

BACTERIAL VAGINOSIS: RISK FACTORS AND VAGINAL LAVAGE CYTOKINES IL-1 β , IL-1RA

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ABSTRACT

Background: To analyse risk factors for bacterial vaginosis (BV); to evaluate concentrations of cytokines IL-1 β and IL-1ra in vaginal lavage of pregnant women and analyze their relation with BV.

Materials and methods: Vaginal lavage samples were obtained from 81 women who were between the 18th and 22nd week of an uncomplicated singleton pregnancy. BV was diagnosed pursuant to Amsel criteria and Nugent scoring. Cytokines were measured by immunoassay.

Results: Living place, education, working and living conditions, gynaecological history increased the risk of BV. Cytokine IL-1 β was not found in the vaginal lavage of women without BV; cytokine IL-1 β was always found in vaginal lavage of women with BV. Cytokine IL-1ra concentration was higher in women with BV.

Conclusion: Social factors, history of gynaecological diseases, peculiarities of sexual life-style are some risk factors for development of BV. Higher levels of cytokines in the vaginal lavage of pregnant women may lead to detection of lower genital tract infection.

INTRODUCTION

A leading hypothesis for the cause of intrauterine infection is the ascent of the pathogenic microorganisms from the lower genital tract. A number of previous investigators have described the relationship between lower genital tract marker and upper genital tract infection and/or inflammation. One of these markers may be bacterial vaginosis (BV). In accordance with the data reported by the majority of authors, BV is diagnosed in every fifth pregnant woman [1]. BV has been linked with preterm labour and delivery, chorionamnionitis,

postpartum endometritis, postcesarean delivery wound infections [2].

The mechanisms linking BV and adverse obstetric sequelae are unclear. It was once believed that pathogenic microorganisms alone were responsible for these poor outcomes. It has recently been established, however, that many of these sequelae are mediated by endogenous host products. It has been demonstrated that the levels of certain cytokines in the lower genital tract are altered in the presence of BV [3]. Interleukin-1 β and Interleukin-1ra are proinflammatory cytokines that have been found to be elevated in the cervical secretions of both pregnant and nonpregnant women with BV [3, 4].

The main purpose of this study was to investigate risk factors of BV and analyse their correlations with concentrations of cytokines IL-1 β and IL-1ra in vaginal lavage of pregnant women.

MATERIALS AND METHODS

Pregnant women who during the period of 2005 - 2006 for the first time visited Vilnius City Šeškinės Polyclinic in order their pregnancy to be followed-up at women's consultation was investigated. All these women visited the consultation till the 12th week of pregnancy. Main screening criteria included: 18-35 years old, the first pregnancy, singleton pregnancy, duration of pregnancy up to 12 weeks.

Attention was also paid to additional screening criteria. The pregnant women with history of harmful habits, serious gynaecological or general illnesses were excluded from the investigation. These screening criteria were met by 261 pregnant women.

All the pregnant women underwent routine clinical, biochemistry, anthropometric tests and cytological examination of cervicovaginal discharge during the first visit; all these women also had to complete an original Questionnaire at this visit.

The examination of 261 pregnant women revealed BV in 50 women (19.6%); the diagnosis was confirmed by means of cytological examination. BV was diagnosed pursuant to Amsel's criteria and Nugent's scoring. These women were included into a case group (3 of them withdrew from the study later).

Vaginal lavage samples were obtained from October 2005 through April 2006 from 81 women who were between the 18th and 22nd week of an uncomplicated singleton pregnancy. Among them, 34 women were of the control group (BV⁻), while 47 ones ill with BV were from the case group (BV⁺).

To collect vaginal lavage, a disposable sterile 10 ml syringe was filled with sterile physiological solution and the cervical os was washed with a stream of solution. Then the pooled lavage was aspirated from the posterior fornix and collected into 15 ml conical polypropylene tube. The tube transported on ice within 4 h of collection to Vilnius University Institute of Immunology. The samples of vaginal lavage were centrifuged at 1500 rpm for 14 min. The resulting supernatant was poured into 1.5 ml micro tube and frozen at - 80°C temperature.

Cytokine IL-1β and IL-1ra measurement

The supernatants of vaginal lavage samples were analyzed undiluted for IL-1β and IL-1ra concentrations by commercial RayBio Human IL-1β and RayBio Human IL-1ra ELISA kits (RayBiotech, Inc, USA) in accordance with manufacturer's recommendations. The values were converted to pg/ml (IL-1β) or ng/ml (IL-1ra) by reference to a standard curve that was generated in parallel to all test samples. The limit of sensitivity was 0.3 pg/ml for IL-1β and 0.1 ng/ml for IL-1ra.

