

Do Microbiological Factors Account for Poor Pregnancy Outcome among Unmarried Pregnant Women in Poland?

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Key Words

Prematurity · *Mycoplasma hominis* · Bacterial vaginosis · Infection · Marital status, unmarried

Abstract

Objective: Being unmarried is a well-known risk factor for poor pregnancy outcome such as preterm delivery and intrauterine growth restriction. The aim of this prospective study was to assess the prevalence and risk of bacterial vaginosis (BV) and selected bacteria isolated from the lower genital tract and to determine the socio-economic and microbiological characteristics that might be responsible for poor pregnancy outcome observed among unmarried pregnant women. **Methods:** The study population comprised 196 pregnant women attending 10 randomly selected outpatient maternity units in the Lodz region, central Poland. Cervicovaginal samples were obtained between 8 and 16 weeks of gestation. Based on Spiegel's criteria, gram-stained vaginal smears were examined for BV and the BV-associated flora was sought by culture. To evaluate the risk factors, relative risk ratios were calculated using EPI INFO software.

Results: Among 196 pregnant women, 40 (20.4%) were unmarried. BV was diagnosed among 55 (28.1%) women studied. In the univariate analysis, unmarried pregnant women were characterized by younger age, primary educational level, poor economic situation and excessive smoking during pregnancy, as compared to married women. The unmarried status was a borderline risk factor for BV (OR = 1.83, 95% CI 0.94-4.9) after adjustment for age, smoking and education. An analysis of the microbiological culture from the lower genital tract revealed that unmarried pregnant women had a higher risk for several types of pathological microflora, as compared to married women. However, this finding was significant only for *Mycoplasma hominis*. The independent risk factors of *M. hominis* were the young age of the subject and a low concentration of *Lactobacillus* spp. **Conclusions:** The observed socioeconomic, demographic and microbiological differences between unmarried and married women could be responsible for the poor pregnancy outcome among unmarried pregnant women in Poland. Unmarried pregnant women should be covered by comprehensive medical care even before pregnancy. Further studies taking into account the role of psychological

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stress, patterns of sexual behavior and substance abuse during pregnancy could help identify the factors responsible for adverse pregnancy outcome among unmarried pregnant women.

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Introduction

Among the socioeconomic and demographic factors associated with poor pregnancy outcome such as preterm delivery and intrauterine growth restriction, the unmarried state during pregnancy is one of the most essential [1, 2]. Some of the epidemiological studies have also suggested that maternal urogenital tract infections are more prevalent in the population of socially underprivileged women [3–5]. Bacterial vaginosis (BV) is a well-known cause of various perinatal complications [6] and is currently characterized as a change from the normal vaginal ecosystem to a reduced concentration of a normally existing aerobic bacteria and *Lactobacillus* species, and an increased concentration of anaerobic bacteria such as *Gardnerella vaginalis*, *Mobiluncus*, *Bacteroides*, *Prevotella* and *Mycoplasma* species [7, 8].

Microorganisms ascending from the lower genital tract produce local inflammation, subclinical chorioamnionitis leading to preterm rupture of membranes and/or preterm labor and possibly preterm birth [9–13]. It is not clear why unmarried pregnant women are at risk for adverse pregnancy outcome. One of the explanations could be the negative socioeconomic, demographic and environmental characteristics of these women recorded during pregnancy. As pathological microflora has a negative influence on the course of pregnancy, the differences in the microbiological environment of the lower genital tract could also account for poor pregnancy outcome among unmarried pregnant women.

The main aim of this prospective study was to assess the prevalence and the risk of BV among unmarried pregnant women in early pregnancy and to determine the socioeconomic and microbiologic characteristics which could be responsible for the poor pregnancy outcome observed among these women.

Materials and Methods

A group of 196 pregnant women was selected randomly from the patients of 10 district maternity units in the Lodz region, central Poland, between January 1, 1998 and December 12, 2000. Only singleton pregnancies between 8 and 16 weeks of gestation were quali-

fied for the survey. Women with chronic diseases diagnosed during the first prenatal visit were excluded from the study. A standard questionnaire covering medical, socioeconomic, demographic, constitutional and environmental aspects was administered to every subject and verified based on medical records.

