Biomedical Research 2017; 28 (1): 118-121 *ISSN 0970-938X*
www.biomedres.info

Analysis of the vaginal microecology in patients with severe vulvovaginal candidiasis.

Wenxiang Wu, Qinping Liao, Zhaohui Liu*

Department of Gynaecology, Peking University First Hospital, Beijing, PR China

Abstract

This study aimed to evaluate the vaginal microecology in patients with severe vulvovaginal candidiasis (SVVC). Vaginal microecology was evaluated using vaginal secretions that were collected from 452 patients with SVVC, who were treated between September 2013 and April 2014. The predominant bacteria were *lactobacilli* (69.91% of the cases). The vaginal flora intensity was level II-III in 81.42% of the cases, and the vaginal flora diversity was level II-III in 77.65% of the cases. Level I cleanliness was exhibited by 276 patients (61.06%), compared to level II cleanliness in 75 patients (16.59%) and level III cleanliness in 101 patients (22.35%). Approximately 67% of the patients had a vaginal pH of <4.5. The patients with SVVC exhibited varying degrees of vaginal microecological disorders, although the predominant bacteria were still *lactobacilli*. Most cases exhibited vaginal intensity and diversity that were within the normal ranges.

Keywords: Severe vulvovaginal candidiasis, Vaginal microecology.

Accepted on May 4, 2016

Introduction

The lower genital tract of women is an important microecological environment, and the predominant bacteria are normally *lactobacilli*. However, this environment is very sensitive, and can vary between menstrual cycles or during different diseases, which can also affect the progression of disease. Furthermore, the vaginal flora in healthy women changes according to age and pregnancy, and the intensity and diversity of the vaginal flora is significantly reduced during pregnancy [1]. However, the menstrual cycle exerts the greatest negative affect on vaginal flora stability [2]. Many studies have used molecular, microscopic, and culture methods to confirm that the vaginal flora's composition continues to change [3,4]. Nevertheless, there is little information regarding the changing microecosystem during different diseases, despite the >150-year history of research regarding flora in the human reproductive tract [5].

Vulvovaginal candidiasis (VVC) is a common gynaecological infection that affects up to 75% of women of child-bearing age at least once during their lifetime, and it is predominantly caused by *Candida albicans* [6-10]. The reported incidence of VVC in the US is 39% [11], and VVC can be divided into uncomplicated and complicated VVC. Uncomplicated VVC refers to mild-to-moderate sporadic VVC that is caused by strains of *Candida albicans* in otherwise healthy non-pregnant women. The severity of each symptom and sign (which include itching, burning, discharge, and erythema) is assigned a score on the following scale: 0=absent, 1=mild, 2=moderate or 3=severe.

Patients with a total severity score of ≥ 7 have severe vulvovaginal candidiasis (SVVC), which is classified as complicated VVC. Recurrent VVC is also classified as complicated VVC, and is defined as four or more episodes of proven infection during the previous 12 months [6]. Patients with SVVC typically exhibit severe clinical symptoms, and the clinical manifestations normally include vulvar or vaginal skin membrane damage. However, there is very little research regarding the vaginal microecological changes in Chinese patients and other patients with SVVC. Therefore, the present study aimed to evaluate the vaginal microecology of 452 Chinese patients with SVVC who were treated in our hospital.

Materials and Methods

General information

This study evaluated 452 Chinese women with SVVC who were 20-45 years old, and were treated in our Gynaecology Department between September 2013 and April 2014. This study was conducted in accordance with the declaration of Helsinki.

This study was conducted with approval from the Ethics Committee of Peking University. Written informed consent was obtained from all participants. During their treatment, all women had a vaginal secretion smear that tested positive for fungus plus vulvar pruritus and thick curdy vaginal discharge, with a mean VVC score of 9.1 ± 1.71.

The inclusion criteria were 1). Age of 20-45 years, healthy, non-pregnant, and non-lactating; 2). Not having condyloma

acuminata or other sexually transmitted diseases; 3). Not receiving vaginal medication during the previous week; 4). No sexual history during the previous 3 days; 5). No special medical disorders; 6). And fulfilling the diagnostic criteria for SVVC: vulvar pruritus, Gram staining that indicated yeasts or pseudohyphae, and a VVC score of ≥ 7.

