Correlation between Bacterial Vaginosis and Adverse Pregnancy Outcome

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Key Words

Bacterial vaginosis · Pregnancy · Adverse outcome of pregnancy

Abstract

Objective: The main purpose of this study was to investigate the role of bacterial vaginosis (BV) in prematurity, premature rupture of membranes (PROM) and other disorders of pregnancy. **Methods:** High vaginal and cervical swabs were taken from pregnant women in the second and third trimesters of pregnancy and from 30 non-pregnant women. Informed consent was obtained from all potential subjects. The specimens were smeared, gram-stained and cultured on a variety of selective and non-selective media which were then incubated at the appropriate atmospheres. Vaginal pH, character of the discharge, and outcome of the amine test were noted. Ultrasound was performed to

confirm gestational age and all pregnant women followed up until postpartum. **Results:** A total of 123 pregnant women were evaluated at the end of the study. BV was diagnosed clinically and bacteriologically in 34 women, giving a prevalence rate of 28%. BV was diagnosed in none of the 30 non-pregnant control patients. Fifty-four percent and 46% of the study population were Kuwaitis and non-Kuwaitis, respectively. The mean parity recorded was 2.24 ± 2.06 . There was a positive correlation between BV and preterm labour (9/34, 27%; CI, $p < 0.05$), PROM (7/34, 21%; CI; $p < 0.05$) and preterm delivery (7/34, 21%; CI, $p < 0.05$). Three (9%) of the women with BV had babies with intra-uterine growth retardation compared with 1 (1%) in the 89 non-BV group ($p < 0.05$). **Conclusion:** The prevalence rate of BV among pregnant women in Kuwait is high and is significantly associated with prematurity and other disorders of pregnancy.

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Introduction

Prematurity is a major problem in obstetrics world-wide and in spite of all efforts to reduce the incidence by focusing on the elimination of some of the aetiological factors, preterm birth remains an important contributor to perinatal morbidity and mortality [1–5]. Research efforts have continued to identify more causes of prematurity with a view to eliminating such problems. Many studies have confirmed the role of bacterial vaginosis (BV) in prematurity. Some of the studies have shown that treatment of BV leads to the reduction of the incidence of prematurity [6–9]. Recent interests have also focused on a probable role for BV in recurrent abortions [6, 10, 11]. In spite of the volume of research in the area of BV in pregnancy [6–9, 12, 13], there still remain many unanswered questions such as the natural course of bacterial vaginosis in pregnancy [13, 14].

As far as we are aware, the prevalence of BV in pregnant women and its associated adverse effect on pregnancy outcome have not been studied in Kuwait. From our personal unpublished observations, prematurity and intra-uterine growth retardation (IUGR) are important obstetric problems in Kuwait. Therefore, the main objective of this study was to establish the prevalence of BV in pregnant women in Kuwait, as well as to investigate the role of BV in prematurity and other disorders of pregnancy.

Material and Methods

A total of 165 pregnant women were initially enrolled in the study, which was conducted at the Maternity Hospital, Kuwait, during the period of 1 year, from January to December 1998. The women were fully counselled and their informed consent obtained. All patients who presented at their first antenatal visit were interviewed and their social, obstetric and medi-

cal history were carefully documented in a designed pro forma. High vaginal and endocervical swabs were taken from the subjects under direct vision, using the Cusco speculum. Ultrasound examination was carried out to confirm the gestational age. At each visit, the progress of pregnancy and associated events were recorded. All the cases were followed up through labour, delivery and during puerperium. Cases of premature labour, premature rupture of membrane, and premature delivery were noted, as were cases of idiopathic IUGR. Vaginal specimens were also obtained from 30 non-pregnant women, who attended the gynaecology clinic for reasons other than genital infections, and were studied for comparison.

Exclusion Criteria

At the end of the study, 42 patients were excluded from the evaluation. These included patients who had conditions that might predispose to prematurity and preterm labour, such as diabetes mellitus, multiple pregnancy, hypertension, kidney disease, cardiac disease, cervical incompetence/cerclage and haemoglobinopathies. Other cases that were excluded were those who had antibiotic treatment for about 2–4 weeks before the commencement of the study.

Specimens

Vaginal specimens (high vaginal swab and cervical swab) were obtained by the obstetricians in our team, under direct vision using unlubricated Cusco's speculum. Duplicate high vaginal swabs and cervical swabs were collected in Amies' transport medium (Unipath, Basingstoke, UK) and *Mycoplasma* transport medium and sent to the Anaerobe Research Laboratory, Department of Microbiology, Faculty of Medicine, where they were processed within 2 h of collection. The characteristic discharge such as quantity, colour and smell were noted and pH determined directly using the pH indicator paper at the time of specimen collection. One to two drops (10%) of KOH was added to a wet smear of the discharge by the examining obstetrician and sniffed; an immediate fishy odour was regarded as a positive amine test and recorded.

