

Prevalence of Cytolytic Vaginosis in Symptomatic Bulgarian Women - Need for Microbiological Study

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Abstract

Cytolytic vaginosis (CV) is a condition where the vagina is overrun with lactobacillus. It occurs when the delicate balance of the vagina normal flora is interrupted. CV is not a frequent condition but can lead to gynecology complications. The objective of this study was to determine the frequency of CV in symptomatic Bulgarian women and to compare it with the frequency of vulvovaginal candidiasis (VVC) and bacterial vaginosis (BV). Vaginal samples of 468 women were collected. Gram-stain smears were prepared. Vaginal pH was determined. Cultures on Sabouraud dextrose agar with Gentamycin and Chromagar candida were performed. Multiplex RT-PCR for detection of *Candida* was done. Based on clinical symptoms and microbiological study, CV was detected in 24 (5.1%) samples. In the remaining 236, 128 (27.4%) showed VVC and 108 (23.1%) BV. In our view clinically CV is commonly misdiagnosed as candidiasis, so special attention has to be paid when a vaginal smear is examined. Wrong diagnosis could be reason for inadequate treatment and increase in patient's suffering. Further investigations regarding the pathogenicity of the disorder are warranted.

Keywords: Cytolytic vaginosis, prevalence, vulvovaginal candidiasis, bacterial vaginosis

Резюме

Цитолитичната вагиноза (CV) е състояние, при което влагалищният секрет изобилства от бактерии от род *Lactobacillus*. Причина за развитието му е дисбаланс в нормалната за тази ниша флора. CV не е често срещано заболяване, но появило се и нелекувано може да доведе до усложнения. Целта на настоящето проучване е определяне честотата на CV спрямо тази на вулвовагиналната кандидоза (VVC) и бактериалната вагиноза (BV) при жени с оплаквания от гениталния тракт. В изследването бяха включени вагинални проби от 468 жени. На всички тях бяха изготвени натривки, оцветени по Грам, и определено вагиналното рН. Материалите бяха култивирани върху Sabouraud декстрозен агар с Gentamycin и върху Chromagar candida. Извършена бе и мултиплексна RealTime-PCR методика за откриване на *Candida spp.* Въз основа на клиничните симптоми и микробиологичното изследване CV бе открита в 24 (5.1%) проби. От останалите 236 проби при 128 (27,4%) се доказва VVC, а при 108 (23,1%) BV. Според нас, висок е процентът на случаите, при които клинично цитолитичната вагиноза бива погрешно интерпретирана като вулвовагинална кандидоза или бактериална вагиноза. Това налага вземане на клиничен материал и извършване на натривка с нейното последващо изследване чрез различни микробиологични методи. Поставянето на грешна диагноза води до неадекватно лечение и може да стане причина за възникването на усложнения при пациента. Необходими са допълнителни проучвания относно патогенезата на цитолитичната вагиноза.

Introduction

Cytolytic vaginosis (CV) is a condition in which the vagina is overrun with lactobacillus. It occurs when the delicate balance of the vagina nor-

mal flora is interrupted.

In healthy women the vagina is colonized by a variety of microorganisms. This ecosystem is balanced, thanks to the dominant effect of micro-

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organisms of the genus *Lactobacillus* spp. Their metabolism provides an acidic pH (3.8 - 4.2) due to lactic acid production. *Lactobacillus* spp. maintain the homeostasis of the vagina and do not allow overgrowth of microorganisms such as *Candida albicans*, *Escherichia coli*, *Gardnerella vaginalis*, *Mobiluncus* spp., *Atopobium vaginæ*, *Megasphera* spp. and other predominantly anaerobic bacteria that are associated with diseases in the lower genital tract of woman (Obset *et al.*, 2001).

In some persons overgrowth of *Lactobacilli* occurs. Then *Lactobacilli* alone or in combination with other bacteria may cause damage to the vaginal epithelial cells leading to lysis of these cells. This dissolution is typical for the condition called CV (Cibley, 1991). The change in the balance of the vaginal ecosystem can lead to the presence of different symptoms especially whitish discharge resembling that of a fungal infection, itching and/or burning (Hillier *et al.*, 1992). These patients may be misdiagnosed as having vulvovaginal candidiasis (VVC) and therefore receive incorrect treatment.

