Original Article

The Significance of the Secretory Immunoglogulin A to *Gardnerella vaginalis* in the Pathogenesis and Diagnosis of Bacterial Vaginosis

Micobiology Section

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ABSTRACT

Background: Although bacterial vaginosis is prevalent, not much progress has occurred in identifying the factors which are responsible and are associated with bacterial vaginosis and its pathophysiology. Studies on the development of better methods for the diagnosis of bacterial vaginosis are still on.

Objective: To estimate the levels of secretory immunoglobulin A (sIgA) antibodies against *G.vaginalis* in vaginal discharge and its correlation with the clinical condition.

Materials and Methods: We developed an indirect ELISA by using whole cell antigen to detect sIgA antibodies against *G.vaginalis* in vaginal washings which were collected from 170 women who attended two hospitals in south India for ante-natal care or any other complaint. The women were categorised into bacterial vaginosis, intermediate and normal by using Nugent's criteria. Statistical analysis was done by using the Chi-Square test, analysis of variance and Scheffe's multiple range tests, as appropriate.

Results: Of the 170 women under study, 42 were cases of bacterial vaginosis, 30 had intermediate flora and 98 were normal women. Of the 42 women with bacterial vaginosis, 16 (38%) showed a significantly high titre and 26 (62%) had low titre. Of the 30 women with intermediate flora, 10 (33.3%) showed a significantly high titre and 20 (66.7%) had low titres of the 98 women with normal flora, 84 (85.7%) showed low titres and 14 (14.3%) showed high titres of sIgA antibodies against *G.vaginalis*. The results of the ELISA test correlated statistically with the cases of bacterial vaginosis when analysed by Chi-Square test ($p < 0.05$). Analysis of variance showed that there was significant variation ($p < 0.05$) between the titers of sIgA antibodies detected in the women categorised by Nugent's criteria.

Conclusion: All the women with bacterial vaginosis or with intermediate flora do not show significant titres of sIgA antibodies to *G.vaginalis*. So, the detection of sIgA to *G.vaginalis* in the vaginal discharge cannot be used as a means for the diagnosis of bacterial vaginosis.

Key Words: Bacterial vaginosis, Diagnosis, *Gardnerella vaginalis*, Pathogenesis, Secretory Immunoglobulin A

KEY MESSAGE

- All women with bacterial vaginosis or with intermediate flora do not show significant titres of sIgA antibodies to *G.vaginalis*.
- The detection of sIgA to *G.vaginalis* in the vaginal discharge cannot be used as a means for the diagnosis of bacterial vaginosis.

INTRODUCTION

Bacterial vaginosis represents a unique and complex change in the flora of the vagina, which is characterised by a reduction in the prevalence and the number of lactobacilli and an increase in the concentration of *Gardnerella vaginalis* and resident anaerobic bacteria [1]. In India, the prevalence of bacterial vaginosis in the general population is 18-20% [2],[3],[4]. Although bacterial vaginosis is prevalent, not much progress has occurred in identifying the factors which are responsible and are associated with bacterial vaginosis and its pathophysiology [5]. Bacterial vaginosis is associated with a variety of obstetric and gynaecological complications such as preterm birth, low birth weight, post partum endometritis and pelvic inflammatory disease (PID) [1],[6].

Nugent's gram's stain scoring system is considered as the gold standard for the diagnosis of bacterial vaginosis [4], [7]. Nugent's criteria divides women into bacterial vaginosis (Nugent's score > 7), intermediate (Nugent's score of 4-6) and normal women (Nugent's score of ≤ 3) [7]. The chromogenic filter paper method for sialidase detection and quantitative PCR are some of the newer methods for the diagnosis of bacterial vaginosis, which are being developed [8],[9].

There are studies on the immune response in the vaginal discharge in cases of bacterial vaginosis. But in these studies, ELISA was done by using purified haemolysin as an antigen [10],[11]. In these studies, a specific immunoglobulin A response was detected in 60% of the women with overt bacterial vaginosis, and in 18.5% of the women with intermediate vaginal flora and 91% of the normal women showed the absence of specific sIgA antibodies. In the present study, we used whole cell antigen ELISA to detect the sIgA antibodies against *G.vaginalis* in the vaginal discharge, with the

intention of getting better results as haemolysin is highly unstable than the whole cell antigen [12].

