Predictive Value of Sialidase in the Diagnosis of Sexually Transmitted Infections: a Cross-section Study Based on Vaginal Microecology Evaluation System

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Abstract

Background: The purpose of this study was to determine whether any indexes of vaginal microecology evaluation system (VMES) could predict sexually transmitted infections (STIs).

Methods: A total of 1032 women, who presented to Gynecology Outpatient Clinic of the First Affiliated Hospital of Xi'an Jiaotong University between November 2016 and November 2020, were included in the study. Incident STIs was defined as any case of Chlamydia trachomatis (CT), Mycoplasma genitalium (MG) and Neisseria gonorrhoeae (NG), and were confirmed using the RNA-based simultaneous amplification and testing (SAT) assay. VEMS is structured in two parts, one is the morphological characterizations encompassing bacterial density, flora diversity, predominant flora, pathogenic microorganisms and indicators of inflammation, the other is the functional indexes, including vaginal pH, cleanliness, hydrogen peroxide (H₂O₂), sialidase, β -glucuronidase, leukocyte esterase, and acetylglucosaminidase. Bacterial vaginosis (BV) was diagnosed by Gram stain (Nugent score). Associations were mainly assessed using logistic regression (LR).

Results: SAT assay detected STIs in 64 (6.2%) of the 1032 samples tested and 136 (13.2%) women had a clinical BV diagnosis using Nugent score. Multivariate logistic regression analysis revealed that women with sialidase-positive were more likely to test positive for STIs (aOR=3.081, 95% CI=1.586-5.984, P=0.001). Of 896 women without clinical BV, significant association was also found for sialidase and STIs (aOR =4.133, 95% CI= 1.140-14.978, P=0.031).

Conclusions: Sialidase may be a useful indicator to help clinicians identify these women who are at risk for STIs, especially in the absence of BV population.

1 Background

Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG) and Mycoplasma genitalium (MG) are common sexually transmitted infections (STIs) and treat mostly in the outpatient clinic in China. In 2016, the estimated number of incident cases was 124.3 million for CT, 30.6 million for NG worldwide[1]. Besides, the prevalence of MG was estimated to be 1.3% among countries with higher levels of development and 3.9% among countries with lower levels of development[2]. STIs is known to cause various complications including infertility, chronic pelvic inflammatory disease, premature delivery, neonatal encephalitis and others[3–5]. However, most carriers are asymptomatic and still transmissible, making the identification and diagnosis of patients with STIs a public health issue[6–8].

Vaginal microecology evaluation system (VMES) has gained popularity in China recently due to its effective, inexpensive and quick to conduct. Wet preparations of vaginal swab samples that are examined microscopically and stained by Gram staining are studied using the tool. It consists of two parts, one is the morphological characterizations encompassing bacterial density, flora diversity, predominant flora, pathogenic microorganisms and indicators of inflammation, the other is the functional indexes, including vaginal pH, cleanliness, hydrogen peroxide (H₂O₂) and activity of sialidase, β -

glucuronidase, leukocyte esterase, and acetylglucosaminidase[9]. In 2016, infectious Disease collaborative group of Obstetrics and Gynecology Branch in Chinese Medical Association proposed the standard VMES and published the expert consensus (Table 1)[10]. The evaluation of the vaginal microecosystem can help the clinician to make a prompt diagnosis and provide new opportunities for the comprehensive management of dysbacteriosis.

Table 1
Vaginal microecology evaluation system

Variable	Normal	Abnormal
Morphological indicators		
Bacterial density	II(10–100/ field)	I(1–10/field)
	III(100–1000/field)	IV(> 1000/field)
Flora diversity	II(4–6 types/ field)	I(1–3 types/ field)
	III(7–9 types/ field)	IV(> 10 types/ field)
Predominant flora [*]	Large Gram-positive rods	Gram-positive cocci
		Large Gram-negative rods
		Small Gram-negative rods
BV (Nugent score)	1–6	≥ 7
AV score	< 3	≥ 3
Pathogen	Negative	Fungus (spore, budding yeast, or hyphal forms) and trichomonads
Functional indicators		
Vaginal PH	≥ 3.8 and <4.5	< 3.8 or ≥ 4.5
H2O2	Negative	Positive
Vaginal preformed enzymes	Negative	Positive for sialidase, β-glucuronidase, leukocyte esterase, and acetylglucosaminidase
[*] Large Gram-positive rods: <i>Lactobacillus</i> ; Gram-positive cocci: <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> ; Gram-negative rods: <i>Escherichia coli</i> .		
AV: Aerobic vaginitis. BV: Bacterial vaginosis. H2O2: hydrogen peroxide		