For the qualitative and quantitative assessment of biocenosis in the lower genital tract, vaginal and cervical swabs were collected from the pregnant women under study. At first, bacteriological tests of cervical swabs were made to screen for *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. The *C. trachomatis* antigen was detected by direct immunofluorescence assay (BioMerieux). For isolation, identification and differential titration of genital mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics, Pasteur) were used. Identification was based on specific hydrolysis of arginine (*M. hominis*) by the species present in the specimen levels of pathogenicity. Titration based on dilution in liquid medium was expressed as the number of cfu/ml specimen (the mean concentration of microorganisms in vaginal fluid). The technique allows titration around the levels of 10^3 and 10^4 cfu/ml. The vaginal swabs were tested for other aerobic and anaerobic bacteria. The swabs were placed in 3 ml prerduced sterile saline. Serial dilutions 1:10 from 10^{-1} to 10^{-8} were prepared. Each of the dilutions made from swabs was inoculated onto appropriate plates.

Sheep blood agar, MacConkey, *D*-Coccosel agar, *Gardnerella* agar, azide blood agar (BioMerieux) plates were used for isolation of aerobic organisms. Schaedler blood agar (BioMerieux) and Rogosa agar (Oxoid Ltd.) were inoculated for anaerobic cultures. After the incubation period, the anaerobic and aerobic bacteria were identified by biochemical tests, API (BioMerieux).

Cervicovaginal swabs were collected from the pregnant women under study and tested for BV by gram stain of vaginal smears according to Spiegel's [8] criteria. Based on microbiological results, 3 groups of pregnant women were distinguished: group I, with normal cervicovaginal flora, predominantly *Lactobacillus* spp. with coagulase-negative staphylococci and viridans streptococci; group II, with intermediate microbial flora, predominantly *M. hominis*, *U. urealyticum*, *G. vaginalis*, gram-negative anaerobic rods, *C. trachomatis* and few *Lactobacillus* spp., and group III, BV.

To evaluate the risk factors, the odds ratios (OR) were calculated. Statistical analysis, by EPI INFO software, was carried out taking into account the ORs and their 95% confidence intervals (CI). Logistic regression models were applied to examine the relationship between the probability of developing BV and *M. hominis* colonization and the risk factors that were found to be significant in the univariate analysis.

Results

Population Characteristics

The average pregnancy duration at the time of microbiological analysis was 12.3 weeks and the mean age of the subjects was 26.1 years. According to gram stain, BV was diagnosed in 55 (28.1%) pregnant women, while grade-I microflora were found in 70 (35.7%) and grade-II (intermediate) in 71 (36.2%) women.

Table 1. Association between selected microorganisms isolated from lower genital tract and marital status during pregnancy

Concentration of microorganisms (cfu/ml)	Marital status				OR, 95% CI
	married (n = 146)		unmarried (n = 40)		
	n	%	n	%	
<i>Mycoplasma hominis</i>					
Culture (-)	127	87.0	28	70.0	Reference group
<10 ⁴	8	5.5	3	7.5	1.70 (0.41–5.26)
≥10 ⁴	11	7.5	9	22.5	3.71 (1.27–10.84)
<i>Lactobacillus</i> spp.					
Culture (-) or <10 ⁵	59	40.4	22	55.0	1.80 (0.84–3.87)
≥10 ⁵	87	59.6	18	45.0	Reference group
Gram stain					
Normal	57	39.0	10	25.0	Reference group
Intermediate	56	38.4	13	32.5	1.32 (0.49–3.58)
BV	33	22.6	17	42.5	2.94 (1.11–7.89)

The mean duration of pregnancy was 39.06 ± 1.83 in group I, 38.66 ± 2.89 in group II, and 38.16 ± 2.94 weeks in group III (p = 0.04). The mean birth weight of the infants was the lowest in group III (2,964 g) compared to group I as the reference group (3,224 g; p = 0.02). The preterm delivery rate (15.7%) and low birth weight rate (23.5%) were the highest in group III compared to group II (11.3 and 11.3%, respectively) and to group I (9.1 and 10.6%, respectively).