Statistical analysis

The statistical analyses were performed using the statistical programmes – *SPSS for Windows (version 8.0)*. Values of parameters with standart deviations and 95% confidence intervals (CI) were calculated. Differences between the groups were tested with the two-sample *t*-test for normal distribution, and the Mann-Whitney *U*-test when normality could not be assumed. The χ^2 (chi-square) test was used to test for differences in categorical variables. Differences were considered significant when the reliability level was $p < 0.05$.

Approval for the study was obtained from the Lithuanian Medical Ethics committee (No. 49, 2005-10-04)

at each participating center and all patients gave their informed consent after oral and written information.

RESULTS

Mean age in both groups was nearly similar (BV⁺ – 27.7 years, BV⁻ – 26.9 years) and did not differ statistically reliably (95% CI = 26.5 – 28.2). A majority of women tested were townswomen (80.2%, 65/81), among them 52.3% (34/65) were with BV⁺. Countryside women BV⁺ made 81.3% (13/16). The women who live at the countryside were BV⁺ more frequently in comparison with women living in towns ($p < 0.05$, $\chi^2=4.4158$). The level of education of women was very different. Three women (3.7%) had incomplete secondary education and all of these with BV⁺, 33 (40.7%) secondary education, 9 (11.1%) incomplete university and 36 (44.4%) university education. The majority of women in the BV⁺ group had secondary education (24/47, 51.1%) and in the BV⁻ group the majority of pregnant women had university education (20/34, 58.8%). BV frequency was reverse to index of the education of pregnant women ($p < 0.05$, $\chi^2=8.5064$).

The women in this study were employed in very different types of work. The majority of women were not exposed to harmful factors when working (BV⁻ group 28/34; BV⁺ group 31/47). The women in the BV⁺ group were statistically reliably more frequently employed in shift works (BV⁻ group $n=1$; BV⁺ group $n=11$; $p < 0.001$), and women in control group were statistically reliably more frequently working with computers (BV⁻ group $n=19$; BV⁺ group $n=6$; $p < 0.001$) and their work was related with emotional stress (BV⁻ group $n=17$; BV⁺ group $n=8$; $p < 0.001$).

The living conditions of women examined were also very different. In BV⁻ group 21/34 (61.8%) of women had perfect living conditions, while the rest – satisfactory, and in BV⁺ group only 12/47 (25.5%) of women had perfect living conditions, 30/47 (63.8%) – satisfactory and 5/47 (10.6%) –unsatisfactory ones ($p < 0.001$, $\chi^2=12.4087$).

The women in both groups used different methods of contraception before pregnancy. The women BV⁺ used oral contraceptives more frequently. Their average duration of usage was 35.2 ± 11.53 months (ranges 12-48 months). Besides, the BV⁺ women used complex methods of birth control (intrauterine devices, spermicide and, especially, oral contraceptives) more frequently, too (Table 1).

Table 1 Contraception methods

Method of contraception	BV ⁻		BV ⁺		χ^2 test	95% CI	P value
	n	%	n	%			
Intrauterine devices	1	2,9	4	8,5	1,0566	0,0348-3,0531	-
Oral hormonal contraception	4	11,8	14	29,8	3,7075	0,0931-1,0605	<0,05
Spermicides	3	8,8	13	27,7	4,4158	0,0658-0,9728	<0,05
Condom	7	20,6	12	25,5	0,2685	0,2623-2,1799	>0,05
Coitus interruptus	6	17,6	11	23,4	0,3943	0,2310-2,1292	>0,05

The mean age of the start of sexual life in pregnant women BV⁻ group was 19.2 ± 2.63 years (age range 15-29 years), while in pregnant women BV⁺ was 18.9 ± 1.96 years (age range 16 - 25 years). Pregnant women ill with BV proved to be more sexually active. Within the first pregnancy period a majority - 36.2% (17/47) had sexual partners 3 - 4 times a week, while control group pregnant women - 1 - 2 times, respectively (47.1%, 16/34). However, statistically reliable difference of sexual activity between groups was not found ($p > 0.05$, $\chi^2 = 6.2790$). The women of BV⁺ group had a higher number of sexual partners within 6 month period before pregnancy. Even 27/47 BV⁺ group women had 2 - 4 sexual partners before pregnancy; while only 4/34 control group women had 2 partners ($p < 0.001$, $\chi^2=18.2939$). During pregnancy the count of sexual partners from both groups did not significantly differ ($p > 0.05$, $\chi^2=3.8550$).