The clinical diagnosis of bacterial vaginosis was made when three of the following four criteria were met: a vaginal pH of ≥ 4.5 , presence of thin homogeneous discharge, presence of 'clue' cells and a positive amine test. The subject was considered normal if at least three of these criteria were not met and neither yeasts nor trichomonads were detected and the vaginal smear was mainly lactobacilli morphotypes.

Table 1. Characteristics of pregnant and non-pregnant healthy women

Characteristic	Pregnant (n = 123)		Non-pregnant (n = 30)	
	n	%	n	%
Vaginal pH				
<4.5	62	50	29	96.7
≥ 5.0	61	49.6	1	3.3
Quality of discharge				
None	49	39.8	30	100
Small	40	32.5	0	0
Moderate	29	23.6	0	0
Profuse	5	4.1	0	0
Malodorous	25	20.3	0	0
Colour/type of discharge				
White	70	56.9	20	66.7
Mucoid/white	25	20.3	7	23.3
Creamy	18	14.6	3	10
Greyish-frothy	6	4.9	0	0
Yellowish-frothy	4	3.3	0	0
Positive amine test	7	5.7	0	0
Presence of 'clue cells'	33	26.8	0	0
Pus cells present	4	3.3	3	10

Microscopic Examination

The microscopic examinations and assessment of the smears for the presence of bacterial vaginosis were carried out by the modified criteria described by Rosenstein et al. [15] and a modified semiquantitative method described by Rotimi et al. [16]. Briefly, in wet films, an estimate of the *Lactobacillus* and *Gardnerella* morphotypes was made into the following schemes: 1⁺ = 1/field; 2⁺ = 1–5/field; 3⁺ = 6–30/field; 4⁺ = >30/field. Thus, a 3⁺–4⁺ *Gardnerella* morphotype with 1⁺ or no *Lactobacillus* morphotype was regarded as grade III, i.e. abnormal. A 2⁺–3⁺ *Gardnerella* morphotype plus 2⁺ *Lactobacillus* morphotype was regarded as intermediate, grade II, and a >3⁺ *Lactobacillus* morphotype plus 1⁺ or no *Gardnerella* morphotype as normal, grade I vaginal flora.

Bacterial Culture and Identification

The following media were used for primary culture: selective brain-heart infusion agar (SBHIA) for *Gardnerella vaginalis*, comprising of brain-heart infusion agar (Unipath) with expired human blood 5%, neomycin 75 mg/l and colistin 10 mg/l [17], selective chocolate agar (Blood Agar Base No. 2; Unipath) with expired human blood 10%, vancomycin 3 mg/l and

colistin 7.5 mg/l, and Thayer-Martin agar (Unipath) for *Neisseria gonorrhoeae*; Sabouraud dextrose agar (Unipath) for cultivation of *Candida* spp.; PPLO agar (Difco Laboratories, Mich., Detroit, USA) and Mycoplasma IST broth (bioMérieux SA, Lyon, France) for *Mycoplasma hominis* and *Ureaplasma urealyticum* and Rogosa agar (Unipath) for cultivating *Lactobacillus* spp.; H₂O₂-producing lactobacilli were detected by quantitative assays on a tetramethylbenzidine agar incubated anaerobically for 5 days [18]. Brucella agar (Difco Laboratoires), supplemented with 5% lysed horse blood, was used for the cultivation of *Mobiluncus* spp. Colonies appearing translucent on Brucella agar, curved gram-negative bacilli on smear, actively motile in wet preparation, and indole-, oxidase- and catalase-negative were identified as *Mobiluncus* spp.

N. gonorrhoeae and *Candida* spp. were identified by standard methods [19]. Isolates that produce tiny β-haemolytic colonies on SBHIA were gram-stained and catalase-tested and those presumptively identified as *G. vaginalis* were confirmed by a battery of biochemical tests [16]. Typical large gram-positive bacilli from non-haemolytic colonies were presumptively identified as lactobacilli. *M. hominis* and *U. urealyticum* were identified by Mycoplasma IST kit according

Table 2. Characteristics of pregnant women with or without BV

Characteristics	Clinical diagnosis	
	with BV (n = 34)	without BV (n = 89)
Demographics		
Kuwaitis, n (%)	16 (47)	50 (56)
Non-Kuwaitis, n (%)	18 (53)	39 (44)
Age, years	29.68 ± 5.3	28.75 ± 5.1
Reproductive history		
Nulliparous	10	23
Previous preterm delivery	6	5
Previous spontaneous abortion	7	12
Infertility	0	0
The mean parity for all women was 2.24 ± 2.06.		

to the manufacturer's standard procedures for the diagnosis of urogenital *Mycoplasma* (bioMérieux).