Of the 196 women studied, 146 (74.5%) were married, 40 (20.4%) were unmarried and 10 (5.1%) were divorced or widows. 30.9% of pregnant women with BV were unmarried, compared to only 14.3% in the reference group. The unmarried status proved to be a significant risk factor for BV in early pregnancy (OR = 2.94, 95% CI 1.11–7.89; table 1). We also evaluated the association between various microorganisms isolated by culture from the lower genital tract of pregnant women and their marital status during pregnancy (table 1). Unmarried pregnant women demonstrated a higher risk of positive culture of *Bacteroides* spp. (OR = 2.59, 95% CI 0.21–23.37); *Mobiluncus* spp. (OR = 3.68, 95% CI 0.47–28.33); *Prevotella* spp. (OR = 1.76, 95% CI 0.37–6.78); *M. hominis* (OR = 3.71, 95% CI 1.27–10.84) and *U. urealyticum* (OR = 2.36, 95% CI 0.89–6.17). The unmarried status was not connected with *C. trachomatis* infection (OR = 0.76, 95% CI 0.29–1.93). However, it constituted a risk factor for the low concentration of protective *Lactobacillus* spp; (OR = 1.80, 95% CI 0.84–3.87).

Since the unmarried status proved to be an important variable connected with BV in early pregnancy, we fo-

cused on this factor and made an additional analysis trying to answer the question as to the socioeconomic and demographic characteristics of unmarried pregnant women in the Polish population. Unmarried pregnant women were characterized by younger age (<20 years), primary educational level, poor economic situation, worse housing conditions and excessive smoking during pregnancy as compared to married women (table 2).

The risk of *M. hominis* colonization was significantly increased not only for the 'unmarried status' variable but also for some factors directly related to this variable, i.e. age <20 years (OR = 2.08, 95%CI 0.68–6.26), primary education (OR = 3.24, 95%CI 1.27–8.45) and smoking during pregnancy (OR = 2.33, 95% CI 0.95–5.70; table 3). The presence of *Lactobacillus* spp. at concentrations above 10⁵ cfu/ml was a strong protective factor. After including all these variables in the logistic regression model, the significant risk factors for *M. hominis* were found to be age <20 years and a low concentration of *Lactobacillus* spp. (table 3).

A similar analysis was performed with respect to the risk of BV. In this case the unmarried status was a significant risk factor for BV, after adjusting for maternal education and smoking during pregnancy. The concentration of *Lactobacillus* spp. was not taken into account as it is an element of the BV diagnosis (table 4).

Table 2. Comparison of selected socioeconomic, demographic and environmental characteristics of married and unmarried pregnant women

Variables	Married (n = 146)		Unmarried (n = 40)		OR, 95% CI
	n	%	n	%	
<i>Age, years</i>					
<20	11	7.5	14	35	2.80 (0.98–8.09)
21–25	44	30.1	20	50	Reference group
26–30	58	39.7	3	7.5	0.11 (0.03–0.44)
>30	33	22.6	3	7.5	0.20 (0.04–0.80)
<i>Education</i>					
Primary	47	32.2	23	57.5	2.19 (0.97–4.96)
College	67	45.9	15	37.5	Reference group
University	32	21.9	2	5.0	0.28 (0.04–1.40)
<i>Employment</i>					
Yes	107	73.3	17	42.5	Reference group
No	39	26.73	23	57.5	1.94 (0.94–3.40)
<i>Economic situation</i>					
Poor	24	16.4	12	30.0	2.62 (0.96–7.17)
Average	48	32.9	15	37.5	1.63 (0.66–4.06)
Good	68	46.6	13	32.5	Reference group
Very good	6	4.1	0	0	Not calculated
<i>Active smoking (cigarettes/day)</i>					
0	121	2.9	20	50.0	Reference group
1–5	14	9.6	8	20.0	3.46 (1.15–10.30)
6+	11	7.5	12	30.0	6.6 (2.33–18.69)