The exclusion criteria were the SVVC being accompanied by trichomonas vaginitis, bacterial vaginosis, mucopurulent cervicitis, condylomata acuminata, or pelvic inflammatory diseases.

Sampling and microecological detection

Samples of typical vaginal discharge were obtained from the lateral vaginal wall using a sterile cotton-tipped swab. A saline wet mount was then created, and light microscopy was used to directly evaluate cleanliness and check for trichomoniasis.

The microecological detection was performed by comprehensively evaluating various characteristics of the microecological system: vaginal flora intensity, vaginal flora diversity, dominant bacterium, systemic inflammatory response, causative bacterium, vaginal pH, and hydrogen peroxide levels (H_2O_2, which reflect the function of *lactobacilli*) (Table 1).

The vaginal microecology was evaluated based on previously described methods [12]. Vaginal flora intensity was defined as the average number of bacteria in the microscopic field (1,000× magnification) and was divided into four levels: level I, 1-10/field; level II, 10-100/field; level III, 100-1,000/field; and level IV, >1,000/field.

Vaginal flora diversity was defined as the number of different bacterial flora that could be identified in the microscopic field (1,000× magnification) and was divided into four levels: level I, 1-3 types/field; level II, 4-6 types/field; level III, 7-9 types/field; and level IV, >10 types/field. The predominant bacterium was defined as the most frequently observed microorganism. The causative microorganism was identified based on the more frequent presence of either fungal hyphae or trichomoniasis. The presence of bacterial vaginosis was evaluated using the Nugent Standard [13] and was defined as ≥ 7 points.

Vaginal pH was evaluated using precision strips (range, 3.8-5.4; normal, ≤ 4.5). Vaginal cleanliness was divided into three levels, based on the ratio of leukocytes/epithelial cells under low microscopic magnification (10×): level I, ratio of <1; level II, ratio of 1; and level III, ratio of >1. Kits from Beijing Ruimeiao Biopharmaceutical Co. were used to detect the H_2O_2 concentrations in the vaginal secretions; a negative reading indicated normal vaginal microbial function, and corresponded to H_2O_2 levels of ≥ 2 μmol/L.

Table 1. *Content of vaginal microecological test.*

Content of vaginal microecological test				
Flora intensity	I (1-10/per field of view)	II (10-100/per field of view)	III (100-1000/per field of view)	IV (>1,000/per field of view)
flora diversity	I (1-3 types/per field of view)	II (4-6 types/per field of view)	III (7-9 types/per field of view)	IV (>10/per field of view)
Predominant bacterium	Gram-positive macro-bacillus	Gram-positive coccus	Gram-negative macro-bacillus	Gram-negative micro-bacillus
Vaginal cleanliness	Level I	Level II	Level III	
H_2O_2	negative	positive		
Vaginal pH	<4. 5	≥ 4. 5		

Statistical analysis

SPSS software (version 10.0; SPSS Inc., Chicago, IL) was used for the statistical analysis.

Results

Vaginal microecology

The predominant bacteria were *lactobacilli* (69.91%), 81.42% of the vaginal flora intensity readings were level II-III, and 77.65% of the vaginal flora diversity readings were level II-III.

Level I cleanliness was observed in 276 patients (61.06%), compared to level II cleanliness in 75 patients (16.59%) and level III cleanliness in 101 patients (22.35%). The majority of the patients (67.26%) had a vaginal pH of <4.5 (Table 2).

Table 2. *Results of microecological test.*

	Cases (%)
Predominant bacterium	
Gram-positive macro-bacillus	316 (69.91%)
Gram-positive coccus	70 (15.49%)
Gram-negative macro-bacillus	12 (2.65%)
Gram-negative micro-bacillus	54 (11.95%)
Vaginal flora intensity	

I	69 (15.26%)
II	152 (33.63%)
III	216 (47.79%)
IV	15 (3.32%)
Vaginal flora diversity	
I	101 (22.35%)
II	246 (54.42%)
III	105 (23.23%)
IV	0 (0%)
H_2O_2	
negative	280 (61.95%)
positive	172 (38.05%)
Vaginal cleanliness	
Level I	276 (61.06%)
Level II	75 (16.59%)
Level III	101 (22.35%)

Discussion

In 2005, an epidemiological survey evaluated 11,853 patients with vaginitis from the gynaecological clinics of 62 Chinese hospitals. That survey revealed that VVC accounted for 39.3% of all vaginal inflammatory diseases, and that the VVC cases could be subdivided into uncomplicated VVC (53.2%), SVVC (20.8%), and VVC during pregnancy (6.6%), and recurrent VVC (12.3%).