Statistical Analysis

The data were analysed by χ^2 statistical test for univariate comparisons of proportions, with or without Yates' corrections, between women with or without bacterial vaginosis, using SPSS statistical package for Windows.

Results

General Characteristics

Of the 165 women enrolled, 123 could be evaluated. The general characteristics and clinical features of the 123 pregnant and 30 non-pregnant women are summarized in table 1. Small to moderate vaginal discharge was present in 74 (60%) of the 123 pregnant women and was malodorous in 25 (20%). The discharge was white in 70 (57%), mucoid and white in 25 (20%) and creamy in 18 (15%). Amine test was positive in only 7 (6%) cases and the presence of 'clue cells' was recorded in 33 (27%) pregnant women.

Table 2 shows the general demographic and reproductive history of the pregnant women. Sixty-six Kuwaitis and 57 non-Kuwaitis were evaluated. BV was diagnosed in 18 (32%) out of the 57 non-Kuwaitis and in only 16 (24%) of the 66 Kuwaitis. An overall prevalence rate of BV among the pregnant women was 28%. None of the control non-pregnant women had BV. There was also no significant difference in the reproductive history of those with BV and those without ($p > 0.05$).

Prevalence of *G. vaginalis* and Other BV-Associated Pathogens in Pregnant Women with and without BV

The association of *G. vaginalis* and other BV-associated pathogens with pregnant women diagnosed as having BV and non-BV is shown in table 3. *G. vaginalis* was present in all (100%) of the 34 pregnant women with BV but in only 7 (8%) of those without BV ($p < 0.0001$). *Lactobacillus* spp. were reported in a significantly higher number of patients without BV than with BV ($p < 0.0001$). Although H_2O_2 -producing lactobacilli and *Mobiluncus*

Table 3. The prevalence of BV-associated pathogens in pregnant women with and without clinical diagnosis of BV

Vaginal pathogens	Women colonized/infected		Total (n = 123)	p value
	BV group (n = 34)	non-BV group (n = 89)		
<i>Gardnerella vaginalis</i>	34 (100)	7 (8)	41 (33)	<0.0001
Lactobacilli	21 (62)	88 (99)	109 (89)	<0.0001
H ₂ O ₂ lactobacilli	2 (6)	9 (10)	11 (9)	NS, >0.05
<i>Mobiluncus</i>	15 (44)	60 (67)	75 (61)	NS, >0.05
<i>Mycoplasma hominis</i>	4 (12)	1 (1)	5 (4)	<0.05
<i>Ureaplasma urealyticum</i>	25 (74)	12 (14)	37 (30)	<0.0001
<i>Candida</i> spp.	14 (41)	20 (23)	34 (28)	<0.05
<i>Peptostreptococcus</i> spp.	26 (77)	35 (39)	61 (50)	<0.001
Staphylococci	6 (18)	38 (43)	44 (36)	<0.001

Number (and percent) of women are given. Staphylococci were mainly coagulase-negative staphylococci.

Table 4. Pregnancy outcome among patients with or without BV

Outcome	BV present (n = 34)	No BV (n = 89)	p value
Preterm labour, <37 weeks	9 (27)	7 (8)	<0.05
Preterm PROM, <37 weeks	7 (21)	6 (7)	<0.05
Low birth weight, <2,500 g	5 (15)	4 (5)	<0.05 ^a
IUGR	3 (9)	1 (1)	<0.05 ^a
Neonatal infections	2 (6)	0 (0)	
Low Apgar score (<7)	2 (6)	0 (0)	
Perinatal mortality	0 (0)	0 (0)	

Number (and percent) of women are given.

^a χ^2 for both were 3.8 and 4.6, respectively, which are significant ($p < 0.05$) without Yates' correction.

spp. were found in a higher number of patients without BV than with BV, the differences were not significant ($p > 0.05$). *M. hominis* was present in 4 (12%) of the 34 women with BV and in only (1%) of the 89 women without BV. *U. urealyticum*, *Candida* spp. and *Peptostreptococcus* were found in a signif-

icantly higher number of patients with BV than without BV ($p < 0.0001$, $p < 0.05$ and $p < 0.001$, respectively). On the other hand, staphylococci were reported in significantly more patients without BV than with BV ($p < 0.001$).

