаници су сврстане у студијску групу (85 жена с инфекцијом грлића материце) и контролну групу (43 жена без ове инфекције). Ниво цитокина у серуму одређиван је комерцијалним тестовима ELISA.
Резултати Није утврђена статистички значајна разлика у нивоима IFN-γ (p=0,632), IL-8 (p=0,712) и IL-10 (p=0,676) у се- руму између две испитиване групе трудница.
Закључак Резултати истрживања су показали да није било статистички значајне разлике у концентрацији цитокина у серуму између трудница са симптомима претећег превременог порођаја са инфекцијом грлића материце и без ње.
Кључне речи: интерферон гама; интерлеукин 8; интерлеукин 10; превремени порођај; бактеријска инфекција; Candida; Chlamydia trachomatis