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Bacterial vaginosis in early pregnancy and adverse pregnancy outcome in Polish women

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ABSTRACT

Objective: To evaluate the impact of bacterial vaginosis and bacterial vaginosis-associated flora at early pregnancy and newborns' low birth weight (LBW) in indigent population of Central Poland.

Design: Prospective cohort study.

Setting: 10 district Outpatients Maternity Units in Lodz region, Central Poland. 179 randomly selected healthy pregnant women between 8 and 15 weeks' gestation.

Interventions: On the basis on Spiegel's criteria Gram-stained vaginal smears were examined for BV and bacterial vaginosis-associated flora.

Main Outcome Measures: The relationship between bacterical vaginosis and bacterical colonization of the lower genital tact during pregnancy and the risk of low birth weight (LBW) delivery.

Result: 26 (14.5%) women each delivered a low birth weight infant. Bacterial vaginosis were more frequently diagnosed among women with LBW as compared to reference group (46.2% vs.25.5%). BV constituted a significant risk factor for LBW (OR=2.51). Women who delivered a LBW infants were more likely to be culture-positive than those in reference group mainly for: Bacteroides spp.(OR=10.02). Gardnerella vaginalis (OR=4.94) and Mycoplasma hominis (OR=2.44). In logistic regression model after controlling for maternal height, pre-pregnancy weight and cigarette smoking only lower genital tract colonization by Gardnerella vaginalis at early pregnancy constituted a significant risk factor for delivering a LBW infant (OR=3.86 CI: 1.46 - 10.20).

Conclusions: Bacterial vaginosis and colonization of the lower genital tract by Gardnerella vaginalis at early pregnancy are the risk factors for LBW and indicates the need for detailed microbiological monitoring of pregnant women at early pregnancy or even before pregnancy.

Key words: bacterial vaginosis, risk factors, infections, pregnancy, low birthweight. microbiology

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INTRODUCTION

Low birth weight (below 2500 grams) constitutes one of the major hazards in perinatology and neonatology. The most important factors influencing the LBW rate is prematurity and intrauterine growth restriction. The pathogenesis and etiology of LBW is multifactorial, but there is now substantial evidence that subclinical infection, ascending into the uterine cavity from the lower genital tract, is associated with preterm labor and birth[1,2,3]. It has been demonstrated that infection, and the ensuing inflammatory response, can stimulate production of prostaglandins and cytokines from decidua and chorioamnion cells leading to the onset of labour[4,5]. It has been documented in many studies[6,7,8] that bacterial vaginosis (BV) is an important risk factor for chorioamnionitis and /or amniotic fluid infection. Many microorganisms from lower genital tract, classified as bacterial vaginosis associated flora, have been implicated in the inflammatory process[9]. All bacterial infections are the outcome of a dynamic interaction between the host's immune system and the virulence determinants of the infecting organism. Hosts have developed specific and non-specific factors to protect themselves from invading pathogens, while these pathogens have evolved mechanism to subvert these defenses and cause the disease process. It is noteworthy that infectious agents display a wide spectrum of virulence: some may cause disease in otherwise healthy individuals, whilst others can only infect the immunocmpromised patients. The endogenous vaginal microbiota, for example, is multitudinous, diverse and dynamic, and is made up of mixed aerobic organisms of varying virulence potential.

Furthermore, within a single species of vaginal microbiota there may be sub-populations of strains with differing pathogenic potential. In pregnancy, however, host factors of relevance for outcome of a particular infection will depend on the stage of gestation, previous maternal exposure and immunity, individual immune response variability, the effectiveness of the placental barrier and the development of fetal immunity.

Thorsen et al.[10] evaluated the association between various microorganisms isolated from lower genital tract in pregnant women with bacterial vaginosis. After controlling for the presence of other microorganisms, strong associations between Gardnerella vaginalis, anaerobic bacteria and Mycoplasma hominis and present bacterical vaginosis were found. Lactobacillus spp.were found to be associated with the absence of BV. Still the prevalence and the impact of various microoganisms isolated from lower genital tract on LBW is unclear, mainly due to observed race, ethnicity and populations differences in vaginal flora ecology and various gestational age at the time of examination[11].