The aim of this study was to determine the prevalence of CV in symptomatic Bulgarian women on the basis of strict microbiological standards and then to compare it with the prevalence of VVC and bacterial vaginosis (BV).

Materials and Methods

For a period of 11 months a total of 468 women of child bearing age were included in the study. A written consent from the women was obtained for recruitment to the study. The presenting clinical symptom was of vaginal discharge. Exclusion criteria for participation in the study were pregnancy, ingestion of hormonal contraceptives and cervical cancer. Two vaginal swabs were taken from each person - one for routine microbiological study, and second for molecular diagnostic procedures. The samples were collected in Amies media and examined afterwards. Gram-stain smears were prepared and their morphological characteristics were observed under the microscope. The smears were assessed for the quantity of lactobacilli, epithelial cell morphology, absence/presence of clue cells and *Candida* spp.. The samples were classified into three major groups, using the Nugent score (range from 0 to 10). The first group included samples with normal vaginal flora (Nugent score 0-3); the second, with transition between normal flora and BV (Nugent score 4-6); the third, with BV (Nugent score 7-10). Vaginal pH was also determined using pH indicator paper (Merck, Germany). The samples

were cultivated in aerobic conditions on non-selective sheep blood agar, MacConkey agar and on Saboraud dextrose agar (SDA) with Gentamycin and Chromagar candida medium (Becton Dickenson) for 72 hours at 35°C. The samples were subjected to total DNA extraction using DNA-sorb-AM nucleic acid extraction kit (AmpliSens) according to the manufacturer's requirements. Multiplex RT-PCR was carried out and *C. albicans*, *C. glabrata* and *C. krusei* were detected (Sacace Biotechnologies, Italy).

Results

A total of 468 women were included in the study. Increased amount of discharge, change in the discharge color - whitish or cheesy, pruritus, burning vulvar sensation, redness, painful sexual intercourse were the most frequent complaints. Based on clinical findings and microbiological results from the microscopy and culture, CV was registered in 24 vaginal swabs (5.1%). The literature criteria for CV are: Gram stain showing abundant amounts of lactobacilli, fragmented epithelial cells, presence of the so-called "naked nuclei", vaginal pH between 4.0 - 4.5 and lack of fungal elements at the microscopy and after cultivation on SDA with Gentamycin and Chromagar candida. All samples were also subjected to mRT-PCR for detection of *C. albicans*, *C. glabrata* and *C. krusei*. Of the 468 women tested, VVC was detected in 128 (27.4%) and BV in 108 (23.1%).

Discussion

Each woman at reproductive age has some vaginal discharge. The secretions produced by healthy vagina cleanse and regulate it and constitute the normal discharge. The normal discharge does not have an offensive odor and is not associated with vaginal complaints such as irritation, itching, or burning. The normal vaginal discharge is white, nonhomogeneous and viscous. It contains epithelial cells in a serous transudate, as well as secretions from the sweat, sebaceous, and Bartholin's glands along with secretions from the cervix. A small number of polymorphonuclear leukocytes may be seen (probably from the cervix). The pH is below 4.5, usually between 3.8 and 4.2. The predominant organisms are large gram-positive rods called lactobacilli. The quantity of normal discharge varies from woman to woman and increases during ovulation, premenstrually and during pregnancy. Changes in normal discharge can occur for many reasons, including menstrual cycle, emotional stress, nutri-

tional status, pregnancy, and usage of medication. Abnormal discharge may be caused by infections of the vagina, but infections or inflammation of the cervix can also lead to an increase in vaginal discharge. It is important to know that in some patients more than one cause is present. A careful history and physical examination may help to separate these conditions. For precision of the etiological diagnosis a microbiological examination is required. In this study symptomatic patients were tested and two of their main complaints with which they presented were increased amount of discharge and/or change in the discharge color.

