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Determination of Immunoglobulin A against *Gardnerella vaginalis* Hemolysin, Sialidase, and Prolidase Activities in Vaginal Fluid: Implications for Adverse Pregnancy Outcomes

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A nested case-control study of low birth weight and preterm delivery was performed with singleton women. Immunoglobulin A (IgA) against the *Gardnerella vaginalis* hemolysin (anti-Gvh IgA) and sialidase and prolidase activities were determined in vaginal fluid at 17 weeks of gestation. Sialidase positivity and bacterial vaginosis with high prolidase activity were associated with 2- and 11-fold increased risks for low birth weight, respectively. No woman with bacterial vaginosis plus a strong anti-Gvh IgA response had an adverse outcome.

Low birth weight (LBW; <2,500 g), resulting primarily from preterm delivery (PTD; spontaneous delivery before 37 weeks of gestation [29]), is the strongest risk factor for neonatal mortality and morbidity (14, 20). Bacterial vaginosis (BV) is associated with adverse pregnancy outcomes (15, 17, 22–24, 26), but few women with BV have LBW or PTD infants (15). Identification of more specific predictive markers than a mere BV diagnosis could indicate which women would benefit from antibiotic intervention (4, 32).

BV is characterized by a decrease in lactobacillus colonization and overgrowth of many anaerobic or facultative species (1, 5, 13, 30), such as *Gardnerella vaginalis*. Immunoglobulins A (IgAs) against the hemolytic *G. vaginalis* toxin (Gvh) and sialidase and prolidase activities have been measured in the vaginal fluid of BV-positive women (3, 5–11, 16, 22, 27, 28, 33). We conducted a nested case-control study to determine whether sialidase and prolidase activities, combined with anti-Gvh IgA, can identify BV- and/or *G. vaginalis*-positive pregnant women at risk for producing LBW and PTD infants.

We selected 579 women from a Danish regional cohort of 2,846 singleton pregnant women prospectively enrolled from 1992 to 1994 (29, 30). (Appropriate informed consent was obtained, and clinical research was conducted in accordance with the guidelines for human experimentation of the authors' institutions.) Eighty-six PTD and 116 LBW cases were suitable for analysis; 40 PTD cases were also LBW cases. A control group of 417 women delivering normal birth weight babies at term (NTD) was randomly selected. The mean gestational age at enrollment was 16 weeks 5 days (range, 7 weeks 4 days to 24 weeks).

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BV was clinically diagnosed by Amsel criteria (1, 30). Vaginal fluid samples were collected with a sterile cotton-tipped swab, inoculated into 1 ml of sterile saline containing 2% calf serum, and immediately frozen to −80°C.

Vaginal flora were determined by culture of cervicovaginal swabs by routine methods (19, 30). The term *Bacteroides* group was used for isolates of *Bacteroides* spp., *Prevotella* spp., *Porphyromonas* spp., and the *Bacteroides fragilis* group. The remaining isolates were collectively assigned to the nonspecified group of anaerobic bacteria. *Mobiluncus* spp. were not identified (19).

Healthy controls were 133 women without bacteria other than lactobacilli. Cutoff values for sialidase, prolidase, and anti-Gvh IgA were determined in these women as follows: an anti-Gvh IgA (6) value below a threshold of 392 millioptical density (mOD) (mean value of the anti-Gvh IgA in healthy controls plus 1 standard deviation [SD]) was considered no response, a value of ≥392 and <784 mOD (two times the cutoff) was considered a low response, and a value of ≥784 mOD was considered a high response.

Sialidase specific activity (9) was expressed in nanomoles of methoxyphenol produced. A value below the +1 cutoff (mean of healthy controls plus 1 SD) was considered no activity, a value of ≥0.19 nmol (>+1 cutoff) was considered positive, and a value of ≥5.00 nmol (>+2 cutoff) was considered high (7).

Prolidase activity (7) was scored as follows: no activity, <22 mOD (mean of healthy controls plus 1 SD); positive, ≥22 mOD (+1 cutoff); high, ≥2,000 mOD (+2 cutoff) (11).

