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RESEARCH ARTICLE



# The role of female intimate hygiene practices in the management of vulvovaginal candidiasis: A randomized, controlled open-label trial

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**ABSTRACT**

This multicenter, observational, controlled open-label trial randomized 200 women with vulvovaginal candidiasis (VVC) to: Group 1, 6-days clotrimazole 2% vaginal cream once-daily plus 15-days concomitant acid pH thymol and zinc-containing cleansing wash (SaugellaActi3) twice-daily; Group 2, 6-days clotrimazole treatment alone. In both groups, pruritus and burning VAS scores improved from baseline at Days 6, 10, and 15. On Day 10 and Day 15, the pruritus score was significantly lower in Group 1 versus Group 2 ($p < 0.005$ at both timepoints), suggesting acid pH thymol and zinc-containing cleansing wash ameliorates VVC-associated pruritus as part of a female hygiene regimen.

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## Background

Vulvovaginal candidiasis (VVC) is a fungal infection associated with a negative impact on quality of life (Denning et al., 2018). An estimated 70–75% of women of childbearing age will experience VVC during their lifetime (Sobel, 2007). This high prevalence is despite the availability of antimycotic agents and modern screening methods. Data evaluating the effects of intimate hygiene practices on the symptoms of VVC are lacking, and there is an unmet need for vulvar hygiene products supported by clinical data (Chen et al., 2017). We hypothesized that the incorporation of an acid pH thymol and zinc-containing cleansing wash into a daily cleansing regimen may protect against vulvovaginal infection by maintaining the vulvovaginal microbiome. To test this, we conducted an observational, randomized, controlled open-label trial in women with moderate–severe VVC, comparing clotrimazole plus an acid pH thymol

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and zinc-containing cleansing wash versus treatment with clotrimazole alone. The results will help to inform physicians regarding the optimal management of the symptoms of VVC.

VVC is an opportunistic fungal infection, that in most cases is caused by Candida species (mainly *Candida albicans*) (Workowski & Bolan, 2015). The symptoms of VVC include genital pruritus, abnormal discharge, vaginal irritation, external dysuria, and dyspareunia (Workowski & Bolan, 2015). The development of symptomatic disease nominally results from overgrowth of yeast that previously colonized the vagina without causing symptoms (Tortelli et al., 2020); however, an estimated 4–9% of women are affected by recurrent VVC, which is a long-term, debilitating condition (Denning et al., 2018; Mendling et al., 2020). In 2010, the global financial cost associated with lost productivity due to recurrent VVC was an estimated $14.39 billion (USD) (Denning et al., 2018).

A diagnosis is based on medical history, clinical symptoms, and the detection of yeast; however, diagnosis can be problematic due to the insensitivity of yeast detection when based on microscopic examination and/or cultures of vaginal secretions (Mendling et al., 2015; Pappas et al., 2016). Due to the potential negative impact on health and quality of life, high prevalence and cost, and unreliable detection methods, VVC is recognized as a serious global challenge to public health, with a large socio-economic and medical impact (Denning et al., 2018).

The severity of VVC varies, ranging from sporadic cases with mild, local symptoms that resolve with oral or topical treatment, to chronic or recurrent infection that is hard to manage with currently available drugs (Denning et al., 2018). The majority of women present with azole-sensitive Candida species, for whom topical or oral azoles nominally offer effective symptom control (Denning et al., 2018; Sobel, 2016). Clinical guidelines recommend topical antimycotic agents (e.g., polyenes, imidazoles, or ciclopirox olamine) as first-line treatment and, if candidosis has spread to the vulvar region, an antimycotic cream (e.g., clotrimazole) (Mendling et al., 2015). The antimycotic effect of azoles can last several weeks, for example, one dose of a clotrimazole 500 mg vaginal tablet is associated with a mycological cure rate of 70–95% for up to two weeks after treatment (Mendling et al., 2020). Topical azoles are well tolerated and are considered to have an acceptable safety profile, although approximately 1–10% of patients report a burning sensation after application (Farr et al., 2021; Mendling et al., 2004).

