Vaginal hydrolytic enzymes, immunoglobulin A against *Gardnerella vaginalis* toxin, and risk of early preterm birth among women in preterm labor with bacterial vaginosis or intermediate flora

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OBJECTIVE: The purpose of this study was to determine whether the microbial hydrolytic enzymes, sialidase and prolidase, and immunoglobulin A against the *Gardnerella vaginalis* cytolysin (anti-Gvh IgA) increase the risk for early preterm birth (≤34 weeks of gestation) among women with bacterial vaginosis or intermediate flora.

STUDY DESIGN: Two hundred eighteen afebrile women in preterm labor with intact membranes had a vaginal Gram stain performed, and sialidase, prolidase, and anti-Gvh IgA concentrations were determined.

RESULTS: Women with bacterial vaginosis or intermediate flora had significantly higher sialidase and prolidase concentrations than women with normal flora. Among women with bacterial vaginosis or intermediate flora, the women with sialidase had a higher rate of early preterm birth (P = .05). Sialidase had a sensitivity of 43% and specificity of 77% for early preterm birth. Prolidase and anti-Gvh IgA did not predict early preterm birth.

CONCLUSION: Women in preterm labor with bacterial vaginosis or intermediate flora and detectable sialidase are at increased risk of early preterm birth. (Am J Obstet Gynecol 2002;187:877-81.)

Key words: Early preterm birth, bacterial vaginosis, *Gardnerella vaginalis,* immunoglobulin A, sialidase, prolidase

Bacterial vaginosis (BV) is characterized by a shift in vaginal flora, with a decrease in the prevalence of *Lactobacillus* and an increase in the prevalence and concentration of anaerobic bacteria, *Gardnerella vaginalis,* and *Mycoplasma hominis.* BV, which is consistently associated with preterm delivery,[1,2] is also associated with first- and second-trimester miscarriage,[3,4] amniotic fluid infection,[5,6] histologic chorioamnionitis,[7] and postpartum endometritis.[8] The consistent associations between BV and these markers of upper genital tract infection provide strong indirect evidence that abnormal vaginal flora can ascend to cause bacterial upper genital tract infection and preterm birth. However, most pregnant women with BV have normal pregnancy outcomes.

The presence and concentrations of microbial hydrolytic enzymes in women with BV vary. Sialidases (or neuraminidases) are glycosylases that are produced in vitro by several BV-associated bacteria.[9] Prolidase (or proline aminopeptidase) is another hydrolytic enzyme produced by BV-associated bacteria, including *G vaginalis* and *Mobiluncus* spp.[10]

The only characterized immunogenic virulence factor found in BV thus far is the *G vaginalis* hemolytic toxin (Gvh).[11,12] The production and concentration of immunoglobulin A (IgA) in response to Gvh in women with BV also vary. Low levels of anti-Gvh IgA in women with BV who are not pregnant are correlated with high levels of vaginal sialidase activity[13] and with cleavage of IgA in vaginal fluid.[14]

It remains to be established whether different profiles of these biochemical markers correspond to different levels of risk for poor pregnancy outcome. In the present study, we examined the hypothesis that an increased concentration of sialidase and prolidase and a decreased con-

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Table I. Demographic and reproductive characteristics and pregnancy outcomes for women with BV and intermediate and normal flora, by Gram stain

Characteristic/outcome	*BV (n = 31)*	*Intermediate flora (n = 66)*	*Normal flora (n = 121)*
Age (y)			
Median	24	23	26
Range	15-36	16-42	15-43
Racial group (No.)			
White	18 (58%)	40 (61%)	81 (67%)
Black	6 (19%)	12 (18%)	20 (16.5%)
Other	7 (23%)	14 (21%)	20 (16.5%)
Cigarette smoker (No.)	4 (13%)	14 (22%)	21 (18%)
Nulliparity (No.)	13 (42%)	27 (42%)	55 (46%)
Previous preterm delivery for parous women (No.)	8/18 (44%)	14/39 (36%)	32/66 (48%)
Weeks of gestation at enrollment (No.)*			
Median	28	30.5	32
Range	20-34	23-34	22-34
Days from enrollment to delivery (No.)			
Median	12	14	21
Range	1-110	1-129	1-129
Weeks of gestation at delivery (No.)*			
Median	31	33.5	36
Range	21-39	23-41	23-41
Infant birth weight (g)†			
Median	1580	2261	2581
Range	482-4107	596-3739	553-4369