In the resource constrained settings of many developing countries, screening tests for STIs using culture, ELISA, and polymerase chain reaction are not practical options because of their costs or procedures[11–13]. Given this, there is a clinical need for a cheap, rapid, and simple assay that would allow clinicians to make an early predictive diagnosis during the initial patient visit. Therefore, we evaluated whether any indexes of VMES that could predict the presence of STIs.

2 Methods

2.1 Subjects and study design

A cross-sectional study was conducted in Gynecology Outpatient Clinic of the First Affiliated Hospital of Xi'an Jiaotong University between November 2016 and November 2020. Prior to data collection, participants ≥ 16 years signed written informed consent, and those < 16 years provided assent and parental consent from their parents/guardians. The study protocol was approved by the ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. Collected information including age, status of their pregnancy, main symptoms (color and amount of discharge, itching, burning pain, urodynia, *etc*), medical history before gynecological examination. All had simultaneous STIs test(including CT, MG and NG)using the RNA-based SAT and vaginal microecology evaluation based on VMES. The exclusion criteria were: (1) Women during menstrual period; (2) women received hormones, antibiotic or immunosuppressive agents within 2 weeks; (3) women had a history of vulva or vaginal medication in the last 7 days. Eligible patients were required to complete a comprehensive gynecologic examination after collecting the basic information. Samples of vaginal fluid from the lateral vaginal walls were obtained using two sterile cotton swabs, one was used for the functional detection of vaginal microecology evaluation, another vaginal swab was streaked on a glass slide and air dried to Gram stain for morphological interpretations. Additionally, a long sterile cotton swab was used to collect the endocervical samples for the test of STIs. All samples were taken to the laboratory for testing immediately.

2.2 Vaginal microecology evaluation system

(1) Microscopic evaluation on Gram-stained vaginal smears:

(A) Bacterial density: it refers to the average number of bacteria in the microscopic field under oil immersion lens. The results were classified into four levels(denoted as + to ++++): grade I (+): 1–9; grade II (++) : 10–99; grade III (+++) : 100 and above; and grade IV (++++): bacteria clustered full of vision. Both “++” and “+++” mean normal.

(B) Flora diversity: it refers to the number of distinguishable bacterial species under oil immersion lens. The results were also divided into four levels (denoted as + to ++++): grade I (+): 1–3 kinds of flora; grade II (++) : 4–6 kinds of flora; grade III (+++) : 7–10 kinds of flora; and grade IV (++++): more than 10 kinds of flora. “++” and “+++” were viewed as normal.

(C) Predominant flora: it refers to the maximum number of microorganism observed in the microscopic field under oil immersion lens. Only Large Gram-positive rods (*Lactobacillus*) was determined as a normal flora.

(D) Vaginal cleanliness evaluation: It was graded by the density of *Lactobacillus*, epithelial cells, white blood cells, other flora into 4 levels, with Grade I-II as normal and grades III-IV as abnormal usually accompanied by inflammation. Specifically, the judgment of vaginal cleanliness were as follows (Table S1) [14]: Grade I was a large number of large Gram-positive rods (indicative of *Lactobacillus* spp.), vaginal epithelial cells, and no other bacteria observed with WBC 0–5/HP under microscopy. Grade II was some *Lactobacillus* spp. and vaginal epithelial cells, some pus cells, and other bacteria observed under microscopy with WBC 10–15/HP. Grade III was a small amount of *Lactobacillus* spp., a large number of pus cells, and other bacteria observed under microscopy with WBC 15–30/HP. Grade IV was no *Lactobacillus* spp. but pus cells and other bacteria observed under microscopy with WBC more than 30/HP[14](Table S1).