The effects of intimate hygiene practices on the symptoms of VVC infection are not well known. Daily washing of the vulva prevents accumulation of vaginal discharge, urine, and fecal contamination (Chen et al., 2017). Evidence suggests certain products that contain spermicides,

β-lactam or other antimicrobials, or practices such as vaginal douching, can upset the vaginal microbiota and may increase risk of infection or serious gynecologic outcomes (Cottrell, 2010; Huang et al., 2014; Yıldırım et al., 2020). However, few evidence-based medical guidelines offer advice regarding female intimate hygiene (Arab et al., 2011). The Middle East and Central Asia Guidelines on Female Genital Hygiene, that were developed by a specialist advisory committee, recommend that in order to maintain a protective layer of lactobacilli to guard against vaginal infection, the genital region should be kept clean, dry and be washed using a mild, hypoallergenic liquid cleansing agent with pH 4.2–5.6 (Arab et al., 2011; Aroutcheva et al., 2001). Similarly, a United Kingdom National Guideline on the Management of Vulval Conditions (British Association for Sexual Health and Human Immunodeficiency Virus [HIV]) suggests that in order to manage vulval conditions, contact with soap, shampoo or bubble bath should be avoided and an emollient soap substitute used instead (British Association for Sexual Health and HIV, 2014).

Recently, there has been an increase in the number of hygiene products for vulvar cleanliness that are available; however, there is an unmet need for rigorous, scientific evaluation of products in systematic trials (Chen et al., 2017). There is a growing consensus that products to improve female intimate hygiene should support intimate health, be hypoallergenic, protect against epithelial dryness, maintain a protective shield against vulvovaginal infection and be specifically formulated and clinically tested for the vulvovaginal area (Chen et al., 2017). The acid pH thymol and zinc-containing cleansing wash SaugellaActi3 (Meda, Viatris Inc.) has antimicrobial and moisturizing properties and a muco-adhesive formulation intended to prolong the effects of active ingredients. We hypothesized the cleansing wash would have a synergistic action when used with a commonly prescribed topical azole, to improve the symptoms of VVC. Specifically, we hypothesized that a daily cleansing routine that incorporated the acid pH thymol and zinc-containing cleansing wash may protect against vulvovaginal infection by maintaining the vulvovaginal microbiome and consequently that the acid pH thymol and zinc-containing cleansing wash plus concomitant topical azole therapy may result in faster resolution of the symptoms and signs of VVC versus topical azole therapy alone.

We conducted an observational, randomized, controlled open-label trial to test the hypothesis. The objective of the trial was to evaluate the effects on two signs and symptoms of VVC (pruritus and burning) of adding an acid pH thymol and zinc-containing cleansing wash to background topical clotrimazole treatment, in patients with confirmed moderate–severe VVC.

## Methods

### Objective

The prespecified objective of this trial was to evaluate the effects of acid pH thymol and zinc-containing cleansing wash plus concomitant topical azole therapy versus topical azole therapy alone, on the resolution of two signs and symptoms of VVC (pruritus and burning).

### Ethics

Study protocols were approved by the appropriate institutional research and ethical committees, and written informed consent was obtained from all participants prior to enrollment.

### Research participants

Female participants aged 18–45 years with VVC were recruited. VVC was confirmed by (1) a Sobel total score ≥2 (the Sobel clinical tool determines vulvovaginal symptom severity on a semiquantitative basis; the Sobel total score is the sum of scores for individual symptoms [pruritus, vulvar or vaginal erythema, edema, excoriation/fissure formation], each scored: 0, absent; 1, mild; 2, moderate; 3, severe) (Sobel et al., 2001) *and* (2) by the presence of blastospores or hyphae on microscopic examination of a fresh sample, *or* a positive vaginal culture for Candida (Sobel, 2007). Women who were pregnant, lactating, postmenopausal, had trichomonas vaginitis, a history of repeated vaginal candidiasis, or had taken contraceptive pills, broad-spectrum antibiotics, or oral vaginal-related vaginitis drugs in the past two weeks were excluded.

### Trial design

A multicenter, clustered, randomized, controlled open-label trial in patients with confirmed moderate–severe VVC was conducted by four public and private medical centers in Italy.

A total of 200 women were randomized using a permutated randomization table (block size 4) to Group 1 or Group 2. In each group, two centers consecutively recruited and enrolled 100 participants.

Patients randomized to Group 1 were treated with clotrimazole 2% vaginal cream once-daily for 6 days plus concomitant acid pH thymol and zinc-containing cleansing wash twice-daily for 15 days. Patients were provided with one canister of cleansing wash and instructed to use it directly

in the vulvar area, and one tube of clotrimazole 2% vaginal cream and instructed to apply it daily before bed. Patients in Group 2 were treated with clotrimazole 2% vaginal cream alone once-daily for 6 days.