All the patients included in this study had not only discharge change but also one or more complaints - pruritus, burning vulvar sensation, redness, painful sexual intercourse. The reasons for similar complaints could be different but they predominantly appear after vaginal infection and disruption of vaginal ecosystem. At reproductive age, a woman's vaginal ecosystem is balanced by the dominant presence of *Lactobacilli*. Their amount in a healthy woman varies between 10^3 and 10^7 . Different studies have shown that these lactobacilli do not allow uncontrolled multiplication of other microorganisms and their attachment to the epithelial cells of the vagina (Sobel, 1985; 1992; 1993; Carvalho, 1996). Lactobacilli, however, alone or in combination with other microorganisms may cause alteration in the structure of the vaginal epithelium. The condition is called CV. Still it is not clear why fragmentation or cytolysis occur (Cerikcioglu and Beksac, 2004). These changes are the reason for the occurrence of pruritus, painful sexual intercourse,

burning vulvar sensation and abnormal vaginal discharge (Wathna *et al.*, 1994). Combinations of similar clinical symptoms as those in CV are found in other vaginal infections (Table 1).

Therefore, the patient's complaints and the clinical signs cannot be a method to rely on to make a diagnosis. In such cases microbiological testing is required. And we did it. Gram-stain smears of all samples tested were prepared and their morphological characteristics were observed under microscope. The smears were assessed for the quantity of lactobacilli, epithelial cell morphology, absence/presence of clue cells and *Candida spp.*. Vaginal pH was also determined. Cultivation in aerobic conditions on non-selective sheep blood agar, MacConkey agar and on Sabouraud dextrose agar (SDA) with Gentamycin and Chromagar candida medium (Becton Dickenson) for 72 hours at 35°C was also performed.

The laboratory tests for the diagnosis of CV that we used included: Gram stain with intermediate cells, destroyed epithelial cells and "naked nuclei" as a result of cell cytolysis, overgrowth of lactobacilli, no candida blastospores and hyphae and a few polymorphonuclear leucocytes; pH between 3.5 and 4.5 and/or negative culture on Sabouraud dextrose agar. On Gram stain the abundance of lactobacilli covering the epithelial cells in CV resembles the well-known "clue cells", which are typical of bacterial vaginosis (Paavonen, 1995). If results from the vaginal pH and the whiff test are used, confusion between bacterial vaginosis and cytolytic vaginosis disappears. In BV the pH is higher than 4.5, there is a characteristic odor of the vagi-

Table 1. Common types of abnormal infectious vaginal discharge and their possible causes

Type of discharge	Other symptoms	What it might mean
Thick, white, cheesy	Swelling and pain around the vulva, itching, painful sexual intercourse	Yeast infection
White, gray, or yellow with fishy odor	Itching or burning, redness and swelling of the vagina or vulva	Bacterial vaginosis
Cloudy or yellow	Bleeding between periods, urinary incontinence, pelvic pain	Gonorrhea
Frothy, yellow or greenish with a bad smell	Pain and itching while urinating	Trichomoniasis
White, cheesy	Itching or burning, redness and swelling of the vagina or vulva	Cytolytic vaginosis

nal swab and the whiff test with 10% KON is positive. Additionally, PCRs for detection of different microorganisms associated with BV (*G. vaginalis*, *A. vaginae*, *Peptostreptococcus* spp., *Mobiluncus* spp., *Prevotella* spp., *Bacteroides* spp., *Fusobacterium* spp., *Eggerthella*-like bacteria, *Megasphaera*, *Leptotrichia*, etc.) could be performed.

BV is possibly the most common form of vaginitis. A positive diagnosis is accepted if three of the following four criteria are present: adherent and homogeneous discharge; positive whiff test (the positive whiff test is caused by aromatization of aromatic amines in the presence of KOH); vaginal pH greater than 4.5 and/or presence of clue cells. Gram stain reveals gram-negative coccobacilli adherent to epithelial cells. If white blood cells are present in large numbers, coexisting trichomonas infection or cervicitis should be suspected, as bacterial vaginosis does not elicit an inflammatory response.