Education and different illnesses of sexual partners of both group women was evaluated according to data of anonymous questionnaire inquiry. 2/3 sexual partners of BV⁻ group women had higher education (22/34, 64.8%), while in BV⁺ group women sexual partners had higher education by less than 1/3 (15/47, 31.9%). Secondary education of partners dominated in investigational group (18/47, 38.3%), while 4 sexual partners even had no secondary education. The difference of both group indices proved to be statistically reliable ($p < 0.05$, $\chi^2=10.5086$). Sexual partners of BV⁺ pregnant women within pregnancy were ill more frequently than partners of respective control group women. Attention is paid to the fact that among sexual partners of BV⁺ women almost every fourth was ill with bladder inflammation (11/47, 23.4%), while sexual partners of BV⁻ women, respectively, - 1/34, 2.9% ($p < 0.05$, $\chi^2 = 6.5460$).

While examining the influence of factors of gynaecological history on the development of BV, it has been detected that data of both groups regarding past gynaecological diseases differed greatly. Approximately one of five women in the control group was previously

ill with gynaecological inflammatory diseases (7/34, 20.6%). Approximately 3 of 4 women (35/47, 74.4%) of the BV⁺ group were formerly ill with gynaecological diseases including pelvic inflammatory disease ($n=16$, $p < 0.05$, $\chi^2=6.9883$), bartholinitis ($n=18$, $p < 0.05$, $\chi^2=7.0205$).

The morbidity was similar in the BV⁺ and BV⁻ groups before pregnancy. A similar count of both group women indicated such diseases: anaemia, allergic rhinitis, myopia ($p > 0.05$). 5/34 of the control group and 17/47 of case group women were ill with gastritis ($p < 0.05$, $\chi^2=4.5943$). Within pregnancy the women of both investigational groups were frequently ill with viral respiratory tract inflammation disease (rhinitis and pharyngitis). Frequently, anaemia, labial herpes, periodontitis, tonsillitis have been also diagnosed. BV⁺ women were ill with the diseases mentioned slightly more frequently. However, differences of morbidity indices were not statistically reliable ($p > 0.05$). Only BV⁺ group women were significantly more ill with labial herpes (9/47, 19.1%) in comparison to the control group (1/34, 2.9%) ($p < 0.05$, $\chi^2=4.7891$).

Main anthropometric data (height, weight and body mass index - BMI) were registered at the beginning, at the end of the pregnancy and upon arrival for delivery. The mean anthropometric findings of healthy women and of BV⁺ women were almost similar: the women were of average height 167.3 cm (ranges 157 - 180 cm); differences of body mass (at the beginning of pregnancy, mean 51 - 70 kg; $p > 0.05$, $\chi^2=2.0098$ and upon arrival for delivery 71 - 80 kg; $p > 0.05$, $\chi^2=1.5859$) and BMI were alike (respectively, mean 21.1 and 22.1). A slightly greater difference of BMI was observed in the subgroups of women who had BMI 30.0-35.0 before delivery. These findings were more frequent in the control group (17.6%), in comparison with BV⁺ group (10.6%); however this difference is not statistically reliable ($p > 0.05$).

All the women BV⁺ had cervicovaginal discharge typical for this pathology; "indicator cells", intermediate

and pathological flora were found, the amine tests were positive. Statistically significant differences ($p < 0.001$) were detected while comparing these results with those of the control group. During pregnancy vaginal discharge pH of all women in BV⁻ group (100%) proved to be less than 4.50, while in BV⁺ group during the first control (pregnancy < 12 weeks) 74.5% (35/47) women pH was > 4.5, during 18-22 weeks – 80.9% (38/47), 28-34 weeks – 40.4% (19/47), 35-40 weeks – 44.7% (21/47) and upon arrival for delivery – 53.2% (25/47). The values of pH in women BV⁺ were found to be increased throughout pregnancy, from the beginning until delivery ($p < 0.001$). A smaller amount of lactobacteria and greater numbers of flora characteristic for this pathology (*Gardnerella vaginalis* and *Mobiluncus* morphologic types) were found to be present in vaginal secretions of BV⁺ women. While comparing with the respective data of the control group in all cases significant differences have been detected ($p < 0.001$ in either case).