MATERIALS AND METHODS

One hundred and seventy women who attended two hospitals in south India for antenatal care or IUD insertion or removal, with complaints of discharge, abdominal pain or any other complaint, formed the study population. Married women between the age group of 21–35 years and women with or without vaginal discharge complaints were included. Women who were menstruating at the time of the specimen collection andwomen who were on medication for any bacterial, fungal, parasitic or viral infection for up to one month prior to the specimen collection were excluded. The study had the approval of the Institutional Ethics Committee.

Vaginal swabs and vaginal washings were collected. The vaginal walls were irrigated with 10 mL of sterile saline after inserting a sterile speculum which was lubricated with water. The resulting fluid was collected by using a sterile syringe and was stored in storage vials at –70°C until use [11]. The diagnosis of bacterial vaginosis was based on Nugent's criteria [7].

G.vaginalis ATCC 14018 was inoculated on human blood agar plates and incubated at 37°C for 48 hrs. The colonies were harvested from human blood agar plates by using 0.15 M sterile saline. The bacterial cells were washed twice with 0.15 M sterile saline. The saline suspension of the bacteria was adjusted to the turbidity OD_{540} of 2.5. Flat bottom, 96 well microtitre plates were coated with this whole cell antigen suspension by incubating at 37°C for 2 hrs and then overnight at 4°C.

ELISA was done as described earlier [10]. Two hundred microlitres of vaginal washings were added to each well and incubated at 37°C for 2 h. The ELISA plate was washed 3 times with the wash buffer – phosphate buffered saline pH 7.3 with 0.05% (v/v) Tween 20 (Hi Media Laboratories, Pvt. Ltd., Mumbai, India) and 100 µl of the conjugate – goat anti human IgA antibody which was conjugated with the alkaline phosphatase enzyme (Sigma, St. Louis), was added. The plate was incubated at 37°C for 1 hr and was washed 3 times with the wash buffer. Then, 100 µl of the substrate –0.1% (w/v) ρ-nitrophenyl phosphate (Sigma, St. Louis) in 0.1M glycine buffer containing 1mmol/L of magnesium chloride ($MgCl_2$) (Hi Media Laboratories, Pvt. Ltd., Mumbai, India) and 1mmol/L of zinc chloride (Hi Media Laboratories, Pvt. Ltd., Mumbai, India), pH 10.6 was added and this was incubated in the dark for 30 min. Then, 16 µl of the stop solution –3 mol/L NaOH solution was added and the absorbance was read at OD_{405} by using an ELISA reader (Biotech, USA, Model ELX 800). All the tests were performed in duplicate. Wells without the vaginal washings served as the blanks. The cut off value was calculated as the mean absorbance of the normal women plus one standard deviation [10].

Statistical analysis was done by using the Chi Square test, Analysis of variance and Scheffe's multiple range tests as appropriate. A two tailed p value of less than 0.05 was considered to be significant. SPSS version 11.5 was used to do the statistical analysis.

RESULTS

Of the 170 women under study, 42 were cases of bacterial vaginosis (Nugent's score of ≥ 7), 30 had intermediate flora (Nugent's score of 4 – 6) and 98 were considered as normal (Nugent's score of 0 - 3) when diagnosed by Nugent's method. Of the 42 women with bacterial vaginosis, 16 (38%) had significantly high titres and 26 (62%) had a low titre of sIgA antibodies against *G.vaginalis*. Of the 30 women with intermediate flora, 10 (33.3%) had significantly high titres and 20 (66.7) had low titres of sIgA antibodies. Of the 98 women with normal flora, 84 (85.7%) showed low titres and 14 (14.3%) showed high titres of antibodies [Table/Fig-1].

