Comparison of a novel treatment protocol with oral fluconazole vs probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 in the treatment of recurrent vulvovaginal candidiasis

Running title: treatment of vulvovaginal candidiasis

Doi: 10.36129/jog.2022.51

Anna Angela Criscuolo1, Lodovico Patrizi1, Maria Vittoria Cannizzaro2, Caterina Schipani2, Arianna Piccolo2, Chiara Pensa2, Chiara Cappelli1, Marco Ciotti3, Ester Del Duca2, Luca Bianchi2, Rosita Saraceno2, Roberto Sorge4, Francesco Sesti1, Giuseppe Rizzo1

1Department of Gynecology and Obstetrics Fondazione Policlinico Tor Vergata, University of Rome “Tor Vergata”, Rome, Italy

2Department of Dermatology, Fondazione Policlinico Tor Vergata University of Rome “Tor Vergata”, Rome, Italy.

3 Department of Laboratory Medicine, Fondazione Policlinico Tor Vergata, Viale Oxford 81, 00133 Rome, Italy.

4Department of Systems Medicine, University of Rome “Tor Vergata”, Rome, Italy.

Corresponding Author:

Prof Giuseppe Rizzo
Università di Roma Tor Vergata
Department of Gynecology and Obstetrics
Viale Oxford 81, 00133 Rome, Italy e-mail: giuseppe.rizzo@uniroma2.it
Abstract

**Objective** The recurrence of vulvovaginal candidiasis represents a therapeutic challenge. The aim of this prospective case-control study on a series of women with recurrent vulvovaginal candidiasis (RVVC) was to evaluate the efficacy of a new treatment schedule based on fluconazole 200 mg oral capsules in comparison with oral capsules of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14.

**Patients and Methods.** The study was conducted from April 2020 to June 2021 at the Units of Gynecology and Dermatology, Tor Vergata University Hospital, Rome. One hundred and one adult female patients enrolled in the study were divided into two groups: group A treated with 200 mg fluconazole capsules on day 1,4,11,26, then once a month for three months; group B, two oral capsules of L. rhamnosus GR-1 and L. reuteri RC-14 daily for 15 days/month for three months. Statistical analysis was performed using the Chi-square test. A $p<0.05$ was considered statistically significant.

**Results.** A complete clinical response was observed in all 50 patients of the group A at 6 months with no relapse. In the group B, a complete clinical response was observed in 29 patients with ten relapses at 6 months. Itch, leucorrhea and vulvodynia showed a statistically significant better outcome in the group A (respectively, $p<0.001$, $p<0.001$, $p<0.002$).

**Conclusion.** The proposed new treatment schedule using fluconazole allows the fungal biofilm to be directed into the different growth stages, with greater efficacy than the administration of probiotics alone.

**Key Words:** Fluconazole, probiotics, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, recurrent vulvovaginal candidiasis.
Introduction

Vaginal fungal infections are common in the female population and are associated with predisposing factors. Fungal infection of the vulva and vagina is estimated to be the second most common cause of inflammation after bacterial vaginosis [1]. About three-quarters of women of reproductive age have at least one episode of vulvovaginal candidiasis [2,3]. Candida albicans is the most commonly identified pathogen and is responsible for 85 to 90% of all cases. Candida albicans is a member of the healthy human microbiota, which asymptomatically colonizes several niches in the body, including, but not limited to, the gastrointestinal tract, the female reproductive tract, the oral cavity and the skin [3]. In immunocompetent individuals, Candida albicans is a harmless diner living in harmony with other members of the microbiota. However, alterations in this delicate balance, due to predisposing factors (e.g. menstrual cycles, sexual intercourse, birth control methods, aging, medication, nutritional or hormonal changes and pregnancy), changes the local environment, and pH shifts or dysregulation of the immune system due to infection or immunosuppressive therapy, may allow Candida albicans to proliferate rapidly and cause infections [4]. Candida albicans forms highly structured biofilms composed of multiple cell types (round yeast cells in the bud, oval pseudo-etal cells, and elongated hyphatic cells) enclosed in an extracellular matrix [5]. Recurrent vulvovaginal candidiasis (RVVC) treatment is difficult and takes months to achieve a successful clinical response [6].