All 34 women of the control group and 47 women of the case group underwent cytokine concentration examination at 18th – 22nd week of pregnancy. Cytokine IL-1 β was not found in the vaginal lavage of the BV⁻ group women; cytokine IL-1 β was always found in vaginal lavage of women BV⁺, mean concentration – 2.0 pg/ml ($p < 0.001$). Cytokine IL-1ra concentration in the control group was found to be of very small levels only; the BV⁺ group showed mean 49879.0 ng/ml ($p < 0.001$) IL-1ra concentration (Table 2).

DISCUSSION

According to investigational results BV has been diagnosed for 50 pregnant women (19.16%). A similar BV frequency during pregnancy (15-20 of pregnant women) has been indicated also by other authors [5-7]. Demography factors, such as age of pregnant women, have no influence on BV beginning. However, such factors as living at the countryside, lower education,

and shift work are closely related to BV frequency ($p < 0.05$, $p < 0.05$ and $p < 0.001$, respectively). These data indicate that the quality of conditions of life as well as BV frequency are closely related – BV women lived under statistically reliably worse conditions of life ($p < 0.001$). Thus, it's not only attitude to hygiene culture but also possibility to satisfy these hygiene needs that is closely related to BV frequency. Similar data are indicated by other authors [8].

According to literature data, the influence of oral contraceptives on BV rise is estimated controversially. Some of the authors, all together, deny correlation between usage of oral contraceptives and BV frequency [9]. However, this study proves to determine statistically reliable index differences for frequency of oral contraceptives and spermicide use (for either cases $p < 0.05$).

Some clinical observations have suggested that, in cases of BV, treatment of the sexual partner may reduce the risk of recurrence; however, the topic is still controversial [10]. In this study, the number of sexual partners, but not the number of intercourses, was associated with an increased risk of BV. Women having more sexual partners before pregnancy were frequently diagnosed as disturbed vagina microflora during investigation ($p < 0.001$). Similar results are indicated by other authors [8].

It's paid attention to the fact that sexual partners of BV⁺ pregnant women were of lower education than those of ones of control group women ($p < 0.05$). This confirms a general tendency – education is related to medical personal culture and its attitude to hygiene requirements. Besides, morbidity of sexual partners during women pregnancy was more frequent in case group. Especially frequent disease of sexual partners was cystitis ($p < 0.05$).

References indicate that treatment with antibiotics is directly related to BV frequency, especially, when antibiotics are prescribed for gynaecological

Table 2 Mean cytokines IL-1 β (pg/ml), IL- 1ra (ng/ml) concentrations of vaginal lavage at pregnancy 18 – 22 weeks

Cytokines	BV ⁻		BV ⁺		P value (according to Wilcoxon - Man-Whitney U test)
	Median (25%,75%)	Min-max	Median (25%,75%)	Min-max	
IL-1 β	0 (0.0; 0.0)	0.0-0.0	2,0 (0.3; 8.6)	0.0-95.9	< 0.05 (statistical value = 741.0000)
IL-1ra	8349.5 (2600.0; 15737.0)	239.0- 54737.0	49879.0 (17501.0; 138615.0)	2600.0- 551470.0	< 0.001 (statistical value = 788.0000)
IL-1ra/ IL-1 β	0 (0.0; 0.0)	0.0-0.0	13835.0 (6059.0; 38611.0)	1360.0- 1634738.0	< 0.001 (statistical value = 595.0000)

inflammatory diseases [1]. Women of case group were more frequently ill with pelvic inflammatory disease and Bartholinitis (in either cases $p < 0.05$). In the past the women were treated with antibiotics. It explains close correlation of former inflammatory gynaecological diseases and BV.

Morbidity of women investigated before and during pregnancy, ill with different general diseases in case as well as in control group was similar. However, attention must be paid to the fact that pregnant women with BV statistically reliable were more frequently ill with gastritis ($p < 0.05$), as it was indicated by other authors [11]. During pregnancy BV⁺ pregnant women were statistically reliably ill with labial herpes more frequently than those of control group ($p < 0.05$).

On the basis of present data we may conclude that anthropometric parameters (height, body mass, BMI) do not effect the rise of BV. Some authors indicate that BV is influenced by obesity [1]. In this investigation obesity at the beginning of pregnancy has been detected only for one pregnant woman in case group.