According to the vaginitis caused by *Candida* strains it is known that this condition may elicit no vaginal discharge, merely causing vulvar and/or vaginal erythema. If a discharge is present, it is usually thick, white and cheesy. The pH is normal. There is no abnormal odor, and the whiff test is negative. The Gram stain reveals normal epithelial cells. There may be a small increase in the number of white blood cells. The bacteria found are the normal lactobacilli. Yeast, as budding forms or pseudohyphae could be detected. *Candida* may be grown on a variety of media. Cultivation on selective Chromagar candida medium gives the possibility of isolation and presumptive identification of yeast and filamentous fungi and differentiation of *C. albicans*, *C. tropicalis* and *C. krusei*. Cultures are more accurate than microscopic examination alone. For recurrent infections candida speciation is recommended.

In this study, on the basis of the results obtained from microscopy and cultivation of all the 468 tested women, CV was detected in 24 vaginal swabs (5.1%), VVC in 128 samples (27%) and BV - in 108 (23.1%). Interesting and important information obtained from the study was that of the 468 symptomatic women in 24 patients a history of repeated therapy with antifungals without effect was documented. This indicated to us that a possible reason for their long-lasting complaints and therapy failure could be non-*C. albicans* infections or CV. And we found that in 5 of them CV was the reason for their long-lasting complaints and in other 13 non-*C. albicans* infection was discovered.

A number of studies and information exist

regarding the incidence of VVC and BV and their role in lower genital tract disorders. VVC comprises 10 - 49% of all vulvovaginal pathologies with discharge (Foxman *et al.*, 2013; Gunther *et al.*, 2014). It is estimated that 20 - 30% of women of reproductive age attending sexually transmitted infection clinics suffer from BV, and that its prevalence can be as high as 50-60% in high-risk populations (e.g., those who practice commercial sex work) (Bautista *et al.*, 2016). But there are fewer reports regarding CV and considerable variation over its prevalence exists ranging from 1.83% to 30.56% (Demirezen, 2003; Hu *et al.*, 2015). Most of them show low prevalence, for example, Batashki *et al.* (2009) detected CV in 3.9%, Suresh *et al.* (2009) reported 7.1%. Of course, the results depend on a good collaboration between the clinician and the microbiologist, the methods applied for its detection, the groups tested, and the experience of the researchers. In this study, the detected prevalence was 5.1%.

Vaginitis is a common condition and is responsible for many office visits and much discomfort to patients. Untreated it may lead to ascending infections and subsequent complications. Because of this, accurate and prompt diagnosis is mandatory. There is no excuse for trying to diagnose the cause of a vaginal discharge without the use of laboratory tests and to start therapy blindly. The treatment of CV includes: 1. Stopping all antibiotics, anti-fungals and including probiotics in the therapy; 2. Avoiding soaps on the genital area and just washing it in warm water; 3. Discontinuation of the use of tampons because they raise vaginal pH; 4. Baking soda sitz baths that lower the acid levels and soothe the vagina; 5. Abstinence from sexual intercourse during symptoms; 6. Reducing the amount of sugar in the diet. There are a variety of effective treatments for VVC. Topical antifungal pessaries or vaginal tablets containing azols (clotrimazole, miconazole or other) are recommended in mild cases as a single doze or for several days. Oral antifungal medicines containing fluconazole or itraconazole may be used if *Candida albicans* infection is severe or recurrent. Boric acid (boron) 600mg as a suppository at night for 14 days may help to acidify the vagina and reduce the presence of yeasts (*C. albicans* and non-*albicans*). The management of BV includes: 1. Avoidance of douching and bathing with detergents; 2. Reduction of vaginal pH to encourage restoration of normal vaginal lactobacilli; 3. Boric acid 600 mg vaginal capsules or pessaries daily for 21 days; 4. Intravaginal clindamycin or

metronidazole; 5. In recurrent cases oral antibiotics (metronidazole or tinidazole) are used.

The cause of a vaginal discharge should be based on what the clinician feels is the likely pathogen after completion of the history, physical examination, and examination of the discharge. The correct therapy (that is different for CV, VVC and BV) and a successful outcome depend on the accuracy of the diagnosis.