For the treatment of vaginal candidiasis, the azole group is considered the most effective drug (econazole, clotrimazole, miconazole, ketoconazole, sertaconazole) [6,7]. Azole compounds are administered locally, using vaginal cream or tablets or suppositories, or orally. Local administration is safe and allows their use during pregnancy [8]. Although various treatments are currently available and effective, the recurrence of candidiasis is common and represents a therapeutic challenge for both gynecologists and dermatologists. The most commonly used drugs for the treatment of vaginal yeast infections are imidazole derivatives. Fluconazole is a trimidazole with antifungal activity. Currently for the treatment of recalcitrant Candida albicans infections, fluconazole 150 mg capsules are administered orally every three days (day 1, 4, 7) followed by a maintenance period in which is administered once a week for six months [6,7,9,10].

As reported in the literature, the intake of lactobacilli reduces the risk of vaginitis, vaginosis and infections of the urogenital system, as it allows rebalancing the intestinal and vaginal flora through the competition of the different administered microorganisms with the pathogenic species, which in many cases appear to be the same saprophytes as Candida [11-13] In addition to the competition between species, other mechanisms of action to resolve the dysbiosis that underlies RVVC are the stimulation of a correct immune and anti-inflammatory response, and normalization of physiological pH (about 4 in vagina, and between 5 and 7 in intestine). The use of probiotic strains, in particular Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14, proved able to increase the effectiveness of an antifungal pharmaceutical agent in the treatment of RVVC[14].

Further The importance of a natural approach using probiotics has been successfully tested in different women conditions such as cystitis or menopause. [15-17]

The aim of this prospective observational case-control study on a series of women with RVVC was to evaluate the efficacy of a new treatment schedule, based on fluconazole 200 mg oral capsules, administered on days 1, 4, 11, 26, and then once a month for three months, compared with oral capsules of Lactobacillus rhamnosus GR-1 (1* 1 x 109 u.f.c.) and Lactobacillus reuteri RC-14 (0,5 x 109 u.f.c.), administered twice daily for 15 days/month for three months.

Patients and Methods
The study has been conducted at the Unit of Gynecology of "Tor Vergata" University Hospital, in collaboration with the Unit of Dermatology and Venereology of the same center, from April 2020 to December 2021. It was a prospective observational case-control study, approved by the local ethics committee with the protocol nr. 80/17 and carried out according to the Helsinki Declaration. Written informed consent was obtained from each registered patient. One hundred one adult female Caucasian patients (18 to 56 years of age, mean age: 31±7) referred to the gynecology clinic, suffering from RVVC, assessed on vaginal swab, were enrolled in the study. The inclusion criteria were age ≥18 years and positive vaginal swab test for Candida albicans with recurrent symptomatic disease. The criteria for exclusion were pregnancy and treatment with systemic or local immunosuppressive drugs. Patients were divided into two different treatment groups. In the first group (group A), fluconazole 200 mg oral capsules were given using the following schedule, one tablet on day 1, 4, 11 and 26, followed by one capsule once a month for three months. The second group of patients (group B) was treated with oral capsules of Lactobacillus rhamnosus GR-1 (1 x 109 u.f.c.) and Lactobacillus reuteri RC-14 (0.5 x 109 u.f.c.), administered twice daily for 15 days/month for three months. Characteristic data of the treated groups (A and B) at baseline (Ti) are showed in Table 1. The vaginal swab test was performed at baseline, and at 6 months after the treatment for confirming the effectiveness of the therapy. Itching and vulvodynia were evaluated using the visual analogue scale (VAS) from zero (absence) to 10 (the worst imaginable). The clinical response was classified as complete (complete remission of all signs and symptoms), partial (improvement or partial remission of signs and symptoms), and no response (no change or worsening of signs and symptoms). Itch and leucorrhea were evaluated at baseline (Ti), and after 6 months (T6) in both groups A and B. The evaluation of vulvodynia was performed at baseline (Ti), and after one (T1) and 6 months (T6). Adverse events were recorded.

Statistical analysis

All data were initially entered into an Excel spreadsheet (Microsoft, Redmond, Washington–United States), and the analysis was performed using the Statistical Package for the Social Sciences Windows, version 26.0 (SPSS, Chicago, Illinois, USA). Descriptive statistics consisted of the mean ± standard deviation (M±SD) for parameter with normal distributions or median and range (min; max) for variables with non-normal distributions, while the occurrences values are reported as percentages. Comparisons of normal data between treatment groups and between pre and post treatment were conducted with Anova one-way or Anova for measures repeated, while dichotomous occurrences were evaluated with Chi-squared test. A p value < 0.05 was considered statistically significant.