The main aim of this prospective study was to determine the relationship between bacterial vaginosis - associated flora at early pregnancy and the incidence of low birth weight (LBW) in newborns from the indigenous population of Central Poland.

Methods

The study population comprised randomly selected 196 pregnant women attending 10 district Outpatients Maternity Units in the Lodz (Central Poland) region. Only singleton pregnancies between 8-16 weeks of pregnancy were qualified for inclusion in the survey. Women with chronic disease diagnosed during the first prenatal visit were not considered in the study. A standard questionnaire covering medical, socio-economic, demographic, constitutional and environmetal features was administered to every subject and verified based on medical records. Full medical data was available for 179 women who finally constituted the study population.

Cervicovaginal samples were obtained from all pregnant women under study. Gram-stained vaginal smear were examined for BV and bacterial vaginosis-associated flora were sought by culture.

Bacterial vaginosis was diagnosed by Gram's stain according to spiegel' criteria[12], and the flora were graded as follows:

grade I - normal - predominantly lactobacillus morphotypes

grade II - intermediate - mixed lactobacillus morphotypes and other morphotypes;

grade III - bacterial vaginosis - few or absent lactobacillus morphotypes, but greatly increased number of G. vaginalis and other bacterial morphotypes.

For isolation, identification and differential titration of genital mycoplasmas from cervical canal the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification was based on specific hydrolysis of urea (U. urealyticum) or arginine (M.hominis) by the species present in specimen, which is indicated by a change in colour of the well containing the relevant substrate, without clouding of the medium. Titration based on the principle of dilutions in liquid medium is expressed as the number of cfu/ml specimen (colony-forming units/ml). The technique allows titration around the levels of 10^3 cfu/ml and 10^4 cfu/ml which are accepted pathogenicity threshold levels.

The vaginal swabs were testing for facultative and anaerobic bacteria. The swabs were placed onto appropriate plates. Sheep blood agar, Mac Conkey, D-Coccosel agar, Gardnerella agar, Azide blood agar (Bio-Merieux) and Staphylococcus Medium 110 (Oxoid Ltd) plates were used for isolation of aerobic organisms. Schaedler blood agar (BioMerieux) and

Table 1. The relationship between BV diagnosed by Gram's stain at early pregnancy and the risk of delivering a LBW infant.

	Newborns' birth weight				OR, 95%CI
	<2500 grams (n=26)		≥2500 grams (n=153)		
BV(-), group I and II	14	53.8	114	74.5	Reference group
BV(+), group III	12	46.2	39	25.5	2.51 (1.07-5.88)

Table 2. The relationship between lower genital tract colonization at early pregnancy and the risk of LBW

	Newborns' birth weight				OR, 95%CI
Culture(cfu/ml)	<2500 (n=26)		≥2500 grams (n=153)		
Bacteroides spp.					
Culture(-)	22	84.6	147	96.1	Reference group
$< 10^5$	1	3.8	4	2.6	1.67 (0.03-17.87)
$\geq 10^5$	3	11.5	2	1.3	10.02 (1.06-123.39)
Mobiluncus spp.					
Culture(-)	24	92.3	143	93.5	Reference group
$< 10^5$	1	3.8	6	3.9	0.67 (0.01-65.28)
$\geq 10^5$	1	3.8	4	2.6	1.49 (0.03-15.88)
Prevotella spp.					
Culture(-)	25	96.2	138	90.2	Reference group
$< 10^5$	0	0	3	2.0	1.84 (0.03-23.88)
$\geq 10^5$	1	3.8	12	7.8	0.46 (0.01-3.39)
G. vaginalis					
Culture(-)	13	50.0	122	79.7	Reference group
$< 10^5$	3	11.5	12	7.8	2.35 (0.37-10.34)
$\geq 10^5$	10	38.5	19	12.4	4.94 (1.66-14.11)
M. hominis					
Culture(-)	19	73.1	130	85.0	Reference group
$< 10^5$	2	7.7	9	5.9	1.52 (015-8.17)
$\geq 10^5$	5	19.2	14	9.2	4.94 (0.61-8.24)
U. urealyticum					
Culture(-)	20	76.9	114	74.5	Reference group
$< 10^5$	3	11.5	16	10.5	1.07 (0.18-4.26)
$\geq 10^5$	3	11.5	22	14.4	0.78 (0.14-2.98)
Ch.trachomatis					
Non-detected	19	73.6	115	75.2	Reference group
Detected	7	26.9	38	24.8	1.11 (0.39-3.09)

