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Alteration in vaginal microflora, douching prior to pregnancy, and preterm birth

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Summary

We aimed to determine the relationship of douching prior to pregnancy and bacterial vaginosis (BV) during pregnancy on preterm birth, addressing individual and joint effects. We used a prospective cohort study and assessed vaginal microflora using gram stains and Nugent's criteria. Douching behaviour was based on self-report about the 12 months prior to pregnancy. Preterm births were categorised as spontaneous or medically indicated. A total of 2561 women provided vaginal specimens and 1492 provided self-reports on douching behaviour.

Bacterial vaginosis assessed at 24–28 weeks' gestation in the absence of douching prior to pregnancy was associated with spontaneous preterm birth (odds ratio = 2.74 [95% confidence interval 1.13, 6.66]) as was douching in the absence of BV (OR = 2.20 [1.29, 3.75]). The combination of BV and douching was unrelated to spontaneous or medically indicated preterm birth. We concluded that acute alterations in vaginal microflora at mid-pregnancy or douching prior to pregnancy were associated with an increased risk of preterm birth, but the combination did not appear to increase the risk further than would be expected.

Keywords

bacterial vaginosis; douching; preterm birth

Introduction

Studies have consistently demonstrated that disturbances of the vaginal microflora as evidenced by gram stain changes are associated with preterm birth.¹ Treatment trials for abnormal vaginal microflora have shown mixed results with the use of antibiotics early in pregnancy demonstrating some effectiveness at reducing risk of preterm birth whereas treatment later in pregnancy does not appear to be effective.^{2–4} Douching is a behaviour that may be based on concerns with hygiene or initiated in an attempt to relieve symptoms

arising from shifts in the vaginal microflora; regardless of the motivation, douching can alter normal flora.^{5,6} Douching has been associated with upper genital tract pathology in non-pregnant women and small studies have identified this behaviour as a risk factor for preterm birth.^{7–11} A recent study among African-American women found douching during pregnancy to be a risk factor for preterm birth, while douching before pregnancy was protective.¹² This confirmed a case–control study of preterm birth in which douching during pregnancy was associated with increased risk, while that behaviour in the 6 months prior to pregnancy was protective.¹³

We analysed data from the Pregnancy, Infection and Nutrition (PIN) Study, a prospective cohort study of preterm births in central North Carolina. We analysed the impact of bacterial vaginosis (BV) and douching behaviour on preterm birth, addressing the individual and joint effects in relation to preterm birth. This is the first study to address the relationship between douching prior to pregnancy, BV and preterm birth.

Materials and methods

The PIN Study was conducted at prenatal care clinics affiliated to the University of North Carolina Hospitals, Wake County Human Services and the Wake Area Health Education Center. As described in detail elsewhere,¹⁴ women were recruited at 24–29 weeks' gestation, and were interviewed by telephone in the subsequent 2 weeks to collect information on health behaviours, symptoms of infections, physical exertion, employment and other potential risk factors for preterm birth. Most patients reside in central North Carolina. Institutional Review Board approval was obtained to carry out this study.

The analyses presented take advantage of a cohort enrolled between August 1995 and February 2001. During that time, 5196 women were identified as eligible by having enrolled for prenatal care prior to 30 weeks' gestation with a singleton pregnancy, having access to a telephone, being able to communicate in English, and planning to continue care and deliver at a study hospital. The exclusion based on language effectively resulted in our inability to recruit most Hispanic women. Therefore, 'white' refers to 'non-Hispanic' in this analysis.

Among those eligible, 3163 (61%) were successfully recruited, defined as willing to provide genital tract specimens even if other components of the study were not completed. Approximately 29% were lost owing to patient refusal, 5% owing to an inability to make contact at the time of their clinic visit and 6% for other reasons. Among the 3163 women recruited, all provided a genital tract swab, and 2961 (94%) yielded adequate slides for gram stain assessment of BV.

