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.

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 vulgar 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 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$_2$O$_2$, 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 < 1; level II, ratio of 1; and level III, ratio of > 1. Kits from Beijing Ruimeiao Biopharmaceutical Co. were used to detect the H$_2$O$_2$ concentrations in the vaginal secretions; a negative reading indicated normal vaginal microbial function, and corresponded to H$_2$O$_2$ levels of ≥ 2 μmol/L.

**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).
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 H2O2, 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 H2O2 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, H2O2 levels may reflect the function of lactobacilli. The present study revealed that most patients with SVVC exhibited normal H2O2 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 H2O2 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


*Correspondence to*

Zhaohui Liu

Department of Gynaecology

Peking University First Hospital

Xicheng District

PR China