Table 3. Logistic regression model for bacteriological findings at early pregnancy and the risk of LBW

Microorganism	OR	95% CI
Bacteroides spp.	6.07	0.77-47.65
G. vaginalis	3.86	1.46-10.20
M. hominis	1.00	0.73-1.38

* after controlling for maternal weight, pre-pregnancy weight and cigarette smoking

Rogosa agar (Oxoid Ltd) were inoculated for anaerobic cultures. After the incubation period, the strains of bacteria were identified by biochemical tests - API (BioMerieux).
Delivery of newborn with birth weight below 2500 grams was classified as LBW.
To evaluate the risk factors, the odds ratios (OR) were calculated. Statistical analysis was carried out using statistical program EPI INFO. The logistic regression model was applied to evaluate the independent role of selected bacteria after controlling for confounding factors.

Results

The mean pregnancy duration at the time of microbiological analysis was 12.3 weeks and the mean age of the subjects was 26.1 years. 24 women (13.4%) were under 20 years old, 45.8% women had pre-pregnancy weight below 55 kilograms and 22.3% had height below1.60m. The examined population was characterized by: primary educational level (40.2%), single marital status (26.3%) and unemployment (40.0%). 27 pregnant women (15.1%) admitted smoking more than 5 cigarettes a day during gestation.
Readings of the vaginal Gram-stains of 179 examined pregnant women showed that 51 (28.5%) of them had bacterial vaginosis, 62 (34.6%) had vaginal flora grade II (intermediate) and among 66 (36.9%) grade I (normal) flora was diagnosed.
26 (14.5%) of the analyzed women delivered a low birth weight newborns, while 153 constituted a reference group (non-LBW). Bacterial vaginosis diagnosed at early pregnancy constituted an important risk factor for delivering a LBW infant (OR=2.51), (Table 1). Almost half of the women in LBW group had BV diagnosed at early pregnancy.
The relations between bacterial vaginosis associated flora at early pregnancy and low birth weight is shown in Table 2. Women who delivered a LBW infants were more likely to be culture-positive than those in reference group mainly for: bacteroides spp. (OR=10.02), Gardnerella vaginalis (OR=4.94) and Mycoplasma hominis (OR=2.44).
The logistic regression model was applied to evaluate the independent role of microorganisems which constituted a risk factor in univariate analysis (Bacteroides spp, Gardnerella vaginalis and Mycoplasma hominis) after controlling for confounding factors such as: maternal height and pre-pregnancy weight and cigarette smoking (Table 3). Only lower genital tract colonization by Gardnerella vaginalis at early pregnancy constituted a sigificant risk factor delivering a LBW infant (OR=3.86 CI: 1.46 - 10.20).