The results of the ELISA test statistically correlated with the cases of bacterial vaginosis when analysed by Chi-Square test ($p < 0.05$). Analysis of variance showed that there was a significant variation ($p < 0.05$) between the titres of antibodies detected in women categorised by Nugent's criteria [Table/Fig-2]. Scheffe's multiple range tests showed that there was a statistically significant difference in the titer of sIgA antibodies to *G.vaginalis*, between normal women and women with bacterial vaginosis ($p < 0.05$) and also between normal women and women with intermediate flora ($p < 0.001$). But, there was no significant difference between the titer of the sIgA antibodies against *G.vaginalis* of women with bacterial vaginosis and those with intermediate flora ($p = 0.735$).

DISCUSSION

We developed an indirect enzyme immunoassay (ELISA) for the estimation of sIgA antibody levels in the vaginal discharge against *G.vaginalis* by using whole cell antigen. The vaginal discharge samples were tested in duplicate and also the ELISA tests were repeated by using both *G.vaginalis* ATCC 14018 and also vaginal isolates as antigens. The intra assay and inter assay variations have been found to be minimum.

We found that only 38% of the women with bacterial vaginosis showed elevated titers of the sIgA antibodies against *G.vaginalis*. The reason for low positivity may be because of IgA depletion as observed in cases of bacterial vaginosis in past studies. In studies which were conducted earlier, many women showed extensive IgA and IgM degradation in the vaginal washings, whereas the IgG titer was intact. The IgA degradation correlated with a high titer of the sialidase enzyme in the vaginal discharge, which was produced by the organisms which were associated with bacterial vaginosis [11],[13].

Nugent's criteria	ELISA for sIgA				
	n	Positive		Negative	
		No. (%)	OD_{405} ± SD	No. (%)	OD_{405} ± SD
Bacterial vaginosis	42	16 (38)*	1.0 ± 0.37	26 (62)	0.40 ± 0.14
Intermediate	30	10 (33.3)	1.2 ± 0.46	20 (66.7)	0.44 ± 0.11
Normal	98	14 (14.3)	0.8 ± 0.25	84 (85.7)	0.36 ± 0.17

[Table/Fig-1]: Detection of sIgA antibodies to G.vaginalis in vaginal discharge samples by ELISA using whole cell antigen in women categorised by Nugent's criteria

*The results of the ELISA tests statistically correlated with the cases of bacterial vaginosis diagnosed by Nugent's method when analysed by Chi–Square test ($p < 0.05$).

Nugent's method	Number	*Mean OD_{405} ± SD
Bacterial vaginosis	42	0.64 ± 0.4
Intermediate	30	0.7 ± 0.5
Normal	98	0.42 ± 0.23
Total	170	

[Table/Fig-2]: Titre of sIgA antibodies to G.vaginalis in vaginal discharge samples by ELISA using whole cell antigen in women categorised by Nugent's criteria.

*Analysis of variance showed that there is significant variation between the titre of antibodies detected in women categorised by Nugent's criteria ($p < 0.05$)

High titres of the sialidase enzyme and low titres of sIgA correlated with an adverse pregnancy outcome, especially preterm delivery and low birth weight. High titres of sIgA and low levels of the sialidase enzyme were followed by a good pregnancy outcome. So, the sIgA antibodies against *G.vaginalis* appear to be protective [11],[13]. There is only one Indian study on the detection of sIgA in the vaginal discharge of women with bacterial vaginosis [14]. This study showed that women with a low BMI (body mass index) did not show elevated titers of sIgA in their vaginal discharge [14]. In our study, most of the women belonged to the low socioeconomic status. It is possible that the women with low titres of sIgA may be having a low body mass index.

It is not certain whether women with intermediate flora can revert back to the normal flora or complicate into bacterial vaginosis. In the present study, 33.3% of the women with intermediate flora showed a significantly high titre of sIgA antibodies. It is possible that women with intermediate flora, who do not have sIgA antibodies, may have high titres of the sialidase enzyme activity in their discharge and hence, may develop bacterial vaginosis. Further studies are needed to prove this aspect of the pathogenesis of bacterial vaginosis.

All women with bacterial vaginosis or with intermediate flora do not show significant titres of the sIgA antibodies to *G.vaginalis*. So, the detection of sIgA to *G.vaginalis* in the vaginal discharge cannot be used as a means for the diagnosis of bacterial vaginosis.

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