*$P < .001$, by Kruskal-Wallis analysis of variance.
†$P = .008$, by Kruskal-Wallis analysis of variance.

centration of anti-Gvh IgA would be associated with an increased risk of early preterm birth among women in preterm labor with BV or intermediate vaginal flora.

Material and methods

The study population included 218 afebrile women who were admitted in premature labor with intact membranes to the University of Washington Medical Center or Swedish Medical Center in Seattle between June 1991 and June 1997. All women provided written informed consent for study participation, and the Institutional Review Boards of all participating hospitals approved the study protocol. Participants were at gestational ages of 20 to 34 weeks by obstetric estimate, which was determined from menstrual dating or from the earliest available ultrasound imaging. Preterm labor was defined as regular uterine contractions at a frequency of <10 minutes, with either documented cervical change or a cervical dilatation of >1 cm or effacement of >50%. The study population was previously been described in detail.[15] Of 310 participants, 11 women (4%) were lost to follow-up, 12 women (4%) were excluded because of major congenital anomalies or higher-order multiple gestation, 8 women (3%) refused speculum examination, 17 women (5%) had the speculum examination omitted because of imminent delivery, and 44 women (14%) did not have sufficient vaginal fluid for analysis. The remaining 218 subjects had vaginal fluid samples available for these analyses.

Vaginal specimens were obtained by saturating six Pur Wrap (Harwood Products Co, Guildford, Me) swabs with fluid from the posterior vaginal fornix. One swab was used for a Gram stain of the vaginal flora. Vaginal flora was characterized by Gram stain as normal flora, intermediate flora, or BV, according to published criteria.[16]

Samples of vaginal fluid to measure biochemical parameters were collected with three saturated swabs, inoculated into 0.9 mL of sterile phosphate-buffered saline solution (sodium chloride 0.9%), and immediately frozen to –70°C. Sialidase activity was determined by incubation of 50 μL of the vaginal sample with 50 μL of the specific substrate at pH 5.0, as previously described.[13] Specific activity was expressed as nanomoles of methoxyphenol that was produced by comparison with a standard curve of pure methoxyphenol. Positive sialidase activity was considered to be any detectable level >0.04 nmol of methoxyphenol.

Prolidase activity was determined as previously described.[14] Absorbance (mOD) was read at 405 nm. Positive prolidase activity was considered as any detectable level >5 mOD. Four samples were not interpretable; therefore, prolidase results were available for 214 of 218 participants.

The anti-Gvh IgA concentration was evaluated according to an enzyme-linked immunosorbent assay procedure that has been previously described.[14] The lower limit of detection was 5 mOD. A cutoff value of 94 mOD was based on the 50th percentile of the anti-Gvh IgA levels