Conclusions

Since vaginitis is an extremely common condition and reasons for its development could be different, the treatment should be based on the patient's history, the physical examination, and the microbiological examination. In this study, the prevalence of CV was 5.1%. Clinically CV is misdiagnosed as candidiasis and the wrong diagnosis could be the reason for inappropriate treatment, unnecessary drug usage and patient suffering. Our results contribute to the few reports in the literature about this condition. In our view further studies are needed regarding the pathogenicity of the disorder, which will define its place among the other reasons for vaginal discharge.

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References

- Batashki, I., D. Markova, N. Milchev (2009). Frequency of cytolytic vaginosis--examination of 1152 patients. *Akush Ginekol.* **48**: 15-16.
- Bautista, C. T., E. Wurapa, W. B. Sateren, S. Morris, B. Hollingsworth, J. L. Sanchez (2016). Bacterial vaginosis: a synthesis of the literature on etiology, prevalence, risk factors, and relationship with chlamydia and gonorrhea infections. *Mil. Med. Res.* **13**: 4.
- Carvalho, G. (1966). DoEderlein bacilli in vaginal smears of post-menopausal women. *Acta Cytopathol.* **10**: 286 - 288.
- Cerikcioglu, N., M.S. Beksac. (2004). Cytolytic vaginosis misdiagnosed as candidal vaginitis. *Inf. Dis. Obstet. Gynecol.* **12**: 13-16.
- Cibley, L. J. (1991). Cytolytic vaginosis. *Am. J. Obstet. Gynecol.* **165**: 1245-1248.
- Demirezen, S. (2003). Cytolytic vaginosis: examination of 2947 vaginal smears. *Cent. Eur. J. Public Health* **11**: 23-24.
- Foxman, B., R. Muraglia, J. P. Dietz, J. D. Sobel, J. Wagner (2013). Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. *J. Low Genit. Tract Dis.* **17**: 340-345.
- Gunther, L. S., H. P. Martins, F. Gimenes, A. L. Abreu, M. E. Consolaro, T. I. Svidzinski. (2014). Prevalence of *Candida albicans* and non-albicans isolates from vaginal secretions: comparative evaluation of colonization, vaginal candidiasis and recurrent vaginal candidiasis in diabetic and non-diabetic women. *Sao Paulo Med. J.* **132**: 116-120.
- Hillier, H. K., M. A. Krohn, S. J. Klebanoff, D. A. Eshenbach (1992). The relationship of hydrogen peroxide producing Lactobacilli vaginosis and genital microflora in pregnant women. *Obstet. Gynecol.* **79**: 369-373.
- Hu, Z., W. Zhou, L. Mu, L. Kuang, M. Su, Y. Jiang (2015). Identification of cytolytic vaginosis versus vulvovaginal candidiasis. *J. Low Genit. Tract Dis.* **19**: 152-155.
- Obset, J., E. Garcia, R. M. Bartolome, A. Andrué (2001). Role of lactobacillus as protector against vaginal candidiasis. *Med. Clin. (Barc)* **117**: 285-288.
- Paavonen, J. (1995). Vulvodynia-a complex syndrome of vulvar pain. *Acta Obstet. Gynecol. Scand.* **74**: 243-247.
- Sobel, J. D. (1985). Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. *Am. J. Obstet. Gynecol.* **152**: 924-935.
- Sobel, J. D. (1992). Pathogenesis and treatment of recurrent vulvovaginal candidiasis. *Clin. Infect. Dis.* **14** (Supp 1): 48-53.
- Sobel, J. D. (1993). Candidal vulvovaginitis. *Clin. Obstet. Gynecol.* **16**: 153-187.
- Suresh, A., A. Rajesh, M. B. Ramesh, R. Yashaswi (2009). Cytolytic vaginosis: A review. *Indian J. Sex. Transm. Dis.* **30**: 48-50.
- Wathna, B., E. Holst, B. Hovelius, P. A. Mardh. (1994). Vaginal discharge - comparison of clinical, laboratory and microbiological findings. *Acta Obstet. Gynecol. Scand.* **73**: 802-808.