There is a variety of normal microbial flora in a healthy woman's vagina, although *lactobacilli* are the predominant bacteria and play a key role in maintaining the normal vaginal environment. However, bacteria that live inside the human body are primarily anaerobic, and technological limitations can limit our ability to identify these anaerobic bacteria. Thus, future technological improvements are needed to identify additional vaginal bacteria.

There are >20 types of detectable vaginal *lactobacilli*, and various molecular methods have confirmed that most healthy women exhibit 1-2 kinds of predominant *lactobacilli*, although some women can exhibit 3-4 kinds. The most common vaginal bacteria in women of child-bearing age include *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus jensenii*, and *Lactobacillus gasseri* [14,15]. In this context, the *lactobacilli* convert the glycogen in vaginal squamous epithelial cells to lactic acid, and create the weakly acidic environment inside the vagina (pH of ≤ 4.5, with a primary range of 3.8-4.4). This environment helps to partially prevent most diseases that are associated with pathogenic microorganisms, as most pathogenic microorganisms experience impaired growth in acidic environments. In addition, *lactobacilli* may prevent the adherence of the pathogenic microorganisms to vaginal epithelial cells through microbial substitution and competitive exclusion. Furthermore, these *lactobacilli* produce various metabolites, such as lactic acid, bacteriocins, and H_2O_2, which may help regulate the production of cytokines during vaginal infections, as the vaginal installation of *lactobacilli* reduces the local production of interleukin-1β and interleukin-6 [16]. Moreover, *lactobacilli* may also help regulate the functions of the normal vaginal flora in various ways, which can help maintain the vaginal microecological balance and defend against reproductive tract infections. Studies have also confirmed that *lactobacilli* can help protect against urinary tract infections [17,18].

The present results revealed that 69% of the patients with SVVC exhibited *lactobacilli* as the predominant vaginal bacteria, and these results are consistent with previous findings [12]. However, 22.35% of the patients also exhibited significant cleanliness abnormalities (level III), which suggests that some patients with SVVC may have other accompanying infections. For example, a previous study [19] found that mixed infections accounted for 25.87% of vaginitis cases, which typically involved bacterial vaginosis and aerobic vaginitis. The patients with aerobic vaginitis generally exhibited level III cleanliness, and the predominant bacteria in the aerobic vaginitis cases were Gram-positive cocci and Gram-negative micro-bacilli. In the present study, approximately 18% of the patients with SVVC had Gram-positive cocci and Gram-negative micro-bacilli as the predominant bacteria; thus, level III cleanliness in SVVC cases may be combined with aerobic vaginitis. Furthermore, the 30% of SVVC cases that exhibited changes in the predominant bacteria should also be treated using antifungal drugs, and physicians should attempt to achieve full recovery of the vaginal microecological environment after treatment. Theses attempts should include monitoring of the predominant bacteria, because it may be responsible for persistent symptoms or unsatisfactory remission if the microecological environment cannot be restored [12].

Vaginal H_2O_2 is mainly produced by *lactobacilli*, such as *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii*, and *Lactobacillus acidophilus*. Thus, as these *lactobacilli* are often the predominant bacteria in healthy women, H_2O_2 levels may reflect the function of *lactobacilli*. The present study revealed that most patients with SVVC exhibited normal H_2O_2 levels (61.95%), which was similar to the proportion of *lactobacilli* as the predominant vaginal bacteria (69.91%). These findings indicate that the functions of *lactobacilli* were mostly normal in the patients with SVVC. However, a small subset of the patients (36 cases, 8%) exhibited abnormal H_2O_2 levels with normal predominant bacteria. This discrepancy may be related to changes in the types or functions of the *lactobacilli*, which might partially contribute to the SVVC, although further studies are needed to confirm this relationship. Nevertheless, some studies have indicated that vaginal dysbacteriosis is related to many genitourinary tract infections [20], and further intervention may be needed if the healthy vaginal flora has changed [21].