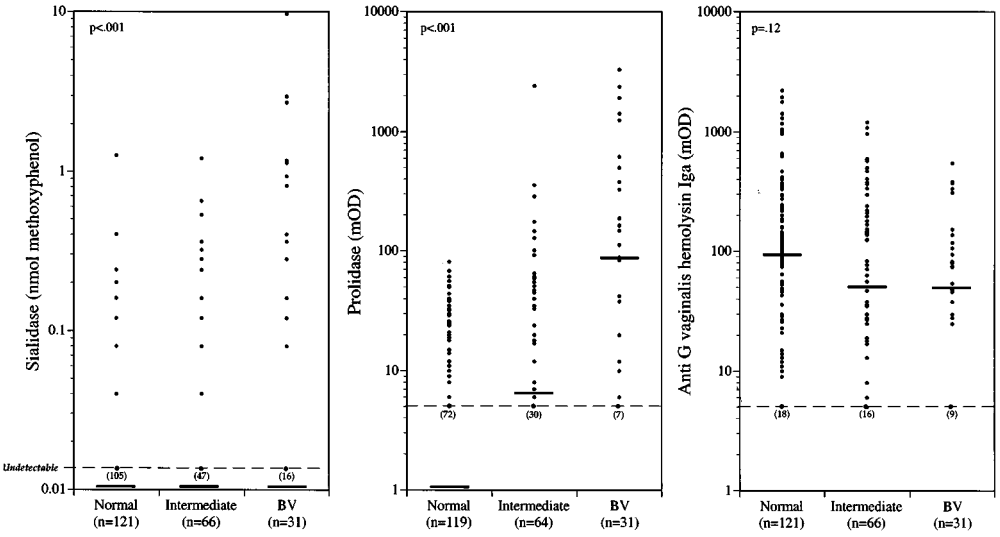

Fig 1. Sialidase, prolidase, and anti-Gvh IgA concentrations, according to vaginal Gram stain category. Statistical significance was determined by Kruskal-Wallis analysis of variance.

that were measured in the 85 women from this data set who had a normal Gram stain and no *G vaginalis* by culture. We defined anti-Gvh IgA values at or below the threshold value of 94 mOD as the low IgA response, and values of >94 mOD were considered as high IgA response.

We compared categoric variables using the χ^2 or Fisher exact test. The Mann-Whitney test and Kruskal-Wallis analysis of variance were used to compare groups for continuous data; the Bonferroni correction for multiple comparisons was used to examine differences between the BV and intermediate flora groups in pregnancy outcomes. Early preterm birth was defined as delivery at ≤34 weeks of gestation. We examined the associations between sialidase, prolidase, and anti-Gvh IgA with early preterm birth among the subset of women with BV or intermediate flora. We also calculated the sensitivity, specificity, and positive and negative predictive values of each of these biochemical markers for early preterm birth among the subset with BV or intermediate flora.

Results

Of 218 participants in preterm labor, 31 women (14%) had BV, and 66 women (30%) had intermediate vaginal flora, by Gram stain criteria. Table I gives a summary of the demographic and reproductive characteristics and pregnancy outcomes, which were stratified by Gram stain findings. Compared with women with normal flora, women with BV or intermediate flora presented and were delivered at an earlier gestational age. There were no statistically significant differences between women with BV and intermediate flora, with respect to gestational age at delivery or infant birth weight.

Fig 1 shows the sialidase, prolidase, and anti-Gvh IgA concentrations for women with normal flora, intermediate flora, or BV by Gram stain criteria. Sialidase and prolidase concentrations were significantly higher among the BV and intermediate flora groups, compared with the normal flora group ($P < .001$, by global Kruskal-Wallis analysis for both enzymes). Anti-Gvh IgA concentrations were lower in the BV and intermediate flora groups than in the normal flora group, but this did not reach statistical significance.

We examined the relationships between sialidase and prolidase concentrations and IgA response by a categorization of anti-Gvh concentrations as low or high, using a cutoff value of 94 mOD (median value for women with a normal Gram stain, Fig 2). For women with BV or intermediate flora, sialidase ($P = .03$) and prolidase ($P = .04$) concentrations were significantly higher among the group with a low IgA response. However, among women with a normal Gram stain, there was no significant difference in sialidase and prolidase concentrations in relation to IgA response (data not shown).

