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Lactobacillus plantarum P17630 for preventing Candida vaginitis recurrence: a retrospective comparative study

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ABSTRACT

Background: Recurrence is a frequent complaint of patients with vulvovaginal candidiasis (VVC). Although the pathogenesis of VVC remains a controversial issue, disruption of the balance between the vaginal microbiota may facilitate overgrowth by Candida. Some probiotic bacterial strains can suppress *Candida albicans*; *Lactobacillus plantarum* P17630 is able to attach to vaginal epithelial cells and significantly reduce the adhesion of *C. albicans*.
Objective: To evaluate the effect of the application of *Lactobacillus plantarum* P17630 in restoring the vaginal microbiota and prevention of relapses among women with acute VVC undergoing conventional (azole) local and main therapy.
Methods: Retrospective comparative study. We recruited 89 women with a diagnosis of VVC, who were placed into two groups on the basis of reported treatment. The control group was treated with a daily dose of 2% clotrimazole vaginal cream at bedtime for 3 days, followed by vaginal application of a capsule containing lubricant once a day for 6 days and then once a week for another 4 weeks. The probiotic group was treated with the same azole-based protocol but followed by vaginal application of a capsule containing *Lactobacillus plantarum* P17630 ($>10_8$ CFU) once a day for 6 days and then once a week for another 4 weeks beginning the day following clotrimazole discontinuation. Clinical and diagnostic patterns were monitored for three months of follow-up.
Results: At the end of study the probiotic-treated women showed a statistically significant increase in Lactobacillus values "+++" (80% versus 40%, $p < 0.001$) and a better subjective resolution of symptoms such as vaginal discomfort described as burning or itching (90% versus 67.5%, $p < 0.03$). Among controls there was a non-significant increase at 3 months of recurrence of infection, but a significant increase of women with value of pH = 5 or >5.
Conclusion: Although the results of different studies are controversial, most have suggested use of probiotics in the prevention or treatment of VVC, and no adverse effects have been reported. Our data with *L. plantarum* P17630 (Gyno-Canesflor - Bayer) confirm the role of this specific strain as a potential empirical preventive agent for reducing vaginal discomfort after conventional treatment of acute VVC and shifting the vaginal milieu toward a predominance of lactobacilli with an improvement of the vaginal pH value.

Introduction

Information on the incidence of vulvovaginal candidiasis (VVC) is incomplete since VVC is not a reportable entity. Most studies suggest a VVC prevalence of 5–15% depending on the population studied [1]. Infection caused by Candida spp. affects 70–75% of women at least once during their lives, and 40–50% of them will experience at least one recurrence. A small subpopulation, probably fewer than 5–8% of these women, will have recurrent vulvovaginal candidosis (RVVC), defined as four or more mycologically proven episodes within 12 months [2–4]. The incidence of VVC increases dramatically in the second decade of life, corresponding to the onset of sexual activity. It peaks in the third and fourth decade, declining in females older than 40 years. Several studies have shown that sexual transmission of candida organisms occurs during vaginal intercourse, although the role of

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nonsexual practice in introducing candida organisms in the lower genital tract has not been appraised. Pregnancy, recent antibiotic use, immunosuppressive illness such as diabetes mellitus or HIV, prolonged or chronic corticosteroid treatment, prolonged wearing of damp clothing, and frequent douching are included as generally-recognized risk factors for acquiring VVC [5].

Although the detailed mechanisms of VVC pathogenesis remain a controversial issue, it seems that when the balance between the microorganisms in the vaginal microbiota is disrupted, overgrowth of Candida is facilitated. Acute pruritus and vaginal discharge are the usual presenting complaints, but neither symptom is specific to VVC. Vaginal soreness, irritation, vulvar burning, dyspareunia and external dysuria are commonly present. Characteristically, symptoms are exacerbated in the week preceding the onset of menstrual flow. The lack of specificity of symptoms and signs precludes a diagnosis that is based on history and physical examination. A positive culture alone of candida should be regarded as a satisfactory basis for diagnosis of VVC. Most patients with symptomatic vaginitis may be readily diagnosed on the basis of microscopic examination of vaginal secretions. A wet mount or saline preparation should routinely be done to identify the presence of yeasts cells and mycelia and to exclude the presence of "clue cells" and motile trichomonads.

Treatment of VVC depends on whether it is categorized as uncomplicated or complicated. The CDC recommends short course treatment with azole topical agents for uncomplicated VVC [6]. Imidazoles, which block fungal ergosterol biosynthesis, have proven effective for vaginal candidiasis monotherapy. Clotrimazole is one of the most commonly used imidazoles for VVC treatment and can persist at inhibitory levels in vaginal secretions for up to 3 days following a single treatment [7].