In conclusion, evaluation of the vaginal microecology might help clinicians understand the changes in the vaginal microenvironment of patients with SVVC. This understanding may help provide a theoretical basis for the study of this disease. However, evaluation of the vaginal microecology may also simply reflect the state at the sampling time, as the vaginal environment is affected by many factors. Therefore, dynamic monitoring of the vaginal microecology may provide more useful information.

References

1. Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, Coarfa C, Raza S, Rosenbaum S, Van den Veyver I, Milosavljevic A, Gevers D, Huttenhower C, Petrosino J, Versalovic J. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PLoS One 2012; 7: e36466.
2. Gajer P, Brotman RM, Bai G, Sakamoto J, Schütte UM, Zhong X, Koenig SS, Fu L, Ma ZS, Zhou X, Abdo Z, Forney LJ, Ravel J. Temporal dynamics of the human vaginal microbiota. Sci Transl Med 2012; 4: 132ra52.
3. Brotman RM, Ravel J, Cone RA, Zenilman JM. Rapid fluctuation of the vaginal microbiota measured by Gram stain analysis. Sex Transm Infect 2010; 86: 297-302.
4. Schwebke JR, Richey CM, Weiss HL. Correlation of behaviors with microbiological changes in vaginal flora. J Infect Dis 1999; 180: 1632-1636.
5. Martin DH. The microbiota of the vagina and its influence on women's health and disease. Am J Med Sci 2012; 343: 2-9.
6. Workowski KA, Berman S, Centers for Disease Control and Prevention (CDC). Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep 2010; 59: 1-110.
7. Sobel JD. Vulvovaginal candidosis. Lancet 2007; 369: 1961-1971.
8. Achkar JM, Fries BC. Candida infections of the genitourinary tract. Clin Microbiol Rev 2010; 23: 253-273.
9. Liu XP, Fan SR, Peng YT, Zhang HP. Species distribution and susceptibility of Candida isolates from patient with vulvovaginal candidiasis in southern China from 2003 to 2012. J Mycol Med 2010; 23: 253-273.
10. Sobel JD, Faro S, Force RW, Foxman B, Ledger WJ, Nyirjesy PR, Reed BD, Summers PR. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. Am J Obstet Gynecol 1998; 178: 203-211.
11. Walker PP, Reynolds MT, Ashbee HR, Brown C, Evans EG. Vaginal yeasts in the era of "over the counter" antifungals. Sex Transm Infect 2000; 76: 437-438.
12. Liu ZH, Zhang D, Zhao M, Wang Y, Bai HH, Xiao BB, Liai QP. The microecological evaluation of vaginal microflora in the women without vaginal infections. Prog Obstet Gynecol 2009; 18: 129-131.
13. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991; 29: 297-301.
14. Lamont RF, Sobel JD, Akins RA, Hassan SS, Chaiworapongsa T, Kusanovic JP, Romero R. The vaginal microbiome: new information about genital tract flora using molecular based techniques. Bjog 2011; 118: 533-549.
15. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA 2011; 108: 4680-4687.
16. Hemalatha R, Mastromarino P, Ramalaxmi BA, Balakrishna NV, Sesikeran B. Effectiveness of vaginal tablets containing lactobacilli versus pH tablets on vaginal health and inflammatory cytokines: a randomized, double-blind study. Eur J Clin Microbiol Infect Dis 2012; 31: 3097-3105.
17. Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, Yarova-Yarovaya Y, Fiedler T, Cox M, Stamm WE. Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis 2011; 52: 1212-1217.
18. Lee JW, Lee JH, Sung SH, Lee SJ. Preventive effects of Lactobacillus mixture on experimental E.coli urinary tract infection in infant rats. Yonsei Med J 2013; 54: 489-493.
19. Fan A, Yue Y, Geng N, Zhang H, Wang Y, Xue F. Aerobic vaginitis and mixed infections: comparison of clinical and laboratory findings. Arch Gynecol Obstet 2013; 287: 329-335.
20. Kovachev SM. Obstetric and gynecological diseases and complications resulting from vaginal dysbacteriosis. Microb Ecol 2014; 68: 173-184.
21. Nardis C, Mosca L, Mastromarino P. Vaginal microbiota and viral sexually transmitted diseases. Ann Ig 2013; 25: 443-456.

*Correspondence to

Zhaohui Liu

Department of Gynaecology

Peking University First Hospital

Xicheng District

PR China