(E) Bacterial vaginosis (BV) was assessed by Nugent score and a score of 7 to 10 was defined as BV[15] (Table S2). Aerobic vaginitis (AV) were assessed by VMES according to the AV score[9, 10](Table S3).

(F) Pathogen: TV (Trichomonas vaginosis) was diagnosed by motile flagellates under a microscope and VVC (vulvovaginal candidiasis) was confirmed when the yeast and(or) pseudohyphae were observed through a microscope.

(2) Functional detection of vaginal microecology evaluation:

(A) Vaginal preformed enzymes: H₂O₂, sialidase, β -glucuronidase, leukocyte esterase, and acetylglucosaminidase were detected following manufacturer's directions (Chaoshi-Bio, Jiangsu China). All these enzymes were classified as positive or negative result.

(B) Vaginal pH: pH paper strips ranging from 3.8 to 5.4 were used to measure the vaginal pH. The normal vaginal pH values range from 3.8 to 4.5.

2.3 Detection of STIs

CT, NG and MG were detected utilizing the RNA-based SAT (simultaneous amplification and testing) assay and STIs was considered positive if any of CT, MG, NG test was positive.

2.4 Statistical analyses

Differences in study population characteristics were tested using the χ^2 , Fisher exact or Mann-Whitney *U* test, accordingly. The unadjusted odds ratios (ORs) were obtained from an unconditional, binary logistic model with only one dichotomous covariate of interest in the model. All variables associated with the outcome (STIs) at $P < 0.1$ were entered into the logistic regression model. Then stepwise backward elimination logistic regression was performed to determine which factors were independently associated with STIs, and the final reduced model was constructed. *P* values < 0.05 were considered significant.

Finally, we also presented the results in the subset of women without BV using the stepwise backward elimination logistic regression. We obtained the unadjusted and adjusted log odds using computer software (IBM SPSS version 25).

3 Results

3.1 Study Population

Of the 1032 women included, all belonged to Han Chinese ethnicity from northwestern China, 563 attended with clinical symptoms of urogenital symptoms such as “itching” “abnormal vaginal discharge” or “cervicitis” and others visited for pre-pregnancy examination, abortion history or pelvic inflammation, *etc.* They ranged in age from 15 to 68 years (mean age = 30) and 18.4% of subjects under 25 years old. 64 (6.2%) participants were diagnosed with STIs and there were 32 cases of CT, 17 cases of MG, and 16 cases of NG.

3.2 STIs and Other reproductive tract infections

Nugent score confirmed that 136 of the 1032 patients had BV and these subjects were more likely to be STIs-positive than subjects without BV. In univariable analyses, we found that BV (Nugent score ≥ 7) was associated with a 1.9-fold increase (OR = 1.942, 95% CI = 1.042–3.618, P = 0.037) in the odds of incident STIs. In addition, the STIs-positive and STIs-negative groups were similar in TV, VVC or AV (Table 2).

Table 2
Subject Characteristics and Predictor Variables Stratified by STIs Status

Characteristic	STIs Results			P-value
	Total (N = 1032)	Positive (%) (N = 64)	Negative (%) (N = 968)	
Age (y)				< 0.001
≤25	192	31(48.4%)	161(16.6%)	
> 25	840	33(51.6%)	807(83.4%)	
Urogenital symptoms				0.310
Yes	563	31(48.4%)	532(55.0%)	
No	469	33(51.6%)	436(45.0%)	
Morphological indicators				
Pathogen				
BV(Nugent Score)				0.034
Positive	136	14(21.9%)	122(12.6%)	
Negative	896	50(78.1%)	846(87.4%)	
VVC				0.376
Positive	170	8(12.5%)	162(16.7%)	
Negative	862	56(87.5%)	806(83.3%)	
TV				0.295
Positive	52	5(7.8%)	47(4.9%)	
Negative	980	59(92.2%)	921(95.1%)	
AV				0.594
Positive	14	1(1.6%)	13(1.3%)	
Negative	1018	63(98.4%)	955(98.7%)	
Bacterial density				0.049
Normal	878	49(76.6%)	828(85.6%)	
Abnormal	153	15(23.4%)	139(14.4%)	