Increased vaginal discharge pH is a typical criterion of BV diagnostics. This is also affirmed by increased pH in BV⁺ group during pregnancy ($p < 0.001$). In one's turn, increased pH induces further growth of anaerobic bacteria as well as disappearance of physiological lactobacteria [12, 13]. In women investigated, ill with BV, during pregnancy in vaginal discharge less lactobacteria and significantly more microorganisms, peculiar to vaginosis have been detected – *Gardnerella vaginalis* and *Mobiluncus* morphotypes. As compared to respective parameters of control group women, in all cases statistically significant difference has been detected. A majority of authors also indicate that *Gardnerella vaginalis* and *Mobiluncus* dominate in bacterial vaginosis ecosystem [12, 14-16].

Special attention during the present investigation was paid to evaluation of cytokine IL-1 β and IL-1ra concentration in the fluid of vaginal lavage. On basis of reference, in vaginal lavage of healthy pregnant women cytokine IL-1 β was not detected at all [15]. According to data of this investigation, in the fluid of vaginal lavage of control group women cytokine IL-1 β has also not been found, while in lavage of pregnant women BV⁺ cytokine IL-1 β has always been detected, average concentration – 2.0 pg/ml ($p < 0.001$). The ability of BV-associated endotoxins to induce cytokine production in monocytic cells may partly explain the increased IL-1 β levels. References indicate that BV⁺ women (pregnant and non pregnant) have significantly

higher cytokine IL-1 α and IL-1 β concentrations in their cervicovaginal discharge than BV⁻ women [12, 14]. Cytokine IL-1ra concentration in secretion of vagina and cervix of pregnant women is an informative index. While this cytokine is antagonist of cytokine IL-1 receptor, the decrease of their ratio of concentration in vaginal discharge indicates disbalance of the cytokines secretion and may be estimated as index of inflammation or intrauterine infection.

Potential limitations of this study should be considered. Firstly, this is a little number from the investigated pregnant women (total $n=261$, later of them 47 with BV⁺ and 34 BV⁻). Secondly, living conditions, sexual life-style, and morbidity, of women and their sexual partners were assessed according to the data of the anonymous questionnaire inquiry, which, in our opinion, could bring in subjectivity and do not fully reflect the real situation.

CONCLUSION

Social factors (education, living conditions), history of gynaecological diseases, peculiarities of sexual life-style are important risk factors for development of BV; however, the age and anthropometric status have no influence on development of BV. The increase of pH of vaginal discharge, decrease of lactobacteria counts and increase of *Gardnerella vaginalis* and *Mobiluncus* counts microorganisms are reliable diagnostic signs of bacterial vaginosis. Reliable correlation between diagnostic criteria of BV and concentration of cytokines IL-1 β , IL-1ra in fluid of vaginal lavage was established. Higher levels of cytokines in the vaginal lavage of pregnant women may lead to detection of lower genital tract infection.

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BAKTERINĖ VAGINOZĖ: RIZIKOS FAKTORIAI IR MAKŠTIES NUOPLOVŲ CITOKINAI IL-1 β , IL-1RA

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Santrauka

Raktažodžiai: bakterinė vaginozė, rizikos faktoriai, citokinas IL-1 β , citokinas IL-1ra.

Įvadas. Šio darbo tikslas – išnagrinėti bakterinės vaginozės (BV) rizikos faktorius, įvertinti citokinų IL-1 β ir IL-1ra koncentraciją nėščių moterų makšties nuoplovoje ir jų sąsają su BV.

Priemonės ir metodai. Paimtos 81 nėščios moters makšties nuoplovos. Tiriamųjų moterų nėštumas – nuo 18-os ir 22-os nekomplikuoto vienvaisio nėštumo savaitės. BV buvo diagnozuojama remiantis Amsel kriterijais ir Nugent vertinimo sistema. Citokinai matuoti imunologinės reakcijos būdu.

Rezultatai. Gyvenamoji vieta, išsilavinimas, darbo ir gyvenimo sąlygos, ginekologinė anamnezė padidino galimybę susirgti BV. Citokinų IL-1 β nerasta moterims, nesergančioms BV; citokinas IL-1 β sergančių BV makšties nuoplovoje visada nustatytas. Citokino IL-1ra koncentracija buvo didesnė sergančiųjų BV nuoplovoje.

Išvados. Socialiniai faktoriai, ginekologinių ligų anamnezė, seksualinio gyvenimo ypatumai turi įtakos BV išsivystymui. Didesni citokinų kiekiai nėščių moterų makšties nuoplovoje gali padėti nustatyti apatinių lytinių takų infekciją.

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