Participants made two study visits. During visit 1, women were screened for VVC, baseline demographics and characteristics were recorded, and diaries and trial products were provided. Following visit 1, women were asked to complete a daily diary to document pruritis and burning until the final visit. Patients marked the severity of pruritis and burning on a 10 cm visual analogue scale (VAS) that ranged from 0 (no pruritis/burning), to 10 (highest pruritis/burning). During visit 2 (15 ± 3 days after visit 1), a physical examination was conducted, diaries were returned, and women were asked about adverse events (AEs) experienced during the treatment period. All AEs, whether volunteered by the participants, discovered during general questioning by the investigators, detected through a physical examination, or by other means, were recorded in a clinical chart.

### *Statistical analysis*

GraphPad StatMate version 2.00 (GraphPad Software, La Jolla California USA) was used to conduct statistical analyses and produce graphical representations of the data. Sample size calculation was carried out using the Simple Interactive Statistical Analysis online platform according to accepted methods (Machin & Campbell, 1987). Based on the personal experience of the principal investigator, the estimated effect size for decrease in burning symptoms was 90% for Group 1 and 75% for Group 2. A significance level of 5% (two-tails test) and a power 1β of 0.8 determined a sample size of 100 participants per treatment group. Efficacy endpoints were mean change in pruritus and burning VAS scores from baseline, at Days 6, 10 and 15. Efficacy analyses were conducted using a per-protocol dataset (patients who completed treatment as planned and received a post-treatment smear and culture test). Chi-square ($\chi^2$) and Student's t (paired and unpaired) tests were used for between-group comparisons of categorical and continuous data, respectively. $p < 0.05$ was considered statistically significant.

## Results

### *Participants disposition, and baseline demographics and characteristics*

A total of 220 participants were recruited, 200 were randomized, 192 completed the trial and were included in the efficacy analysis (Group 1,

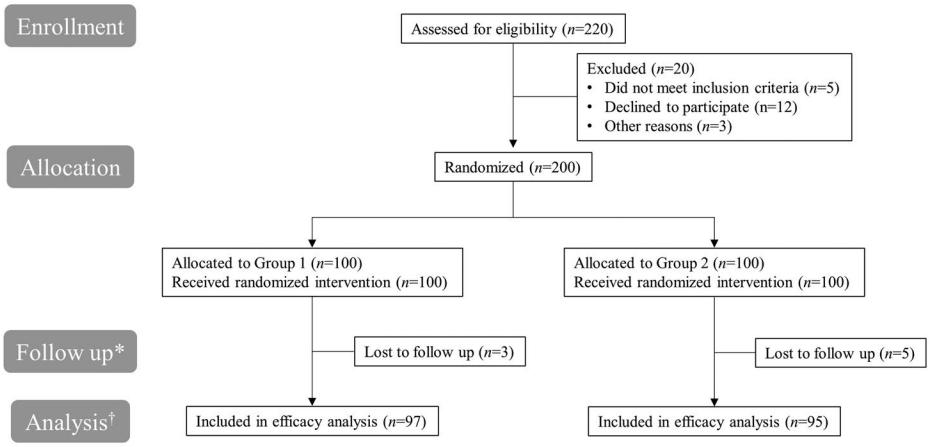


**Figure 1.** Participants disposition. *Visit 2 (Day 15). †Included participants who completed the study per protocol and received a post-treatment smear and culture test.

**Table 1.** Baseline demographics and participants characteristics.

| | Group 1 | Group 2 |
|---|---|---|
| Age (years) | 27.5 (6.3) | 27.3 (6.3) |
| BMI (kg/m$^2$) | 21.5 (5.2) | 22.3 (4.8) |
| Sobel total score | 8.2 (1.1) | 8.3 (1.1) |

Data are mean (standard deviation). Sobel total score is a semi-quantitative measure of vulvovaginal symptom severity, based on the sum of scores for individual symptoms (pruritus, vulvar or vaginal erythema, edema, excoriation/fissure formation) each scored: 0, absent; 1, mild; 2, moderate; 3, severe (Sobel et al., 2001). BMI, body mass index.

$n = 97$; Group 2, $n = 95$), and eight were lost to follow-up (Figure 1). Baseline demographics and characteristics were comparable (Table 1).

### *Efficacy*

In both groups, pruritus and burning VAS scores improved from baseline at Days 6, 10 and 15 (Figure 2). On Day 10 and Day 15, the pruritus VAS score was significantly lower in Group 1 versus Group 2 ($p < 0.005$ at both timepoints). The burning VAS score was numerically lower in Group 1 than Group 2 at all timepoints, but comparisons did not reach statistical significance.