Questions about douching behaviour in the 12 months prior to pregnancy were added to the telephone interview in August 1997, after which 1773 women were recruited. The analysis was restricted to women who answered questions about douching and symptoms of yeast infection (84%), had a livebirth at a study hospital where preterm subtype could be assessed (98% of all births), and had a readable gram stain (99%), resulting in 1492 women available for analysis. Patterns of participation have been reported in detail elsewhere¹⁴ and suggest that those recruited were generally similar to those eligible but not recruited, particularly with respect to pregnancy outcome.

Gestational age at delivery was based on ultrasound estimates completed prior to 22 weeks' gestation; otherwise, last menstrual period (LMP) was used. Among these women, 80% had both ultrasound and LMP, and 82% of the pregnancies were dated by ultrasound. Preterm birth was defined as delivery prior to 37 completed weeks of gestation. All preterm births occurring at study hospitals were reviewed by one of two study clinicians and births occurring after preterm labour or preterm premature rupture of the membranes (rupture more

than 4 hours prior to the onset of labour) were classified as spontaneous while the remainder were classified as medically indicated.

Specimens of vaginal fluid were collected with cotton swabs at the time of speculum examination during the 24- to 29-week visit. Specimens were obtained from the posterior vaginal apex. Study staff touched swabs to pH strips (EMD Chemicals, Gibbstown NJ, colorpHast strips, 4.0–7.0 range) and recorded the result. Swabs were smeared on glass slides and allowed to air dry. Slides were gram stained using an automated device at a commercial laboratory (LabCorp, Burlington, NC). Gram stains were scored using Nugent's criteria¹⁵ by one of three readers.

Maternal demographic information (race, marital status, age, education, household income and parity), lifetime number of sexual partners, history of sexually transmitted infections, yeast infections during pregnancy, and smoking behaviour during months 1–6 of pregnancy were ascertained during the phone interview conducted by trained research staff. A poverty index was calculated using the number of children and adults supported by the household income, and expressed as a percentage of the level that defined poverty in 1996.¹⁶ Douching history before and during pregnancy was obtained through the telephone interview (questionnaire available upon request). Pre-pregnancy weight and height were obtained from medical chart data, and body mass index was calculated as underweight (<19.8), normal weight (19.8–26.0), overweight (>26.0–29.0) and obese (>29.0).

For analysis, logistic regression was used to examine the relationship between women's characteristics and BV and between those characteristics and douching. Multinomial logistic regression^{17,18} was used to investigate the relationship between BV and the outcomes of spontaneous preterm birth, medically indicated preterm birth, or term birth, including the interaction of BV and douching habits. All variables in Tables 1 and 2 were considered for inclusion in the final model using backward selection and fitting a model with the BV and douching variables. We adopted a rigorous procedure for determining which potential confounders from Tables 1 and 2 should be included in the final model, deleting only variables that had $P > 0.20$ for association with both spontaneous and medically indicated preterm birth and that did not change any single effect estimate for the BV + douching variables by 10% or more. Analyses were conducted in SAS version 8 and Stata version 8.2. Results for all preterm births in the aggregate (not shown) were similar to those reported for the preterm birth spontaneous and medically indicated subtypes, which constituted 98% of all preterm livebirths.

Results

A total of 2961 women provided vaginal specimens, 85% of whom had normal vaginal flora (Nugent score ≤ 6). Women were more likely to have BV if they were black (odds ratio [OR] = 2.58 [95% confidence interval (CI): 1.82, 3.66]), smoked during months 1–6 of pregnancy (OR = 1.63 [1.14, 2.33]) or had a pH level >4.4 (OR = 12.41 [9.04, 17.0]) (Table 1).

Of the 1492 women who answered questions about douching behaviour, few ($n = 32$, 2.2%) reported douching during pregnancy. Therefore, the analyses only address douching behaviour in the 12 months prior to pregnancy. The majority of black women (62.0%) douched in the year prior to pregnancy while 70.3% of white women did not, thereby making black race a strong predictor for douching (OR = 2.68 [1.98, 3.64]). Single marital status (OR = 1.68 [1.22, 2.31]), <16 years of education, obesity (OR = 1.47 [1.08, 2.00]), smoking (OR = 2.09 [1.52, 2.88]) and pH > 4.4 (OR = 1.59 [1.20, 2.10]) were associated with self-reported douching prior to pregnancy (Table 2).