STIs: Sexually transmitted infections, BV: Bacterial vaginosis; TV: Trichomonas vaginalis; VVC: vulvovaginal candidiasis, AV: Aerobic vaginitis; H2O2: hydrogen peroxide

Characteristic	STIs Results			P-value
	Total (N = 1032)	Positive (%) (N = 64)	Negative (%) (N = 968)	
Flora diversity				1.000
Normal	985	61(95.3%)	924(95.6%)	
Abnormal	46	3(4.7%)	43(4.4%)	
Predominant flora				< 0.001
Normal	669	28(43.8%)	641(66.2%)	
Abnormal	363	36(56.3%)	327(33.8%)	
Vaginal cleanliness				0.225
Normal	775	44(68.8%)	731(75.5%)	
Abnormal	257	20(31.3%)	237(24.5%)	
Functional indicators				
Vaginal PH				0.012
Normal	237	6(10.3%)	231(24.8%)	
Abnormal	752	52(89.7%)	700(75.2%)	
H2O2				1.000
Positive	982	60(96.8%)	922(96.8%)	
Negative	32	2(3.2%)	30(3.2%)	
Sialidase				< 0.001
Positive	109	18(28.1%)	91(9.4%)	
Negative	923	46(71.9%)	877(90.6%)	
Leukocyte esterase				0.078
Positive	669	48(75.0%)	621(64.2%)	
Negative	363	16(25.0%)	347(35.8%)	
β-glucuronidase				1.000
Positive	2	0(0.0%)	2(0.2%)	

STIs: Sexually transmitted infections, BV: Bacterial vaginosis; TV: Trichomonas vaginalis; VVC: vulvovaginal candidiasis, AV: Aerobic vaginitis; H2O2: hydrogen peroxide

Characteristic	STIs Results			
	Total (N = 1032)	Positive (%) (N = 64)	Negative (%) (N = 968)	P-value
Negative	1030	64(100.0%)	966(99.8%)	0.001
Acetylglucosaminidase				
Positive	76	12(18.8%)	64(6.6%)	
Negative	956	52(81.3%)	904(93.4)	
STIs: Sexually transmitted infections, BV: Bacterial vaginosis; TV: Trichomonas vaginalis; VVC: vulvovaginal candidiasis, AV: Aerobic vaginitis; H2O2: hydrogen peroxide				

3.3 Other Characteristics Associated with STIs by univariable analysis

In univariable analysis, younger age (15-25y), abnormal bacterial density, abnormal predominant flora, vaginal pH > 4.5, sialidase-positive and acetylglucosaminidase-positive were strongly associated with the positive result of STIs ($P < 0.05$), a nonsignificant trend was observed between positive STIs test results and leukocyte esterase ($P = 0.078$), no associations were found between STIs and urogenital symptoms, flora diversity, H2O2, β -glucuronidase, cleanliness ($P > 0.1$) (Table 2).

3.4 Multivariate logistic regression analysis

Because the individual markers (bacterial density and predominant flora) that connected to the Nugent score had independent associations with STIs, the individual markers rather than the composite variable "BV" were used in the final logistic regression model. After adjustment for predominant flora, bacterial density, vaginal PH, Leukocyte esterase and acetylglucosaminidase, the multivariate logistic regression model revealed that sialidase-positive and younger age (15-25y) were independently associated with STIs. Sialidase-positive was significantly associated with an increased odds of STIs (aOR = 3.08; 95% CI = 1.586–5.984, $P = 0.001$). Further, younger subjects(15-25y) were 5.1 times more likely to be infected with STIs (aOR = 5.042, 95% CI, 2.890–8.798, $P = 0.001$) (Table 3).