Of 218 women, 105 women (48%) had an early preterm birth. Delivery at ≤34 weeks occurred in 58 of 97 women (60%) with BV or intermediate flora compared with 47 of 121 women (39%) with a normal Gram stain ($P = .003$). We examined the associations between sialidase, prolidase, and anti-Gvh IgA response and early preterm birth in the subset of women with BV or intermediate flora. Early preterm birth occurred in 25 of 34 women (74%) with BV or intermediate flora and sialidase detected in vaginal fluid compared with 33 of 63 women (52%) with BV or intermediate flora but no detectable sialidase ($P = .05$). Among women with BV or intermediate flora, sialidase had a sensitivity of 43%, specificity of 77%, positive predictive value of 74%, and negative predictive value of 48% for early preterm birth

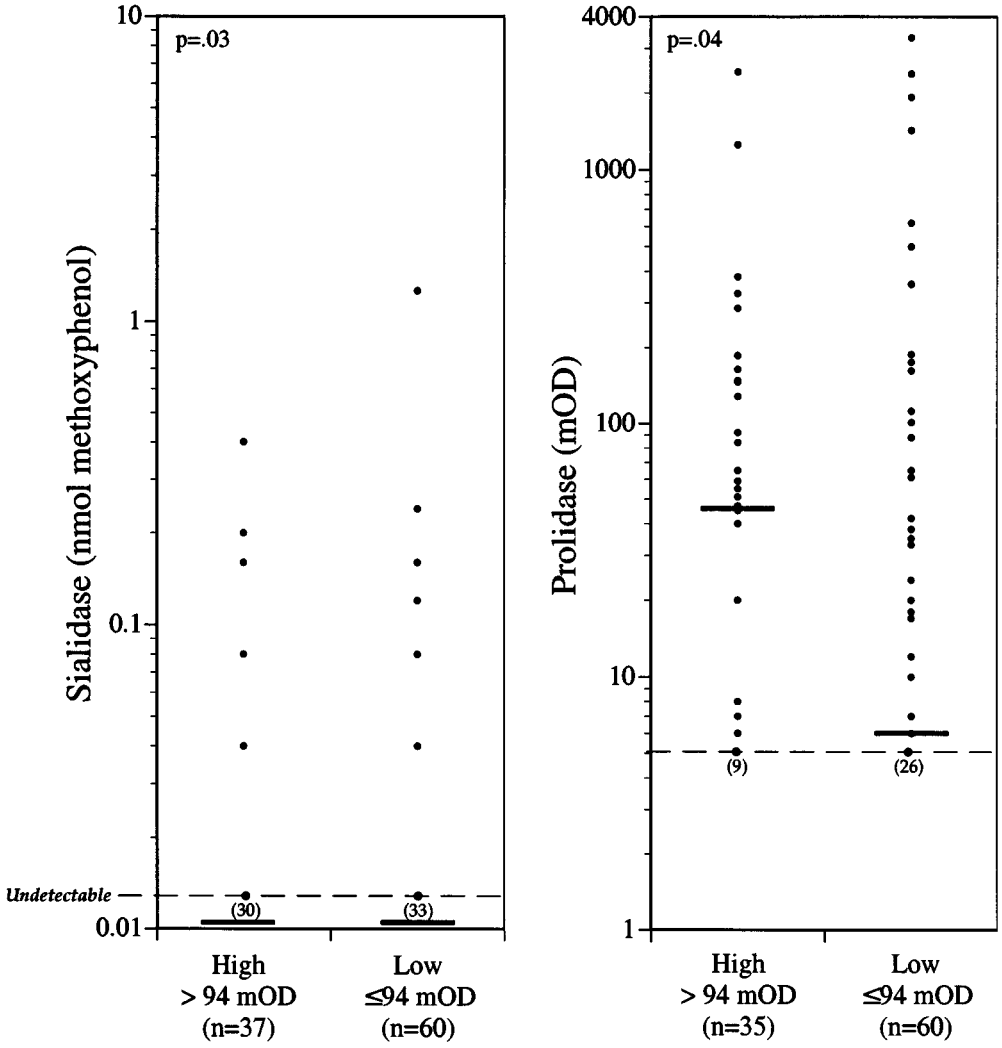

Fig 2. Sialidase and prolidase concentrations, according to anti-Gvh IgA category, among women with BV or intermediate flora. Statistical significance was determined by the Mann-Whitney test.