Of the 1420 women with all required data, 86 (6.1%) were classified as spontaneous preterm birth and 68 (4.8%) were classified as medically indicated (Table 3). BV on gram stain in the absence of douching (OR = 2.74 [1.13, 6.66]) and douching in the absence of BV (OR = 2.20 [1.29, 3.75]) were each associated with spontaneous but not medically indicated preterm births. The combination of BV and reporting douching in the year prior to pregnancy was essentially unrelated to spontaneous preterm birth (OR = 1.42 [0.57, 3.55]). Similar results were seen for preterm births in the aggregate (data not shown). We repeated the analyses for preterm births before 34 weeks only and found no significant effects (BV in the absence of douching, OR = 2.56 [0.46, 14.16], BV and douching, OR = 1.82 [0.29, 11.24] and douching in absence of BV, OR = 0.97 [0.30, 3.11]). Only delivery at the academic health centre (OR = 3.17 [1.56, 6.45]) was associated with medically indicated preterm birth (Table 3). Results for BV and douching in relation to medically indicated preterm birth were imprecise, and provided a limited and non-significant suggestion that BV in the absence of douching might be associated with increased risk (OR = 2.12 [0.80, 5.63]). When douching was eliminated from the statistical model and analysis conducted on the $n = 2499$ women with complete data on BV and other covariates in Table 3, we found no association between BV and either spontaneous or medically indicated preterm birth.

In order to shed further light on the frequency of douching and spontaneous preterm birth, we examined spontaneous preterm birth rates as a function of never douching, douching monthly or less frequently, and douching more than once per month, stratified by BV diagnosis. Among women without BV, spontaneous preterm birth rates were 3.7%, 9.4% and 7.5% among women never douching, women douching monthly or less frequently, and women douching more than once per month; medically indicated preterm birth rates were 4.3%, 4.2% and 8.2% respectively ($P = 0.37$ for association using Fisher's exact test). Among women with BV, spontaneous preterm birth rates were 10.6%, 7.8% and 3.7% among women never douching, women douching monthly or less frequently, and women douching more than once per month; medically indicated preterm birth rates were 8%, 3.4% and 0% respectively ($P < 0.01$).

Discussion

Our finding that BV at mid-pregnancy is moderately associated with an increased risk of spontaneous preterm birth is consistent with previous reports.^{1,3} The risk ratio of 2 is similar to the result of a meta-analysis of BV and preterm birth by Guise *et al.*¹⁹ We report the largest cohort study ever completed to explore douching prior to pregnancy and not unexpectedly found that douching at this time was linked to spontaneous preterm birth.

What is perplexing to us and defies explanation under current paradigms is our finding that women with BV at 24–29 weeks who douched prior to pregnancy were not at an increased risk for preterm birth. In fact, there is some suggestion that douching is protective among these women. We would have expected these women to be the highest risk group given the effects of each separately. Thus, in these data, BV and douching have complex joint effect patterns on preterm birth and point to an area worthy of future research. We note that our study was underpowered to look at effects of BV on spontaneous preterm birth stratified by douching history, and that a much larger study would be needed to answer this question. Perhaps the concept of BV as a marker of upper reproductive tract infection is altered by a history of douching. In the absence of douching, BV may serve as an appropriate marker of upper reproductive tract infection. In the presence of douching, even before pregnancy when it may change the presence of or susceptibility to infection, BV may be a poor proxy for such infection. Others have drawn similar conclusions about how alterations in vaginal microflora and preterm birth are linked in a complex fashion that spans aetiological possibilities from stress²⁰ to mutations in genes coding for immune modulators.⁵

Limitations of our study include the statistical imprecision in the effect estimates when preterm birth is categorised into spontaneous or medically indicated preterm births, as well as uncertainty regarding whether this breakdown is an appropriate way to classify preterm births.²¹ Gram staining is a 19th century technology that looks at overall patterns rather than subtle differences and may lump a diverse group of vaginal micro-environments into single categories. It also does not reflect activation of the immune system. For example, how one handles 'intermediate' changes in the flora as assessed by Nugent's criteria in analyses is in debate, with some considering these flora to be normal and others abnormal. Douching information is available from retrospective report of the year ending at the date of LMP and this may not be a relevant time period for examination of the effect of douching on BV status during pregnancy and the subsequent association with preterm birth. If douching does affect preterm birth risk only when douching occurs close to conception, then we have very low power to detect such an effect.