Table 3

Univariable and Multivariable Logistic Regression Analyses of Characteristics Associated STIs

Characteristic	Univariable		Multivariable	
	OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
Age (y)		0.000		0.001
> 25	1		1	
≤ 25	4.709(2.803,7.909)		5.042(2.890,8.798)	
Urogenital symptoms		0.311		
No	1			
Yes	0.770(0.464,1.277)			
Morphological indicators				
Pathogen				
BV (Nugent Score)		0.037		
Negative	1			
Positive	1.942(1.042,3.618)			
VVC		0.378		
Positive	1			
Negative	0.711(0.332,1.519)			
TV		0.300		
Negative	1			
Positive	1.661(0.637,4.332)			
AV		0.883		
Negative	1			
Positive	1.166(0.150,9.057)			
Bacterial density		0.052		
Normal	1			
Abnormal	1.824(0.995,3.341)			
Flora diversity		0.928		
STIs: Sexually transmitted infections, BV: Bacterial vaginosis; TV: Trichomonas vaginalis; VVC: vulvovaginal candidiasis, AV: Aerobic vaginitis; H2O2: hydrogen peroxide; CI: confidence interval; OR: odds ratio				

Characteristic	Univariable		Multivariable	
	OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
Normal	1			
Abnormal	1.057(0.319,3.504)			
Predominant flora		0.000		
Normal	1			
Abnormal	2.520(1.511,4.203)			
Vaginal cleanliness		0.227		
Normal	1			
Abnormal	1.402(0.810,2.426)			
Functional indicators				
Vaginal PH		0.016		0.069
Normal	1		1	
Abnormal	2.860(1.213,6.745)		2.277(0.937,5.534)	
H2O2		0.976		
Normal	1			
Abnormal	0.976(0.228,4.182)			
Sialidase		0.000		0.001
Normal	1		1	
Abnormal	3.771(2.099,6.777)		3.081(1.586,5.984)	
Leukocyte esterase		0.081		
Normal	1			
Abnormal	1.676(0.938,2.997)			
β-glucuronidase		0.999		
Normal	1			
Abnormal	0.999	(0.000,0.000)		
Acetylglucosaminidase		0.001		0.053

STIs: Sexually transmitted infections, BV: Bacterial vaginosis; TV: Trichomonas vaginalis; VVC: vulvovaginal candidiasis, AV: Aerobic vaginitis; H2O2: hydrogen peroxide; CI: confidence interval; OR: odds ratio

Characteristic	Univariable		Multivariable	
	OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
Normal	1		1	
Abnormal	3.260(1.656,6.415)		2.183(0.991,4.806)	
STIs: Sexually transmitted infections, BV: Bacterial vaginosis; TV: Trichomonas vaginalis; VVC: vulvovaginal candidiasis, AV: Aerobic vaginitis; H2O2: hydrogen peroxide; CI: confidence interval; OR: odds ratio				

3.5 The multivariate logistic regression analysis in the subset of BV negative group

In addition, we analyzed the subset (n = 896) of BV-negative group. In these subjects, 7 of 23 (30.435%) with a positive sialidase tested positive for STIs, whereas only 43 of 873 (4.926%) without elevated sialidase were positive for STIs. After controlling for potentially confounding factors, a similar significant trend was seen between sialidase and STIs (aOR = 4.133; 95% CI = 1.140-14.978, P = 0.031) (Table 4).

Table 4
Multivariable Logistic Regression Model for Predictors of STIs in the subset of women without BV.

Characteristic	OR	95% CI	P Value
Age of 15–25 years	4.756	(2.524,8.960)	< 0.001
Vaginal PH	2.292	(0.932,5.634)	0.071
Sialidase positivity	4.133	(1.140,14.978)	0.031
Acetylglucosaminidase positivity	2.54	(1.081,5.968)	0.032
CI: confidence interval; OR: odds ratio.			

4 Discussion

The study focused on the relationship between STIs and indexes of vaginal microecology evaluation system in Xi'an district. We found that sialidase is strongly associated with STIs which suggests that information from VMES can be used to predict the presence of STIs. Vaginal microecology tests are inexpensive and widely available tools especially in China that may potentially help clinicians estimate which patients are at risk for STIs.