Table 3. Risk of *Mycoplasma hominis*-positive culture isolated from lower genital tract according to sociodemographic and bacteriological characteristics associated with unmarried status

Variables	<i>Mycoplasma hominis</i>				OR 95% CI	Adjusted OR 95% CI
	positive (n = 31)		negative (n = 155)			
	n	%	n	%		
<i>Marital status</i>						
Married	19	40.4	127	81.9	Reference group	
Unmarried	12	59.6	28	18.1	2.86 (1.15–7.09)	1.31 (0.45–3.86)
<i>Age, years</i>						
<20	11	35.5	14	9.0	9.27 (2.49–38.62)	4.67 (1.48–14.7)
21–30	13	41.9	112	72.3	Reference group	
>30	7	22.6	29	18.7	2.08 (0.68–6.26)	2.54 (0.83–7.7)
<i>Education</i>						
Primary	20	64.5	50	32.3	3.24 (1.27–8.45)	1.28 (0.54–3.05)
College	9	29.0	73	47.1	Reference group	
University	2	6.5	32	20.6	0.51 (0.05–2.67)	0.53 (0.16–1.78)
<i>Active smoking (cigarettes/day)</i>						
No	19	61.3	122	78.7	Reference group	
Yes	12	38.7	33	22.3	2.33 (0.95–5.79)	1.24 (0.45–3.43)
<i>Lactobacillus spp., cfu/ml</i>						
Culture (–) or <10 ⁵	24	77.4	57	36.8	Reference group	
>10 ⁵	7	22.6	98	63.2	0.17 (0.06–0.45)	0.147 (0.05–0.40)

Table 4. Risk of bacterial vaginosis according to sociodemographic and bacteriological characteristics associated with unmarried status

Variables	BV				OR 95% CI	Adjusted OR 95% CI
	positive (n = 50)		negative (n = 136)			
	n	%	n	%		
<i>Marital status</i>						
Married	33	66.0	113	83.1	Reference group	
Unmarried	17	34.0	23	16.9	2.53 (1.14–5.64)	1.89 (0.94–4.90)
<i>Age, years</i>						
<20	10	20.0	15	11.0	2.82 (1.01–7.82)	1.28 (0.44–3.69)
21–30	22	44.0	93	68.4	Reference group	
>30	8	16.0	28	20.6	1.21 (0.47–3.26)	1.09 (0.42–2.80)
<i>Education</i>						
Primary	23	46.0	47	34.6	1.33 (0.63–2.84)	1.11 (0.49–2.50)
College	22	44.0	60	44.1	Reference group	
University	5	10.0	29	21.3	0.47 (0.13–1.46)	0.29 (0.16–1.55)
<i>Active smoking (cigarettes/day)</i>						
No	35	70.7	106	77.9	Reference group	
Yes	15	30.0	30	22.1	1.51 (0.69–3.33)	0.93 (0.32–2.70)

Discussion

Preterm delivery is a major cause of perinatal morbidity and mortality. BV has emerged as an important risk factor for chorioamnionitis, amniotic fluid infection and preterm labor and delivery [10–13]. According to Goldenberg et al. [14], BV accounted for 40% of the attributable risk of spontaneous birth before 32 weeks of pregnancy. Kurki et al. [15] examined 790 women before 16 weeks of gestation and found a 6.9-fold increased risk of preterm birth among women with BV. Hay et al. [16] reported a similar association. Hillier et al. [17], in a cohort study of 10,397 pregnant women, determined the impact of BV diagnosed at 23–26 weeks of gestation. In multivariate analysis, the presence of BV was related to preterm delivery of a low birth weight infant (OR = 1.4). Presently, there is substantial evidence that subclinical infection, ascending into the uterine cavity from the lower genital tract, is associated with preterm labor and birth [10, 11]. Carey et al. [18], in a randomized, placebo-blinded trial, failed to reduce preterm delivery among women with BV diagnosed between 16 and 24 weeks of gestation and treated with two 2-gram doses of metronidazole. The treatment may have failed because diagnosis and treatment were started too late in pregnancy to prevent preterm birth or because the duration of metronidazole ther-

apy needed to be longer to be effective or another antibiotic should have been used.