### *Safety*

The side effect profile was comparable across both groups. No serious AEs required discontinuation of treatment in either group. Two patients in Group 1 reported burning sensation of mild severity, which resolved after 2–3 days.

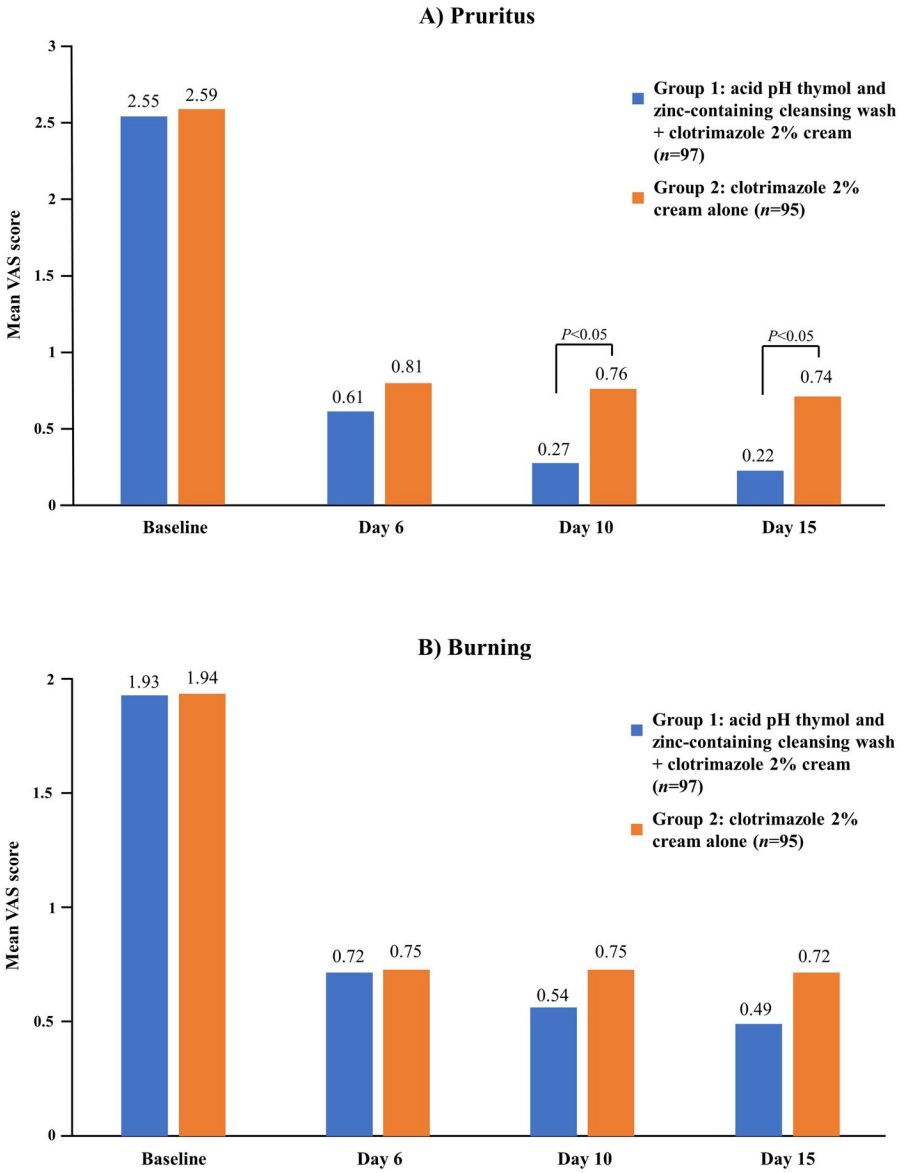


**Figure 2.** Mean pruritus and burning VAS scores at baseline and Days 6, 10, and 15. Data are participants who completed treatment per protocol; VAS score data are self-reported and range from 0 (no pruritis/burning), to 10 (highest pruritis/burning). Acid pH thymol and zinc-containing cleansing wash: SaugellaActi3. VAS, visual analogue scale.