Univariate comparisons of proportions were carried out by using Fisher's exact test. *P* values of <0.05 were considered statistically significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the risk for LBW and PTD. Two-tailed Spearman rho coefficients were used to examine the correlations between continuous variables. The SPSS software package was used for analyses of data. Because

TABLE 1. Association between sialidase or prolidase activity levels and LBW or PTD

Measured parameter(s) and/or vaginal colonization	% Positive[a]		
	NTD (n = 116)	LBW (n = 116)	PTD (n = 86)
BV	14.6	20.7, 1.5 (0.9–2.6)	12.8, 0.9 (0.4–1.7)
G. vaginalis	36.2	42.2, 1.3 (0.8–2.0)	36.0, 1.0 (0.6–1.6)
BV and sialidase activity			
≥+1 (cutoff)	9.1	15.7, 1.9 (1.0–3.4)[b]	7.1, 0.8 (0.3–1.9)
≥+2	1.0	3.5, 3.7 (0.9–15.1)	2.4, 2.5 (0.4–13.8)
G. vaginalis and sialidase activity			
≥+1 (cutoff)	11.0	18.3, 1.8 (1.0–3.2)[b]	11.8, 1.1 (0.5–2.2)
≥+2	1.0	3.5, 3.7 (0.9–15.1)	2.4, 2.5 (0.5–13.8)
Sialidase activity			
≥+1 (cutoff)	15.3	28.7, 2.2 (1.4–3.6)[b]	20.0, 1.4 (0.8–2.5)
≥+2	1.0	3.5, 3.7 (0.9–15.1)	2.4, 2.5 (0.4–13.8)
BV and prolidase activity			
≥+1 (cutoff)	13.5	18.6, 1.5 (0.8–2.5)	12.0, 0.9 (0.4–1.8)
≥+2	0.2	2.7, 11.3 (1.2–109.6)[b]	1.2, 5.0 (0.3–81.5)
G. vaginalis and prolidase activity			
≥+1 (cutoff)	23.4	31.0, 1.5 (0.9–2.3)	26.5, 1.2 (0.7–2.0)
≥+2	0.7	4.4, 6.4 (1.5–27.0)[b]	3.6, 5.2 (1.0–26.0)[b]
Prolidase activity			
≥+1 (cutoff)	33.7	41.6, 1.4 (0.9–2.1)	34.9, 1.1 (0.6–1.7)
≥+2	1.0	4.4, 4.8 (1.3–18.0)[b]	3.6, 3.9 (0.8–17.6)

[a] Where two values are given, the second value is the OR. Values in parentheses are 95% CIs.
[b] $P < 0.05$.

of the low number of adverse pregnancy outcome cases, a multivariate analysis was not performed.

Table 1 shows that positive sialidase (>+1 cutoff) was significantly associated with LBW among women with BV, among women positive for *G. vaginalis*, or overall. Only high prolidase values (>+2 cutoff) were significantly associated with LBW among women with BV, among women positive for *G. vaginalis*, or overall. A similar trend was found for PTD, but only the OR for *G. vaginalis* positivity plus high prolidase activity was significant.

Table 2 shows that the LBW risk appeared much lower in women colonized by *G. vaginalis* with a high anti-Gvh IgA response; the PTD risk also tended to be lower. The LBW risk was elevated two- to threefold in all subgroups of women who had a low or no anti-Gvh IgA response. Considering *G. vaginalis* and nonspecified anaerobes, the risk for LBW was nearly fivefold higher for women with low or no anti-Gvh IgA response. However, a high anti-Gvh IgA response appeared protective, as no cases of either LBW or PTD were found in any subgroups of women positive for *G. vaginalis* plus other microorganisms. No woman had a high anti-Gvh IgA response and sialidase or prolidase activity of ≥+2.

In BV-positive women, anti-Gvh IgA was inversely correlated with sialidase ($r_s = -0.227$; $P = 0.031$); prolidase showed a similar trend ($r_s = -0.169$; $P > 0.05$).

We observed that very high levels of prolidase activity may be associated with LBW. Prolidases are proteolytic enzymes that facilitate matrix remodeling and cellular infiltration and can modulate immune mediators (12, 21, 31). Several bacteria, including *G. vaginalis*, are able to produce prolidases in vitro (12, 27, 28).