Table II. Sensitivity, specificity, and positive and negative predictive values of sialidase, prolidase, and anti-Gvh IgA for the prediction of delivery at ≤34 weeks, among women with BV or intermediate flora

	Positive values/ women tested (No.)	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
Sialidase detected	34/97 (35%)	43	77	74	48
Prolidase detected	60/95 (63%)	62	36	58	40
Anti-Gvh IgA ≤94 mOD	60/97 (62%)	60	36	58	37

(Table II). Prolidase and a low anti-Gvh IgA concentration were not predictive of early preterm birth.

Comment

Women with BV or intermediate flora in this cohort had preterm labor at an earlier gestational age and were delivered at an earlier gestational age compared with women with normal vaginal flora. These observations agree with other studies that show an association between BV and preterm birth.[1,2]

We also confirmed that pregnant women with BV or intermediate flora have higher concentrations of sialidase and prolidase, two microbial hydrolytic enzymes that are produced by BV-associated bacteria, than do women with normal vaginal flora. In addition, pregnant women with BV or intermediate flora appear to have lower anti-Gvh

IgA concentrations than do women with normal flora, although this finding did not reach statistical significance. Women with BV or intermediate flora and a low anti-Gvh IgA response have significantly higher concentrations of sialidase and prolidase than do women with BV or intermediate flora and a normal anti-Gvh IgA response. This lends support to the concept that BV encompasses a spectrum of not only microbes but also microbial products and host response.

Among women with BV or intermediate flora, those women with sialidase had the highest rate of early preterm birth in this cohort. It is biologically plausible that sialidase might increase the risk for preterm birth. Sialidases cleave the terminal sialic acid from various glycoproteins, including immunoglobulins, especially IgA. Thus, sialidases may potentially hamper the local mucosal immune response.[15] In addition, desialylation can alter the mucin plug both by altering the charge potential of glycoproteins and by favoring further degradation by proteases.[16] Persistent sialidase activity after antibiotic treatment in pregnant women was associated with an increased risk of preterm birth in one cohort.[17] However, another study did not demonstrate an association between midtrimester cervical sialidase concentrations and preterm birth.[18]

We defined a low anti-Gvh IgA concentration in our cohort as below the median value for women with a normal vaginal Gram stain and no *G vaginalis* on culture (≤94 mOD) and a high anti-Gvh concentration as >94 mOD. This is a much lower cutoff value for anti-Gvh IgA than has been used in previous studies of nonpregnant women.[11-13] There is no published information about cervical or vaginal IgA concentrations among pregnant women in preterm labor, so it is difficult to put this information into context.

Women with BV or intermediate flora and a low anti-Gvh IgA concentration have increased vaginal sialidase and prolidase concentrations. It is plausible that sialidase contributes to the local degradation of IgA, thus facilitating the entry of vaginal pathogenic bacteria into the upper cervix and uterus. However, because we sampled sialidase and IgA simultaneously, the association between sialidase and low anti-Gvh IgA response cannot be interpreted as causal in this data set.

These data suggest that sialidase is associated with an increased risk of early preterm birth among women with BV or intermediate flora. However, although sialidase has fairly good specificity, it does not have high sensitivity for early preterm birth. Thus, sialidase in isolation will probably have limited usefulness as a clinical screening test. Stated differently, although sialidase appears to increase the risk for early preterm birth, this enzyme is not sufficient to explain the entire association between BV or intermediate flora and early preterm birth. This implies that there are probably other, as yet uncharacterized, virulence and/or host immune factors that contribute to adverse reproductive outcomes among some women with BV. Cervical leukocytes and epithelial cells represent another potential site that is influenced by the microbes that are present in BV and by their products. It is critical that we learn more about the factors that contribute to the pathogenicity of BV if we hope to devise effective prevention and treatment strategies.