Association of BV with Pregnancy Outcome

The pregnancy outcome differed significantly for patients with and without BV (table 4). Preterm labour prior to 37 weeks gestation occurred among 9 patients (27%) out of 34 with BV and 7 (8%) patients without BV ($p < 0.05$). Preterm premature rupture of membranes (PROM) also occurred more commonly in patients with (21%) than without (7%) BV ($p < 0.05$). None of the patients who had preterm labour, PROM or premature delivery had a past obstetric history of previous preterm delivery. Patients with BV had more newborn babies with low birth weight of less than 2,500 g than those without BV (15 versus 5%; $p < 0.05$). Nine percent of patients with BV when compared to 1% of those without BV had pregnancies with IUGR ($p < 0.05$). Neonatal infections and neonates with low Apgar scores (<7) were observed in 2 patients with BV and not in those without. Perinatal mortality was not recorded in either of the groups. Cervical infection with *Mycoplasma* spp. was not independently associated with adverse pregnancy outcome or postpartum fever.

Discussion

In this study, the overall prevalence of BV among the pregnant population was 28%. Of these, 47% were Kuwaitis and 53% non-Kuwaitis. The prevalence of BV among the Kuwaiti women was actually 24% while among the non-Kuwaitis it was 32%. Even though this difference in prevalence rate was not statistically significant it is noteworthy that more Kuwaitis ($n = 66$) than non-Kuwaitis ($n = 57$) were evaluated. The prevalence of 28% BV cases in pregnancy in this study is relatively high. The reported prevalence of this disorder in the literature averages about

10–30% in pregnant women [20, 21]. However, a prevalence rate almost as high as ours (26%), and a rate nearly twice our findings (52%), had been reported by Thomason et al. [22] and Govender et al. [23], respectively.

Prematurity is a major problem in obstetric practice, where it is responsible for 70–80% of all perinatal deaths, excluding congenital anomalies [20]. Preterm onset of labour and PROM are the main causes of such conditions. A past history of preterm labour is one of the most important risk factors in the recurrence of preterm labour and delivery in a subsequent pregnancy. In a much earlier report, Minkoff et al. [24] found no association of BV with adverse pregnancy outcome. However, several other studies have found strong association of this disorder with adverse pregnancy outcome [5, 8, 11, 19, 25–27]. Our findings are in concordance with these previous reports. A relatively high proportion of women with BV had preterm labour (27%) and PROM (21%). These observations are statistically significant and much higher than the rates in most of these previous reports. It is worthy of emphasis to note that a past obstetric history of previous preterm delivery was not recorded in any of our patients with preterm labour and PROM, thus underscoring the role of BV in these adverse outcomes of pregnancy. The absence of postpartum complications in the presence of PROM, in this study, is an important clinical finding and worthy of note. It is possible, and highly likely, that the prophylactic use of antibiotics routinely prescribed for women with PROM prevented the establishment of intra-uterine and postpartum infections. Our study also found that BV was associated with low birth weight and IUGR in a significant proportion of our pregnant women. Although the figure for the IUGR is small, this finding is nonetheless interesting and worthy of further research as the role of BV in this complication is not

well understood nor is it well documented in other reports.

The mechanisms involved in the development of BV and preterm onset of labour and PROM are also not very clear. However, it can be speculated that since bacterial lipase and protease produced by the vaginosis-associated pathogens could reduce chorioamniotic membrane strength [20], it is conceivable that this weakness in the membrane could lead to its rupture. High level of phospholipase A₂ production has been detected in *G. vaginalis*, *Peptostreptococcus*, and other anaerobes associated with BV [28]. This compound is known to initiate prostaglandin production by releasing arachidonic acid from its esterified forms and prostaglandin is a potent initiator of onset of labour. This mechanism may explain the role of BV in the observed increased incidence of preterm labour. An extension of this study in an animal model is required to confirm this line of thinking.

In conclusion, the data generated from this study indicates that, (i) BV is common in our pregnant female population in Kuwait with high prevalence rate of 28%, and (ii) BV is sig-

nificantly associated with preterm labour, PROM, low birth weight and IUGR. The association of BV with adverse pregnancy outcome is a potentially preventable cause of prematurity. Therefore, should BV be actively sought and treated during pregnancy to prevent an adverse pregnancy outcome? This approach may not be cost-effective. However, since this disorder and other vaginosis-associated infections are an important cause of neonatal morbidity, and since safe and effective therapy during pregnancy is available, treatment of syndromic maternal genital infections, like BV and others, is warranted. An extension of this study is essential to evaluate the influence of treatment of BV on these disorders in pregnancy.

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