Our study demonstrates that the rates of STIs acquisition are increased in the BV affected patient and younger women, which is consistent with some previous findings about STIs[16–18]. BV is characterized by a reduced number of *Lactobacillus spp.* and an increased diversity of anaerobic bacterial species,

including *Gardnerella vaginalis*, *Atopobium vaginae*, *Prevotella spp.*, etc. There are strong biological arguments in favor of this hypothesis that BV is risk factor for STI acquisition. Firstly, *Lactobacillus* species protect against STIs by producing antimicrobial compounds, including organic acid to lowers the vaginal pH, H₂O₂, target-specific bacteriocins and possibly biosurfactants[19]. Thus, the lack of *Lactobacillus* species would result in greater susceptibility to infections. Secondly, vaginal strict and facultative anaerobes appear to produce cytokines, nitrosamines and inflammatory mediators, which are supposed to be related to cervical inflammation[20], and mucin-degrading enzymes that reduce the infection barrier[21, 22].

We identified the main factor associated with STIs was sialidase. Sialidase is an enzyme involved in the pathogenesis of several diseases by cleaving sialic acid from terminal glycans of glycoproteins and thus leading to impairment of the local immune response[23–25]. Not surprising that elevated sialidase represents an abnormal state and associated with higher risk, should these women be exposed to an STI pathogen. In former study, Briselden et studied 50 vaginal fluid specimens from women with BV and found that elevated levels of sialidase activity were detected in those with BV (84%) than without BV ($P=0.001$)[26]. Meanwhile, the results of our study also showed a strong correlation between BV and sialidase. Furthermore, increased sialidase activity has been detected in the vaginal fluid of BV patients[26, 27], and is the basis of a marketed quick test for diagnosis of BV[28]. Therefore, then we also focused on the subjects of BV-negative group. We find that women with sialidase-positive have higher prevalence of STIs compared with those without sialidase positivity even in the BV negativity group, and 30% of those with an elevated sialidase were infected with STIs in subset of women without BV, which demonstrating sialidase-positive associated with the vulnerability to STIs regardless of the BV. Therefore, sialidase may be a better predictor of STIs than BV.

However, these findings may be somewhat limited by lacking some other possible determinants and confounders for the acquisition of STIs, such as sexual risk behavior, previous STIs, condom use. Another limitation of this study is that the cross-sectional design limits our ability to interpret the associations we found and we cannot determine whether STIs cause an elevated vaginal sialidase or if women with high vaginal sialidase are more susceptible to STIs. Besides, the low prevalence of STIs in this study came as a surprise, compared to the prevalence in other parts of the world[1]. It might be explained that more than four in five of participants over age 25. Thus, the study should be repeated with a larger group of women to confirm our results due to the lack of positive samples.

5 Conclusions

Overall, our study supports that sialidase is a feasible option to predict the people who need to be further NAATs examined, especially in the absence of BV. In view of this, the VMES may offer great potential for prediction in diagnosis of infectious disease. It is noteworthy that the quick prediction and diagnosis of STIs is key to propose a comprehensive approach to sexual and reproductive health.

6 Abbreviations

STIs: Sexually transmitted infections; VMES: vaginal microecology evaluation system; CT: *Chlamydia trachomatis*; MG: *Mycoplasma genitalium*; NG: *Neisseria gonorrhoeae*; BV: Bacterial vaginosis; TV: *Trichomonas vaginalis*; VVC: Vulvovaginal candidiasis; AV: Aerobic vaginitis; SAT: simultaneous amplification and testing; H2O2: hydrogen peroxide

7 Declarations

7.1 Ethics approval and consent to participate

This study was conducted in accordance with the declaration of Helsinki. Prior to data collection, participants ≥ 16 years signed written *informed consent*, and those <16 years provided assent and *parental consent* from their parents/guardians. The study protocol was approved by the ethics committee of the First Affiliated Hospital of Xi'an Jiaotong University.

7.2 Consent for publication

Not applicable.

7.3 Availability of data and materials

The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

7.4 Competing interest

The authors declare that they have no competing interests.

7.5 Funding

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7.6 Authors' Contributions

LY designed of the work; ZHY, WXW, WWH performed the case and sample collection, analysis, and interpretation of the data; LY, HJT have drafted the work or substantively revised it; ARF reviewed and approved the final manuscript. All authors have read and approved the final manuscript.

7.7 Acknowledgements

Not applicable.

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