REFERENCES

1. Gravett M, Nelson H, DeRouen T, Critchlow C, Eschenbach D, Holmes K. Independent association of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. JAMA 1986;256:1899-903.
2. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. N Engl J Med 1995;333:1737-42.
3. Ralph SG, Rutherford AJ, Wilson JD. Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. BMJ 1999;319:220-3.
4. Llahi-Camp JM, Rai R, Ison C, Regan L, Taylor-Robinson D. Association of bacterial vaginosis with a history of second trimester miscarriage. Hum Reprod 1996;11:1575-8.
5. Watts DH, Krohn MA, Hillier SL, Eschenbach DA. The association of occult amniotic fluid infection with gestational age and neonatal outcome among women in preterm labor. Obstet Gynecol 1992;79:351-7.
6. Newton ER, Piper J, Peairs W. Bacterial vaginosis and intra-amniotic infection. Am J Obstet Gynecol 1997;176:672-7.
7. Hillier S, Martius J, Krohn M, Kiviat N, Holmes K, Eschenbach D. A case-control study of chorioamnionic infection and chorioamnionitis in prematurity. N Engl J Med 1988;319:972-8.
8. Watts DH, Krohn MA, Hillier SL, Eschenbach DA. Bacterial vaginosis as a risk factor for post-cesarean endometritis. Obstet Gynecol 1990;75:52-8.
9. Briselden AN, Moncla BJ, Stevens CE, Hillier SL. Sialidases (neuraminidases) in bacterial vaginosis and bacterial vaginosis-associated microflora. J Clin Microbiol 1992;30:663-6.
10. Schoonmaker JN, Lunt BD, Lawellin DW, French JI, Hillier SL, McGregor JA. A new proline aminopeptidase assay for diagnosis of bacterial vaginosis. Am J Obstet Gynecol 1991;165:737-42.
11. Cauci S, Monte R, Ropele M, Missero C, Not T, Quadrifoglio F, et al. Pore-forming and haemolytic properties of the *Gardnerella vaginalis* cytolysin. Mol Microbiol 1993;9:1143-55.
12. Cauci S, Scrimin F, Driussi S, Ceccone S, Monte R, Fant L, et al. Specific immune response against *Gardnerella vaginalis* hemolysin in patients with bacterial vaginosis. Am J Obstet Gynecol 1996;175:1601-5.
13. Cauci S, Driussi S, Ceccone S, Monte R, Lanzafame P, Pitzus E, et al. Immunoglobulin A response against *Gardnerella vaginalis* hemolysin and sialidase activity in bacterial vaginosis. Am J Obstet Gynecol 1998;178:511-5.
14. Cauci S, Monte R, Driussi S, Lanzafame P, Quadrifoglio F. Impairment of the mucosal immune system: IgA and IgM cleavage detected in vaginal washings of a subgroup of patients with bacterial vaginosis. J Infect Dis 1998;178:1698-706.
15. Pilatte Y, Bigno J, Lambré CR. Sialic acids as important molecules in the regulation of the immune system: pathophysiological implications of sialidases in immunity. Glycobiology 1993;3:201-17.
16. Wiggins R, Hicks SJ, Soothill PW, Millar MR, Corfield AP. Mucinases and sialidases: their role in the pathogenesis of sexually transmitted infections in the female genital tract. Sex Transm Infect 2001;77:402-8.
17. McGregor JA, French JI, Jones W, Milligan K, McKinney PJ, Patterson E, et al. Bacterial vaginosis is associated with prematurity and vaginal fluid mucinase and sialidase: results of a controlled trial of topical clindamycin cream. Am J Obstet Gynecol 1994;170:1048-60.
18. Andrews WW, Tsao J, Goldenberg RL, Hauth JC, Mercer B, Iams J, et al. The preterm prediction study: failure of midtrimester cervical sialidase level elevation to predict subsequent spontaneous preterm birth. Am J Obstet Gynecol 1999;180:1151-4.