Discussion

Bacterical vaginosis is a clinical syndrome based on altered genital microflora in which together with reduced concentrations of Lactobacillus species Gardnerella vaginalis anaerobic species like: Prevotella, Bacteroides, Peptostreptococcus and Mobiluncus and genital mycoplasmas become predominant in vaginal secretions. Bacterial vaginosis affects 10-40% of pregnant women[13], In our study BV was diagnosed among 28.5% of pregnant women at 8-16 weeks of gestation by Gram's stain using Spiegel's criteria. Almost half of the women (46.2%) in LBW group had BV diagnosed at early pregnancy. This relatively high incidence of BV could be explained by negative socio-economic characteristic of the Central Poland indigent population under study and by use of relatively sensitive diagnostic method of BV. According to Tam et al.[14] sensitivity of Gram stain method was significantly higher than that of clinical criteria (91% vs. 46%). The Gram stain method has both a low false-negative (4%) and high negative predictive values (96%).
The loss endogenous vaginal lactobacilli is important in the acquisition of the bacterial vaginosis. A cohort study of 182 women attending a genitourinary medicine clinic in the Unied States found that the acquisition of bacterial vaginosis was independently associated with a specific lack of vaginal H_2O_2-producing lactobacilli (HR 4.0, $P<0.001$). This relationship was not seen for women with candidiasis and trichomoniasis[28]. Close analysis of the H_2O_2 - producing lactobacilli has shown them to be capable of reacidifying the vagina following coital vaginal neutralisation by male ejaculate. During bacterial

vaginosis there is a loss of vaginal acidity (pH>4.5) that correlates with the loss of these lactobacilli. The question remains as to what stimulates the lactobacillus loss.

The development of bacterial vaginosis may centre on hormonal changes. A new episode of bacterial vaginosis often occurs after menstruation when oestradiol levels increase[30,31]. Models using the mice have shown that increased levels of oestradiol, characteristic of pregnancy, are associated with increased levels of genital colonisation by mycoplasmas[32].

Moreover, fourty percent of preterm births are associated with an infective aetiology, but it is unclear how much of this could be classified as bacterial vaginosis. Prospective studies have reported attributable risks of between two and ten for bacterial vaginosis in pregnancy leading to preterm delivery rising to over 30 in women with a history of a previous preterm birth[33,34]. It has been suggusted that the production of endotoxins by the bacterial vaginosis microflora stimulate susceptibility, in certain women, to the cascade of cytokines and prostaglandins that initiate labour[35]. Many studies have only found a statistically significant association when restricting analysis to women considered as at a high risk of pretem delivery. This is has been highlighted by several randomised controlled trials, investigating the potential health gains to be made from treating bacterial vaginosis during pregnancy. The picture may become clearer through the results of the multicentre ORACLE trial which aims to investigate the association of subclinical infection with preterm labour and the effect of broad spectrum antibiotic treatment on the rate of preterm birth[36].