Unmarried status has been repeatedly indicated as a risk factor for preterm delivery and intrauterine growth restriction in the studies concerning the Polish population [1, 19], and to a much lesser extent in other European communities. The ‘unmarried status’ variable is related to some other factors that negatively influence the course of pregnancy, such as cigarette smoking, lower educational and economic status, higher incidence of pathological microflora of the lower genital tract, and more risky sexual behavior before and during pregnancy, as well as the psychological distress connected with the social situation of pregnant women. We can only speculate that in highly developed European countries, the better economic and social situation of pregnant women as well as healthier sexual behavior profiles protect them against negative pregnancy outcomes.

According to Kramer et al. [20], in the case of preterm birth, the socioeconomic gradients in BV and cigarette smoking appear to explain some of the socioeconomic disparities. Several studies confirmed that the unmarried status is a risk factor for BV [3, 19, 21], which is concordant with our findings, while others underline a higher risk for specific bacterial colonization of the lower genital tract during pregnancy among single pregnant women. In

a cross-sectional study conducted by Faye-Kette et al. [3], *U. urealyticum* was more frequently found in unmarried pregnant women and when pregnancy was <20 weeks. In our analysis, 25% of unmarried pregnant women had a positive culture for *U. urealyticum*, compared to 13% in the control group (OR = 2.36). Fonck et al. [21] observed that in nonpregnant women, gonococcal and chlamydial infection was significantly associated with such factors as being unmarried, <20 years of age, and having multiple partners within the previous 3 months. Among the pregnant women, only young age constituted a risk factor. No such excess risk for unmarried women was found in our study.

With regard to other socioeconomic factors connected with BV, tobacco smoking [4, 19, 22] and unemployment [5, 19] were mentioned. Wessel et al. [23] noted that bacterial infection among pregnant women was related to their young age and unmarried status. In another study [24], the woman's age, marital status, number of pregnancies, smoking, and alcohol or drug abuse were not associated with the development of infection, while black race and older gestational age at the first prenatal visit constituted significant risk factors. In our analysis, after adjustment for age, education and smoking, an increase in BV risk of borderline significance was found for the unmarried status.

It is not clear whether and how smoking could negatively affect the microbiology of the lower genital tract. A possible solution was proposed in the study conducted by Pavlova and Tao [25]. They postulated that smoking might reduce the number of vaginal lactobacilli. We observed that more than half of the unmarried pregnant women had a decreased concentration of *Lactobacilli* spp. According to Thorsen et al. [26], *Lactobacilli* spp. were associated with the absence of BV and there is a microbial foundation for BV, possibly due to an intermicrobial interaction in which *G. vaginalis*, *M. hominis* and anaerobic bacteria dominate. Our analysis of pathological microorganisms isolated from the lower genital tract during pregnancy revealed a higher incidence as well as a risk of positive culture among unmarried women, as compared to the married ones. The highest risk was noted for *Bacteroides* spp, *Mobiluncus* spp; *G. vaginalis*, *M. hominis* and *U. urealyticum*. Two thirds of these women had a positive culture for at least one of the pathologic bacteria. In our analysis, smoking was not a factor responsible for the high prevalence of BV among pregnant women. As we did not analyze the impact of psychological stress, risky sexual behaviors and substance abuse, except tobacco smoking, during pregnancy, we cannot consider these factors direct-

ly responsible for BV. However, such an explanation is plausible. According to the data reported by Mensch and Kandell [27], unmarried pregnant adolescents display a four times higher incidence of illicit drug abuse, excluding marijuana.