Studies of douching with more detailed measures of timing would shed further light on this possibility. Another limitation is that while we have data on lifetime sexual practices, we have no information on current activity. Finally, generalisability is limited by the fact that we sampled from hospitals rather than a defined community population, and our sample does contain more low-risk African-American women (who are more likely to douche, and are more likely to have BV) than the general population.²² Nevertheless, our findings encourage further examination of vaginal microflora and feminine hygienic behaviours in relation to preterm birth including assessment of the effect of douching on BV over time.

Our final caveat pertains to how we recruited our cohort. This project was not geographically defined but recruited from two academic health centres. When compared with area women giving birth, social, demographic and behavioural profiles were different. Women from the clinics had lower rates of preterm birth and, contrary to area births, there was no black/white difference in risk of preterm births. This raises questions that are inherent in our study design about the widespread applicability of our findings.²³

Conclusion

Bacterial vaginosis in the absence of douching prior to pregnancy and prior douching in the absence of BV are associated with spontaneous preterm birth, while the combination is not.

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Table 1

Characteristics of women by bacterial vaginosis (BV) status at 24–29 weeks' gestation: the Pregnancy, Infection and Nutrition Study, 1995–2001 ($N = 2961^a$)

	Total <i>N</i>	Positive <i>n</i> (%)	Adjusted odds ratio ^b for BV & 95% confidence interval
Total cohort	2961	439 (14.8)	
Maternal race			
Black	1233	275 (22.3)	2.58 [1.82, 3.66]
White/other	1728	164 (9.5)	1.00 Reference
Marital status			
Married	1418	130 (9.2)	1.00 Reference
Not married	1539	308 (20.0)	1.33 [0.92, 1.92]
Missing	4		
Maternal age (years)			
<20	478	91 (19.0)	1.15 [0.71, 1.86]
20–34	2182	311 (14.3)	1.00 Reference
35+	301	37 (12.3)	1.26 [0.76, 2.08]
Maternal education (years)			
<12	622	110 (17.7)	0.77 [0.43, 1.40]
12	931	165 (17.7)	0.86 [0.52, 1.41]
13–15	689	104 (15.1)	0.81 [0.50, 1.32]
16+	710	56 (7.9)	1.00 Reference
Missing	9		
Poverty index			
<50% of poverty level	214	46 (21.5)	1.22 [0.68, 2.20]
50–99% of poverty level	563	98 (17.4)	1.19 [0.75, 1.87]
100–199% of poverty level	727	130 (17.9)	1.27 [0.84, 1.92]
200%+ of poverty level	1010	97 (9.6)	1.00 Reference
Missing	447		
Pre-pregnancy body mass index			
Underweight	424	56 (13.2)	0.79 [0.50, 1.27]
Normal weight	1255	171 (13.6)	1.00 Reference
Overweight	321	50 (15.6)	0.84 [0.52, 1.36]
Obese	742	118 (15.9)	0.95 [0.67, 1.34]
Missing	219		
Parity			
0	1343	179 (13.3)	1.00 Reference
1	915	143 (15.6)	1.27 [0.89, 1.82]
2+	694	117 (16.9)	1.1 [0.73, 1.67]
Missing	9		
Number of lifetime sex partners			
1	329	26 (7.9)	1.00 Reference
2	258	37 (14.3)	1.43 [0.74, 2.80]