Results

All 101 female patients completed the study. Vaginal swab tests at baseline demonstrated the presence of Candida albicans in the totality of the patients. Vaginal discharge associated with vulvodynia was initially present in all 101 (100%) patients recruited in the study. The median itch and vulvodynia VAS score at the time of the first visit was nine for both groups. Follow up visits were scheduled at 1 and 6 months from beginning of therapy. An improvement of all symptoms was observed significantly more in group A than in group B (Table 2). Figure 1.a shows the decrease of itch score (VAS) in groups A and B pre and after treatment (6 months). It was significantly lower in group A. The percentages of patients with leucorrhea in groups A and B pre and after treatment (6 months) are showed in Figure 1.b. At 6 months, none of the patients in group A presented leucorrhea against 41.2% of the patients in group B. Figure 1.c shows the percentages of patients with vulvodynia in groups A and B, at one and 6 months from the beginning of the treatment. At 6 months, 12% of the
patients in group A complained of vulvodynia against 39.2% of the patients in group B. None of the 101 patients in the two groups of treatments reported side effects during the period of treatment, and none needed hospitalization.

Discussion

Candida albicans is responsible for 85-90% of all vulvovaginitis, and affects women of all ages. Although various treatments are currently available and effective, the recurrence of vulvovaginal candidiasis is common, and represents a therapeutic challenge for both gynecologists and dermatologists. Recurrent vulvovaginal candidiasis can have a significant effect on the quality of life. The treatment is based on the administration of topical and systemic antifungal drugs, but existing treatment schedules are often unsatisfactory [5,7,9]. Azole compounds are the first choice for the treatment of uncomplicated-candida vulvovaginitis [7,18]. In some studies, it has been reported that topical sertaconazole is the most effective anti-yeast drug for controlling inflammation and itching due to Candida albicans infection [19-21]. In recent years, imidazole derivatives have become the most commonly used drugs to treat vaginal yeast infections [22-25].

The use of probiotic strains, in particular Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14, proved able to increase the effectiveness of an antifungal pharmaceutical agent in the treatment of vulvovaginal candidiasis. In this regard, a Brazilian study [14] conducted on a cohort of 55 patients, randomized into two groups, positive for Candida spp. (C. albicans in 90% of cases) and with at least one characteristic symptom (leucorrhoea, itching, burning, dysuria, dysuria) has validated this hypothesis. In fact, in patients treated with fluconazole 150 mg single-dose therapy combined with probiotic at a dose of 2 billion per day for 4 weeks, greater efficacy was demonstrated than in patients receiving fluconazole monotherapy, resulting in a significant reduction of associated symptoms (10.3% vs 34.6%; p=0.03), and a significant reduction of candida in culture (10.3% vs 38.5%; p=0.014). The same combination therapy was then implemented in the treatment of a subgroup of patients with a history of recurrent vulvovaginal candidiasis, once again proving effective in egativizing vaginal swab (80.0% vs 18.2%) and in improving symptomatology.

Likewise, Witt et al. [25]. found that adding Lactobacillus gasseri to maintenance itraconazole therapy provided additional benefit in achieving a culture-negative status within 12 months compared to itraconazole alone. Similarly, Pendharkar et al. [26]. found that adding a vaginal probiotic containing Lactobacillus rhamnosus and Lactobacillus gasseri to maintenance fluconazole therapy significantly affected the 6-month or 12-month cure rate of patients with RVVC.

In our study, fluconazole was administered to patients with RVVC using a different treatment regimen compared to the classical regimen. Fluconazole 200 mg oral capsules were administered discontinuously to follow the biorhythm of Candida albicans, and to target not only the hyphale, but also vegetative forms of fungi [27]. This new treatment showed to be safe and free of side effects.

In addition, the new dosage has shown a powerful activity and greater efficacy than the daily administration of probiotics, as demonstrated by the improved outcome of patients treated with the new protocol.

We have hypothesized that the increased efficacy of this new schedule of fluconazole can be attributed to the “its intermittent administration, by inhibiting the passage of Candida albicans from the hypha form to the spore form (defined as C. Albicans biorhythm) [27]. In fact, it is known that alkaline pH and temperature above 37°C facilitate the switching of C. Albicans to the more virulent (hyphal) or resistant (spore) forms [28].