Bacterial vaginosis is a well known cause of perinatal complications mainly: preterm delivery, low birth weight, preterm ruptures of membrances, chorionamnionitis and postpartum endometritis[15,16,17]. Pathogenic mechanisms include an ascending route of infection and inflammatory process due to microbial products and fetal and/or maternal responses with production of cytokines and prostaglandins[1]. Wasiela et al,[18] showed that certain bacteria strains isolated from genital tract and associated with bacterial vaginosis, such as: U. urealyticum, M. hominis, Bacteroides capillosus and Prevotella disiens were able to activate human placenta mast cells to mediators release. Ureaplasma urealyticum and Mycoplasma hominis are capable of TNF-alpha induction and could stimulate inducible nitric oxide synthase production from murine macrophages, it is also possible that they could either induce or act as a catalyst and augment inflammation which in turn leads to spontaneus preterm delivery[19]. Hillier has shown that bacterial vaginosis is associated with the more frequent occurence of various bacteria including: M.hominis, U.urealyticum, G.vaginalis and some anaerobic strains[20]. The distribution of bacterial species could differ between the populations. According to Royce et al.[11] there are great race/ethnicity differences in vaginal flora during pregnancy diagnosed between 24 and 29 weeks of gestation. Blacks were more likely to have PH above 4.5, no lactobacilli and positive culture for Mobiluncus spp. (12.0% vs. 1.3% among whites). Those differences may play a role in larger proportion of preterm deliveries among black women compared with white pregnant women. In our study univariate analysis revealed that women who delivered a LBW infants were more likely to be culture-positive than those in reference group mainly for: Bacteroides spp.(OR=10.02). Gardnerella vaginalis (or=4.94) and Mycoplasma hominis (or=2.44). Kovavusarach et al.[21] in case-control study determined microorganisms related to premature rupture of the membranes (PROM) in term pregnant women. Candida albicans and Klebsiella pneumonie were the only two significant differences demonstrated between PROM and reference group. There was no difference in prevalence of Garderella vaginalis between study and control group (14.1% vs 16.45). In our study the overall prevalence of Gardnerella vaginalis colonization at early pregnancy was 24.5% (50.0% in LBW Group and 20.2% in control group) what is in concordance with results obtained by Mendoza-Gonzales et al.[22] in non pregnant Mexican population. In multivariate analysis, lower genital tract colonization by Gardnerella vaginalis at early pregnancy, constituted a significant risk factor for delivery of a LBW infant (OR=3.86 CI: 1.46 - 10.20) in our study. According to Cauci et al.[23] there is a correlation between IgA degradation, detected in vaginal washings, and absence of immune response to Gardnerella vaginalis cytolysin. The authors concluded that patients with BV having extensive IgA degradation in their secretion together with high sialidase activity could incur more dangerous infections and adverse pregnancy outcome probably not only coloization by selected microorganisms but also impairment of the mucosal immune system are responsible for poor perinatal outcome observed among pregnant women with pathological microflora.

Leszezynski et al.[24] analyzed the incidence of Bacteroides fragilis among 120 term pregnant women. 6.6% of these women were culture positive what is close to overall prevalence of Bacteroides spp. obtained in our study - 5.6%. The incidence of Bacteroides colonization among women who delivered LBW infants was much higher - 15.4% (OR=10.02). In multivariate analysis after controlling for confounding factors the risk of delivering newborns with birth weight below 2500 grams for women with positive culture for Bacteroides at early pregnancy was still relatively high (OR=6.07 CI: 0.77-47.65). The lack of statistical significance was probably due to small number of analyzed cases.

The mechanisms of preterm delivery with low birthweight infants due to infection is however, not fully understood. Nevertheless, studies have indicated that during pregnancy and parturition, the extra cellular or matrix of the decidua, cervix and the fetal membranes are subject to extensive remodelling which inturn leads to cervical ripening, fetal membrane rupture and placental and membrane separation from maternal tissue[25]. There is evidence of infection in most cases of spontaneous birth that occur prior to 30 weeks of gestation[26]. With the release of endotoxins and exotoxins from bacterial infections there is stimulation and production within gestational tissue (decidua and fetal membranes) of inflammatory cytokines such as interleukin (IL)-1, tumour necrosis factor-alpha, IL-6, IL-8 and granulocyte colony-stimulating factors[25]. In addition, these inflammatory cytokines stimulate gestational tissue to produce protaglandins (PG.). In the extrauterine sites, PGE_2 regulates the release of inflammatory cytokines, acts synergistically with IL-8 to augment neutrophil chemotaxis and stimulates the release of metalloproteinases the latter can degrade connective tissue such as chorioamniotic membranes, leading to rupture[27], and they could also remodel the collagen in the cervix and soften it. Further research is required into the clinical utility of inflammatory cytokines and matrix metalloproteinases as markers to spontaneous preterm delivery.

In conclusion, results of this study suggest that bacterial vaginosis and colonization of the lower genital tract by Gardnerella vaginalis and probably bacteroides spp. at early pregnancy constitute a risk factor for LBW which indicates the need for detailed microbiological monitoring of all pregnant women at early pregnancy or even before pregnancy.

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