	Total N	Positive n (%)	Adjusted odds ratio ^b for BV & 95% confidence interval
3+	1755	271 (15.4)	1.31 [0.77, 2.22]
Missing	619		
Self-reported sexually transmitted infections during pregnancy ^c			
None	2383	346 (14.5)	1.00 Reference
1	255	45 (17.7)	1.20 [0.73, 1.97]
2+	54	9 (16.7)	0.77 [0.25, 2.33]
Missing	269		
Self-reported yeast infection during pregnancy			
None	1982	305 (15.4)	1.00 Reference
1	576	88 (15.3)	0.82 [0.57, 1.17]
2+	138	8 (5.8)	0.15 [0.05, 0.42]
Missing	265		
Smoked (months 1–6)			
Yes	694	120 (17.3)	1.63 [1.14, 2.33]
No	1971	276 (14.0)	1.00 Reference
Missing	296		
pH			
4.4	2042	93 (4.6)	1.00 Reference
>4.4	900	344 (38.2)	12.41 [9.04, 17.0]
Missing	19		

^aSample size for frequencies. Adjusted OR model $N = 2050$ owing to missing values.

^bAdjusted for all variables in the table.

^cSexually transmitted infections were reported as of 24–29 weeks' gestation and include: genital/vaginal warts, genital herpes, chlamydia, trichomonas, gonorrhea, syphilis.

Table 2

Characteristics of women by douching behaviour in the 12 months prior to pregnancy: the Pregnancy, Infection and Nutrition Study, 1995–2001 ($N = 1492^a$)

	Total <i>N</i>	Douched <i>n</i> (%)	Adjusted odds ratio ^b for douching and 95% confidence intervals
Total cohort	1492	624 (41.8)	
Maternal race			
Black	560	347 (62.0)	2.68 [1.98, 3.64]
White/other	932	277 (29.7)	1.00 Reference
Marital status			
Married	806	210 (26.0)	1.00 Reference
Not married	686	414 (60.4)	1.68 [1.22, 2.31]
Maternal age (years)			
<20	202	107 (53.0)	0.57 [0.37, 0.90]
20–34	1102	472 (42.8)	1.00 Reference
35+	188	45 (23.9)	0.92 [0.59, 1.45]
Maternal education (years)			
<12	279	175 (62.7)	5.04 [3.01, 8.44]
12	417	234 (56.1)	4.44 [2.91, 6.76]
13–15	316	151 (47.8)	2.88 [1.91, 4.36]
16+	480	64 (13.3)	1.00 Reference
Poverty index			
<50% of poverty level	122	67 (54.9)	0.86 [0.51, 1.45]
50–99% of poverty level	268	161 (60.1)	1.21 [0.81, 1.80]
100–199% of poverty level	389	202 (51.9)	1.04 [0.74, 1.47]
200%+ of poverty level	651	161 (24.7)	1.00 Reference
Missing	62		
Pre-pregnancy body mass index			
Underweight	222	79 (35.6)	0.92 [0.61, 1.37]
Normal weight	679	241 (35.5)	1.00 Reference
Overweight	158	73 (46.2)	1.40 [0.92, 2.14]
Obese	405	220 (54.3)	1.47 [1.08, 2.00]
Missing	28		
Parity			
0	667	265 (39.7)	1.00 Reference
1	474	195 (41.1)	0.89 [0.65, 1.22]
2+	343	162 (47.2)	0.87 [0.60, 1.26]
Missing	8		
Number of lifetime sex partners			
1	213	57 (26.8)	1.00 Reference
2	153	63 (41.2)	0.87 [0.51, 1.50]
3+	1081	484 (44.8)	1.02 [0.67, 1.55]
Missing	45		

	Total N	Douched n (%)	Adjusted odds ratio ^b for douching and 95% confidence intervals
Self-reported sexually transmitted infections during pregnancy ^c			
None	1321	533 (40.4)	1.00 Reference
1	138	76 (55.1)	0.68 [0.44, 1.06]
2+	29	13 (44.8)	0.54 [0.22, 1.32]
Missing	4		
Self-reported yeast infection during pregnancy			
None	1128	422 (37.4)	1.00 Reference
1	290	166 (57.2)	1.70 [1.23, 2.36]
2+	73	35 (48.0)	1.09 [0.61, 1.96]
Missing	1		
Smoked (months 1–6)			
Yes	352	212 (60.2)	2.09 [1.52, 2.88]
No	1126	403 (35.8)	1.00 Reference
Missing	14		
pH			
4.4	1057	391 (37.0)	1.00 Reference
>4.4	425	229 (53.9)	1.59 [1.20, 2.10]
Missing	10		

^aThe sample size is smaller for the douching variables than for bacterial vaginosis because questions about douching were added to the study protocols at a later time. Adjusted OR model $n = 1335$ owing to missing values.