## Discussion

To our knowledge, this is the first study to evaluate the effects of individual components of a female hygiene regimen on the signs and symptoms of VVC. This trial demonstrated that an acid pH thymol and zinc-containing cleansing wash had a statistically significant beneficial effect on pruritis, that was additional to the effect of clotrimazole, in

women with confirmed VVC. Improvements in pruritis and burning were observed in both treatment groups and, contrary to our prespecified hypothesis, occurred over a similar time frame in both treatment groups. By Day 10 a statistically significant beneficial effect on pruritis was associated with the acid pH thymol and zinc-containing cleansing wash (Group 1), that was additional to the effects of treatment with clotrimazole alone (Group 2).

The importance of pH in maintaining the health of the vulvovaginal ecosystem, by modifying risk of irritation and infection, is well established (Arab et al., 2011; Aroutcheva et al., 2001; Chen et al., 2017). Potentially, use of a cleanser in association with a pharmacological regimen could improve the course of the VVC.

The improvement in pruritis observed with the addition of an acid pH thymol and zinc-containing cleansing wash to background clotrimazole 2% vaginal cream suggest that cleansers containing thymol and zinc may provide additional benefits versus pH-altering therapies that do not contain active ingredients and/or could be attributed to the action of the muco-adhesive formulation. These hypotheses need to be tested and confirmed under trial conditions.

Thymol is derived from oils of the thyme plant and classified as a monoterpene (Braga et al., 2007; Sánchez et al., 2004). In vesicular membrane models, thymol is able to penetrate the phospholipid bilayer and generate structural asymmetries (increased curvature) through electrostatic tension (Sánchez et al., 2004). It has an antimicrobial action that prevents *Candida albicans* forming filamentous structures (an important determinant of virulence and ability to cause disease) (Braga et al., 2007). *In vitro*, thymol reduces the adhesiveness of harmful bacteria such as *Gardnerella vaginalis* to human vaginal cells and inhibits the formation of biofilms following bacterial infection in mice (Braga, 2005; Braga et al., 2010; Yuan et al., 2020). Excessive zinc is toxic to bacteria and fungi (including *Candida albicans*) and used by certain immune cells to kill pathogens (Botella et al., 2011; Crawford et al., 2018).

The formulation of the acid pH thymol and zinc-containing cleansing wash includes xanthan gum and carrageenan that prolong the time the vulvar epithelium is in contact with active ingredients (Benvenuti et al., 2019). The formulation also contains selected surfactants and emollients to prevent vulval irritation and has been tested for daily use in women hypersensitive to detergents (Benvenuti et al., 2019; Cappelli et al., 2019). In cultures of yeast obtained from vaginal swabs of women with vaginitis, the formula had an inhibitory, antimicrobial effect on growth of *Candida albicans* and *Candida glabrata* (Gasparri et al., 2019).

Little has been published in the medical literature regarding the potential role of intimate female hygiene in the management of symptoms of

vulvovaginal infection and in overall urogenital health. The current trial demonstrated that co-administration of an acid pH thymol and zinc-containing cleansing wash, with background clotrimazole, had an additional beneficial effect on pruritus in women with VVC versus clotrimazole alone.

The trial had several limitations. The absence of a control (i.e., placebo) cleansing wash in Group 2 could not eliminate a potential placebo effect in Group 1, where patients received an additional treatment versus those in Group 2. Note, as antimycotic agents are the gold standard treatment for VVC, it would have been unethical to evaluate the acid pH thymol and zinc-containing cleansing wash without background clotrimazole. A limited number of self-reported endpoints were evaluated and it would have been interesting to evaluate mycological resolution of infection. Although the baseline demographics and characteristics were balanced between the two treatment groups, no stratification was performed to minimize potential confounding factors. No follow-up period was conducted to monitor the effects of withdrawal. Lastly, an intention-to-treat analysis was not conducted, introducing the possibility of bias in respect to estimations of efficacy. There were benefits to the study design and methods, that increase the relevance of the data regarding the management of patients with VVC. Firstly, there was a relatively large sample size in each of the two groups. Secondly, the use of the acid pH thymol and zinc-containing cleansing wash with background clotrimazole reflects the expected usage in a real-world setting, where use of concomitant antimycotic agents is likely to be commonplace. In summary, these data demonstrate that an acid pH thymol and zinc-containing cleansing wash, when incorporated into a female intimate hygiene regimen, had a statistically significant beneficial effect on pruritus after 10 days, that was additional to the effects of clotrimazole treatment alone. Consequently, an acid pH thymol and zinc-containing cleansing wash may be an appropriate adjuvant to pharmacological therapy for the management of VVC.

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## Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Disclosure statement

The authors declare this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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