In one study, fluconazole 200 mg oral capsules has already been administered to patients with RVVC, but at greater intervals together with a probiotic, showing 15.6% of recurrence rate during therapy and 9.8% of relapses during follow-up [11]. On the contrary, with our innovative regimen schedule, patients treated unsuccessfully for years and with chronic relapsing infections achieved a complete response.
without relapse at six months since the end of therapy. Moreover, it is worth noting that the shorter treatment schedule, as long as the reduced number of capsules administered, has a positive reflection on both quality of women life and cost savings compared to probiotics.

However, one limitation of our study is the small number of patients enrolled. It is advisable that in the future more patients will be enrolled in a multicenter study to further validate the results of our study.

In conclusion, the present study suggests that a new regimen schedule of fluconazole 200 mg oral capsules is effective in the treatment of RCCV, probably by targeting the fungal biorhythm.

Authors Contribution

All Authors made substantial contributions to the concept and design, analysis and interpretation of data, and drafting and revisions.

Funding

No funding were obtained for this study

Disclosure of interests

No author has any conflict of interest to declare.

Ethical approval

IRB approval was obtained by the Fondazione Policlinico Tor Vergata Ethical Committee, before to start (protocol nr. 80/17).

informed consent

Each patient enrolled in this study gave informed consent to participate in the study and to allow data collection

Data sharing

Available after reasonable request to the corresponding Author

REFERENCES


Legends to Figure:

Figure 1a.

Difference of itch score (VAS) in groups A and B pre and after treatment (6 months)
Figure 1b.

Percentage of patients with leucorrhea in groups A and B pre and after treatment (6 months)

Figure 1c.

Percentage of patients with vulvodynia in groups A and B after treatment (1 and 6 months)
Table 1.

Characteristic data of the treated groups (A, B) at baseline (Ti).

<table>
<thead>
<tr>
<th>Group (Nr. pts)</th>
<th>p-value A vs B</th>
<th>Treatment schedule</th>
<th>Demographics data (M ± S)</th>
<th>Comorbidities Nr. pts (%)</th>
<th>Contraception Nr. pts (%)</th>
<th>Age : $p=0.694^\text{^}$ BMI : $p=0.258^\text{^}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (50)</td>
<td></td>
<td>Fluconazole, 200mg capsule on day 1, 4, 11, 26, and once a month for 3 months;</td>
<td>Age: 32 ± 8 ys BMI: 23.46 ± 4.63 Kg/m2</td>
<td>Smoke: 13 pts (26.0%) Chronic constipation: 20 pts (40.0%)</td>
<td>OC: 18 pts (36.0%) Condom: 25 pts (50.0%)</td>
<td></td>
</tr>
<tr>
<td>B (51)</td>
<td></td>
<td>2 capsules (L. rhamnosus GR-1 and L. reuteri RC-14) per day for 15 days/month for 3 months;</td>
<td>Age: 31 ± 7 ys BMI: 22.50 ± 3.76 Kg/m2</td>
<td>Smoke: 17 pts (33.3%) Chronic constipation: 24 pts (47.1%)</td>
<td>OC: 13 pts (25.5%) Condom: 32 pts (62.7%)</td>
<td></td>
</tr>
</tbody>
</table>

^ anova test one-way; * chi-square test; oral contraceptive: OC
Table 2.

Symptoms in groups A and B at baseline (Ti) and at 6 months (T6).

<table>
<thead>
<tr>
<th>Group</th>
<th>Itch (VAS) (M ± S)</th>
<th>Leucorrhea</th>
<th>Vulvodynia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ti pre-treatment</td>
<td>T6 6 months</td>
<td>Ti pre-treatment</td>
</tr>
<tr>
<td>A</td>
<td>(50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4.6±1.6)</td>
<td>(0.1±0.4)</td>
<td>40pts (80.0%)</td>
</tr>
<tr>
<td></td>
<td>40pts (80.0%)</td>
<td>0pts (0.0%)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>(51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4.5±1.4)</td>
<td>(1.1±1.3)</td>
<td>40pts (78.4%)</td>
</tr>
<tr>
<td></td>
<td>31pts (60.8%)</td>
<td>20pts (39.2%)</td>
<td></td>
</tr>
<tr>
<td>p-value A vs B</td>
<td>p=0.633^</td>
<td>p=0.002^</td>
<td>p=0.846*</td>
</tr>
<tr>
<td>p-value A vs B</td>
<td>p=0.276*</td>
<td>p=0.002*</td>
<td></td>
</tr>
</tbody>
</table>

^ test anova for repeated measures; * chi-square test