In 22.5% of unmarried pregnant women in early pregnancy, *M. hominis* was diagnosed, compared to 7.5% of married women (OR = 3.71). However, after adjustments for age, education and smoking, only the age <20 was identified as an independent risk factor of *M. hominis* infection during pregnancy. Although the causative role of *M. hominis* infection in prematurity was excluded in some of the studies [28, 29], another found this relation very likely [30]. According to Hillier et al. [17] among women with BV, the highest risk of preterm delivery of a low birth weight infant (<2,500 g) was found among those with both vaginal bacteroides and *M. hominis* (OR = 2.1). The presence of *M. hominis* was associated with preterm delivery of low birth weight infant (OR = 1.6) even in the absence of BV. The high prevalence of *M. hominis* infection among unmarried pregnant women and its impact on pregnancy outcome should be investigated in further studies.

The unmarried status constitutes one of the major sociodemographic risk factors for adverse pregnancy outcome. The socioeconomic and bacteriological differences found between the unmarried and married women could to some extent be an explanation of this phenomenon. Based on our findings we concluded that the unmarried status during pregnancy may be regarded as an independent risk factor for BV, while the risk of *M. hominis* is related rather to the young age of women in this group and the low concentration of *Lactobacilli* spp. in the lower genital tract (fig. 1). Both the bacteriological factors could account for the adverse pregnancy outcome observed among single pregnant women in the Polish population. The sociodemographic factors associated with unmarried status (young age and smoking) may, through some specific mechanisms, potentiate this effect. Our data imply that in patients at a young age, the bacteriological background of an increased risk of *M. hominis* colonization may be involved.

The results of the present study may enhance the obstetric risk assessment for preterm delivery by providing the characteristics of pregnant women who are susceptible to BV in early pregnancy as well as help understand the mechanisms that translate social adversity into the pathophysiology of pregnancy.

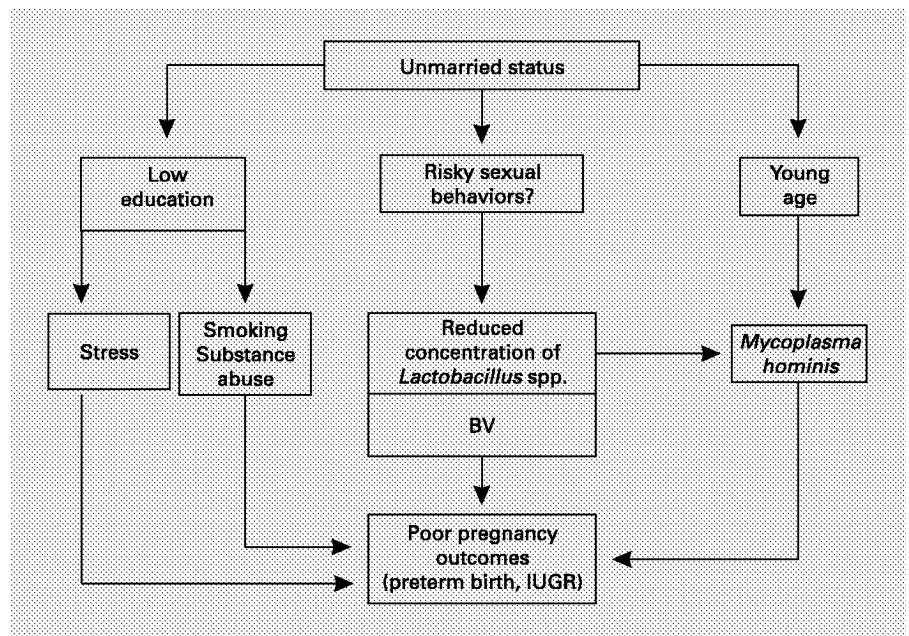


Fig. 1. Risk factors in unmarried pregnant women.

Conclusions

Unmarried pregnant women are characterized by younger age, lower educational level, unemployment, poor economic situation and excessive smoking during pregnancy.

The unmarried status in Poland is independently associated with BV, which is recognized as a risk factor of poor pregnancy outcome. The association of the unmarried status with *M. hominis* resulted mostly from the lower age of unmarried women and the lower concentration of *Lactobacillus spp.*

Further studies taking into account the role of psychological stress, patterns of sexual behavior and substance abuse during pregnancy may help identify the factors responsible for adverse pregnancy outcome among unmarried pregnant women.

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