^bAdjusted for all variables in the table.

^cSexually transmitted infections were reported as of 24–29 weeks' gestation and include: genital/vaginal warts, genital herpes, chlamydia, trichomonas, gonorrhea, syphilis.

Table 3

Bacterial vaginosis (BV), douching prior to pregnancy, and spontaneous or medically indicated preterm birth: adjusted odds ratios and 95% confidence intervals ($N = 1420$)

	Term births		Spontaneous preterm birth		Medically indicated preterm birth	
	N	n	AOR [95% CI]	n	AOR [95% CI]	
Poverty						
<50%	108	6	0.82 [0.34, 2.14]	5	1.05 [0.37, 2.98]	
50% to <100%	224	25	1.71 [0.93, 3.15]	16	1.56 [0.78, 3.12]	
100% to <200%	350	24	1.06 [0.58, 1.92]	13	0.80 [0.40, 1.63]	
200+%	584	31	1.00 Reference	34	1.00 Reference	
			P= 0.21		P= 0.38	
Race						
Black	452	34	0.80 [0.48, 1.33]	28	1.67 [0.93, 3.00]	
White/other	814	52	1.00 Reference	40	1.00 Reference	
			P= 0.39		P= 0.09	
Site						
UNC	838	58	1.53 [0.91, 2.57]	57	3.17 [1.56, 6.45]	
Wake	428	28	1.00 Reference	11	1.00 Reference	
			P= 0.11		P< 0.01	
pH						
>4.4	350	36	1.57 [0.95, 2.60]	18	0.84 [0.45, 1.54]	
4.4	916	50	1.00 Reference	50	1.00 Reference	
			P= 0.08		P= 0.57	
BV/douche combination						
Positive BV, douched	99	8	1.42 [0.57, 3.55]	3	0.65 [0.18, 2.40]	
Positive BV, did not douche	60	8	2.74 [1.13, 6.66]	6	2.12 [0.80, 5.63]	
Negative BV, douched	410	42	2.20 [1.29, 3.75]	25	1.25 [0.69, 2.25]	
Negative BV, did not douche	697	28	1.00 Reference	34	1.00 Reference	
			P= 0.01		P= 0.32	
Partners						
1	189	6	1.00 Reference	9	1.00 Reference	

	Term births	Spontaneous preterm birth		Medically indicated preterm birth		
		N	n	AOR [95% CI]	n	AOR [95% CI]
Yeast (during pregnancy)	2	128	12	3.00 [1.12, 7.99]	4	0.65 [0.20, 2.11]
	3+	914	67	2.20 [0.97, 4.94]	53	1.31 [0.66, 2.60]
	Missing	35	1	<i>P</i> = 0.08	2	<i>P</i> = 0.35
	0	978	52	1.00 Reference	55	1.00 Reference
	1	224	29	2.32 [1.40, 3.85]	12	1.02 [0.52, 2.00]
	2+	63	5	1.52 [0.56, 4.11]	1	0.31 [0.04, 2.35]
	Missing	1		<i>P</i> < 0.01		<i>P</i> = 0.52
	STD (during pregnancy)					
	0	1126	77	1.00 Reference	65	1.00 Reference
	1	115	6	0.63 [0.26, 1.53]	1	0.15 [0.02, 1.09]
2+	21	3	1.85 [0.50, 6.76]	2	1.94 [0.41, 9.26]	
			<i>P</i> = 0.36		<i>P</i> = 0.01	
Total	1266	86		68		

Variables in final model included percentage of poverty, race, site, pH, BV, douching, BV/douching interaction, number of partners, yeast during pregnancy, and STD during pregnancy. All reported ORs have been fully adjusted for these covariates. Variables considered in an initial model but eliminated after testing for confounding include maternal status, maternal age, maternal education, pregravid body mass index, parity, and smoking during pregnancy.

AOR, adjusted odds ratio; UNC, University of North Carolina; STD, sexually transmitted diseases.