We observed that detection of any sialidase activity is a marker for increased LBW risk, regardless of knowledge of the vaginal microbial condition. Sialidases are enzymes involved in the pathogenesis of several diseases by cleaving sialic acid from various glycoproteins and thus altering the immune response (25, 33). Persistent sialidase activity after antibiotic treatment has been associated with increased risk for LBW and PTD (22). Other authors found no statistically significant associations (2).

Synergistic relationships between *G. vaginalis* and anaerobes have been observed previously (7, 9, 15, 30). In this study, concomitant *G. vaginalis* and anaerobe overgrowth was associated with a high risk of poor pregnancy outcomes, especially when women had low or no anti-Gvh IgA response.

In contrast, a high anti-Gvh IgA response appeared protective against LBW or PTD. High enzymatic activities are inversely correlated with a high anti-Gvh IgA response causing impairment of the host mucosal defense (10, 11).

Our findings suggest an association between alteration of the vaginal ecology (BV and/or *G. vaginalis*) and poor pregnancy outcomes comprising two extreme profiles: (i) women with low or no anti-Gvh IgA response plus high sialidase and/or prolidase activity (11.0% of women with BV [10 of 91]), in which the host vaginal immune defense is overwhelmed by microbial virulence factors, who have a higher risk for a poor outcome, especially LBW; and (ii) women with a strong anti-Gvh IgA response and low enzymatic activity (8.8% of women with BV

TABLE 2. Association between anti-Gvh IgA response levels and LBW or PTD

Vaginal colonization and/or anti-Gvh IgA response	% Positive[a]		
	NTD (n = 417)	LBW (n = 116)	PTD (n = 86)
G. vaginalis	36.2	42.2, 1.3 (0.8–2.0)	36.0, 1.0 (0.6–1.6)
No anti-Gvh IgA	22.5	29.6, 1.4 (0.9–2.3)	24.7, 1.1 (0.7–1.9)
No or low anti-Gvh IgA	29.5	40.0, 1.6 (1.0–2.4)[b]	34.1, 1.2 (0.8–2.1)
High anti-Gvh IgA	6.7	2.6, 0.4 (0.1–1.2)	2.4, 0.3 (0.1–1.4)
G. vaginalis, BV	12.9	20.7, 1.7 (1.0–3.0)[b]	10.5, 0.8 (0.4–1.7)
No anti-Gvh IgA	8.4	16.5, 2.2 (1.2–3.9)[b]	8.2, 1.0 (0.4–2.3)
No or low anti-Gvh IgA	11.0	20.9, 2.1 (1.2–3.7)[b]	10.6, 1.0 (0.4–2.0)
High anti-Gvh IgA	1.9	0.0	0.0
G. vaginalis, anaerobes	9.8	19.0, 2.1 (1.2–3.8)[b]	11.6, 1.2 (0.6–2.5)
No anti-Gvh IgA	5.8	14.8, 2.8 (1.5–5.5)[b]	9.4, 1.7 (0.7–3.9)
No or low anti-Gvh IgA	7.4	19.1, 2.9 (1.6–5.3)[b]	11.8, 1.7 (0.8–3.5)
High anti-Gvh IgA	2.4	0.0	0.0
G. vaginalis, *Bacteroides* spp.	5.0	6.9, 1.4 (0.6–3.2)	4.7, 0.9 (0.3–2.8)
No anti-Gvh IgA	2.6	6.1, 2.4 (0.9–6.3)	3.5, 1.4 (0.4–4.9)
No or low anti-Gvh IgA	3.6	7.0, 2.0 (0.8–4.9)	4.7, 1.3 (0.4–4.1)
High anti-Gvh IgA	1.4	0.0	0.0
G. vaginalis, nonspecified anaerobes	5.0	15.5, 3.5 (1.8–6.7)[b]	8.1, 1.7 (0.7–4.1)
No anti-Gvh IgA	3.1	12.2, 4.3 (2.0–9.5)[b]	7.1, 2.4 (0.9–6.4)
No or low anti-Gvh IgA	3.8	15.7, 4.7 (2.3–9.5)[b]	8.2, 2.2 (0.9–5.6)
High anti-Gvh IgA	1.2	0.0	0.0

[a] Where two values are given, the second value is the OR. Values in parentheses are 95% CIs.
[b] $P < 0.05$.

[8 of 91]) who are not at risk for an adverse outcome. Determination of markers in vaginal fluid could help in the planning of prevention strategies (18).

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