Natural defense mechanisms against infections have been described in the vaginal milieu. These include indigenous microbial flora such as Lactobacilli, which are believed to interfere with pathogens by various mechanisms, including competition for adhesion receptors (interference and co-aggregation), competition for nutrients, and the production of antimicrobial substances (H_2O_2, lactic acid, or bacteriocins) [8–10]. Extending the concept of lactobacilli as endogenous defense mechanisms, both oral and local probiotics have been examined and have shown some efficacy in the context of urogenital health [11]. Lactobacilli have been proposed for the treatment and prevention of urinary tract infections, bacterial vaginosis [12], vulvovaginal candidiasis [13], and even for the prevention of HIV and sexually transmitted infections [14]. It has been suggested in some studies that lactobacilli are quite common even in the vaginal epithelium of women with VVC, which would tend to argue against their role as probiotic actors in naturally-occurring cases of VVC. The mechanisms of probiosis ascribed to Lactobacillus species include immunomodulation in the host, restoration of normal vaginal flora, and interference with pathogen colonization [11]. Adherence on the vaginal epithelium is an important virulence factor of *C. albicans*. Co-aggregation of lactobacilli with Candida may also be important for the prophylaxis against vaginal infections by preventing the binding of Candida to the receptors of the vaginal epithelium.

Lactobacillus plantarum is one of the commonly-isolated species in vaginal fluid, together with other lactobacilli such as *L. acidophilus*, *L. jensenii*, *L. gasseri*, *L. crispatus* [9,15–17]. *Lactobacillus plantarum* P17630 is able to adhere to human vaginal cells thereby interfering with adherence of *Candida albicans* [18]. Clinical studies have also shown efficacy of *Lactobacillus plantarum* P17630 in the prevention of vaginal infections [19]. The aim of the present study was to investigate the possible advantages of long-term vaginal administration of *Lactobacillus plantarum* after standard vaginal clotrimazole treatment, and to identify possible applications of a different therapeutic strategy for outpatients.

Materials and methods

Eighty-nine women with confirmed acute VVC attending the Outpatients Service of the Cervico-Vaginal Pathology Unit in the Department of Gynecology and Obstetrics, IRCCS Burlo Garofolo, University of Trieste, between January and November 2013 were eligible for inclusion in this retrospective comparative study. The study was approved by Institutional Review Board of the Institute for Maternal and Child Health—IRCCS "Burlo Garofolo". Diagnosis of acute VVC was defined as meeting cultural and microscopic wet mount criteria in symptomatic women. Exclusion criteria were pregnancy, age younger than 18 years or older than 45 years, presence of other urogenital infections (BV or aerobic vaginitis, trichomoniasis) or anamnestic findings of RVVC, habit of cigarette smoking, and use of hormonal contraceptive methods, prolonged or recent antibiotic or corticosteroid therapy (in the last 30 days) or immunosuppressive illness, such as diabetes mellitus or HIV.

A total of 40 women observed between January and December 2013 were treated with a daily dose of 2% clotrimazole vaginal cream bedtime for 3 days, according to the CDC guidelines, followed by vaginal application of a capsule containing a vaginal lubricant (gelatin with hyaluronic acid and glycerine) once a day for 6 days and then once a week for another 4 weeks beginning the day after clotrimazole discontinuation (group A). In the second semester of 2013 we introduced into our clinical practice the policy to advise women to use *Lactobacillus plantarum* P17630 (Gyno-Canesflor, Bayer) as a adjuvant therapy in cases of acute VVC. A total of 40 women observed between July and November 2013 were treated with the same azole-based protocol but followed by vaginal application of a capsule containing *Lactobacillus plantarum* P17630 ($>10_8$ CFU) once a day for 6 days and then once a week for another 4 weeks beginning the day after clotrimazole discontinuation (probiotic group or group B). According to our routine practice women were invited for a follow-up at 30, 60 and 90 days after the end of clotrimazole therapy

Assessment

Clinical assessment, pH measurement, saline wet mount and potassium hydroxide testing for the microscopic examination of each patient were performed by the same gynecologist. Resolution was defined as absence of VVC symptoms and by negative culture or microscopic disappearance of hyphae. During recruitment (Visit 0), and on scheduled follow-up visits (Visits 1, 2 and 3 at 1, 2 and 3 months after the end of therapy, respectively), patients underwent physical examination. After inspection of the vulva and vestibule, an unmoistened sterile speculum was inserted into the vagina so that vaginal walls, fornices, and the cervix could be evaluated for erythema, color, and viscosity of discharge. The pH value of the vaginal walls and of the lateral fornices was measured by colorimetric paper with 8 comparison colors for pH values between 3.6 and 6.1 (Rottapharm-Madaus, Monza, Milan, Italy). Vaginal samples were collected from lateral fornices by a wooden Ayre's spatula; the samples were mixed with saline or 10% potassium hydroxide on two different slides and immediately observed microscopically. A "whiff test" with 10% potassium hydroxide was performed on each sample. Wet mount examination allowed for detection of clue cells, BV-associated pleomorphic bacteria, lactobacilli, yeasts (hyphae and spores), trichomonas and white blood cells. Gram-stained slides were classified according to the Nugent criteria, being scored as negative (scores 0–3), intermediate (scores 4–6) or consistent with BV (scores 7–10).