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Original article

Recurrent vulvovaginal candidosis: focus on the vulva

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Summary

Recurrent vulvovaginal candidosis is a frequent disease with a serious impact on women's quality of life. Mostly, recurrences are caused by identical Candida strains suggesting *C. albicans* persistence in the female anogenital area. Objectives of the presented work were to identify the site of *C. albicans* persistence, to determine clinical symptoms and signs related to *C. albicans* positive vulvar cultures and to introduce a new therapeutic approach in women with RVVC. Women with an acute, culture-confirmed episode of RVVC at time of visit were included in this prospective case series. Swabs were obtained from both vagina and inter-labial sulcus. Women received a combined 20-day regimen of 100 mg oral fluconazole and ciclopiroxolamin cream topically. Follow-up visits were at 3, 6, 9 and 12 months. Of 139 women, 105 (76%) had at least one *C. albicans* positive culture from the external vulva. Vulvar positive cultures correlated with pruritus (OR 5.4; $P < 0.001$), vulvar edema (OR 3.8; $P = 0.03$) and fissures (OR 2.4; $P = 0.03$). Recurrence rates were 27%, 33% and 34% (at 6, 9, 12 months, respectively). The external vulva appears to represent a site of *C. albicans* persistence and source of endogenous re-infection in patients with RVVC. The combined treatment compared favorably with published fluconazole maintenance regimens.

Key words: Vulvovaginal candidosis, recurrent vulvovaginal candidiasis, vulvovaginitis, *Candida albicans*, fluconazole, ciclopiroxolamin.

Introduction

Recurrent vulvovaginal candidosis (RVVC) defined as four or more culture-confirmed episodes in a 12-month period, is estimated to occur in up to 8%[1] of women of child-bearing age. RVVC can considerably disturb a woman's social and sexual life.[2]

To date, little is known about the pathophysiology of RVVC. In most cases of RVVC, no risk factors can be identified,[3] and recurrences are caused by identical *Candida albicans*[4] strains, suggesting *Candida albicans* persistence in the female anogenital area. Treating RVVC remains challenging; long-term prophylaxis with 150 mg fluconazole once weekly for 6 months results in 91% relapse-free patients at the end of treatment, but symptomatic relapses occur in 57% of patients within 6 months after the cessation of treatment.[5] The main goals were: (1) to investigate in which part of the female anogenital area *Candida albicans* persists despite prolonged oral therapy and (2) to introduce a combined treatment of oral fluconazole and topically applied ciclopiroxolamin.

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Materials and methods

Our case series comprised of non-pregnant women with recurrent vulvovaginal complaints who attended the

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outpatient clinic for Infectious Diseases in Obstetrics and Gynecology of the University Medical Center of Freiburg, Germany, between September 2005 and June 2008. The ethics committee of the University of Freiburg, Germany, stated that this case series is not liable for review by any Institutional Review Board. Eligible women had active, acute vulvovaginal candidosis with *Candida albicans* isolates from either vagina and/or external vulva and met the criteria for RVVC (four or more culture-confirmed episodes in a 12-month period). Clinical symptoms were documented, and colposcopic assessment, pH measurement and microscopy with methylene blue staining from vaginal smears were performed. At the beginning, women (n = 10) were subjected to skin biopsies from the interlabial sulcus. However, skin biopsies showed low diagnostic sensitivity and were painful, and therefore were deemed unsuitable for routine diagnostics. From all women, swabs were obtained from both the vagina and the interlabial sulcus of the external vulva and cultured on CAN 2 chromogenic agar (bioMérieux, Nuertingen, Germany). Antifungal susceptibility testing was not routinely performed as fluconazole resistance in RVVC remains extremely rare.[6,7] Exclusion criteria were pregnancy, co-infection with *Staphylococcus aureus*, previous vulvar skin disease such as lichen sclerosus or atopic dermatitis, and systemic chronic diseases, e.g. known seropositivity for the human immunodeficiency virus (HIV), diabetes, hepatic diseases or renal insufficiency.

After enrolment, all women were treated with fluconazole 100 mg once daily for 20 consecutive days in combination with ciclopiroxolamin cream applied topically to the interlabial sulci of the external vulva and perianally once daily for 4 weeks. Follow-up visits were at 3, 6, 9 and 12 months. At each follow-up visit extensive history with focus on recurrences and additive treatment was conducted and recorded, colposcopic assessment and microscopy from vaginal smears were performed and swabs from both vagina and interlabial sulci were obtained. Recurrences were defined as mycologic recurrences (*Candida albicans* positive cultures) at follow-up visits. Patients with recurrences were considered as non-responders and remained in this subgroup until the end of the observation period. Time-to-recurrence data with an endpoint at 12 months were assessed using the Kaplan–Meier method. On the basis of the culture results for *Candida albicans*, we compared women with positive swabs with women with negative swabs from the external vulva. Association between variables of the two subgroups is described using OR and 95% CI. P values of <0.05 were considered to be statistically significant. Statistical analysis was conducted using EpiInfo 6.0 (CDC, Atlanta, GA, USA).

Results

A total of 139 women, with a history of RVVC and active, acute vulvovaginal candidosis with *C. albicans* positive cultures from either vagina and/or external vulva at the time of visit, were included in this case series. Invasion of the stratum corneum of the keratinised epithelium of the external vulva was demonstrated in three out of 10 skin biopsies from the interlabial sulcus, which were performed at the beginning (Fig. 1). Of the 139 women with *C. albicans* positive cultures, 97 (70%) had *C. albicans* positive vulvar and vaginal cultures, 34 (24%) had positive cultures from the vagina only and eight (6%) from the interlabial sulcus (external vulva) only.

Compared with women with negative vulvar cultures, women with *Candida albicans* positive vulvar cultures more often significantly displayed the following clinical symptoms: Pruritus (OR 5.4; 95% CI 2.0–14.9; $P < 0.001$), vulvar oedema (OR 3.8; 95% CI 1.0–16.8; $P = 0.03$) and fissures (OR 2.4; 95% CI 1.0–5.8; $P = 0.03$). Vulvar erythema and excoriation were found more often in the vulva-positive than in the vulva-negative subgroup (43% vs. 29% and 33% vs. 29%, respectively). However, these differences were not statistically significant.

Of 139 women enrolled, 17 women (12%) were lost to follow-up during the 12-month observation period. At the 3-month follow-up visit, 102 of 122 women

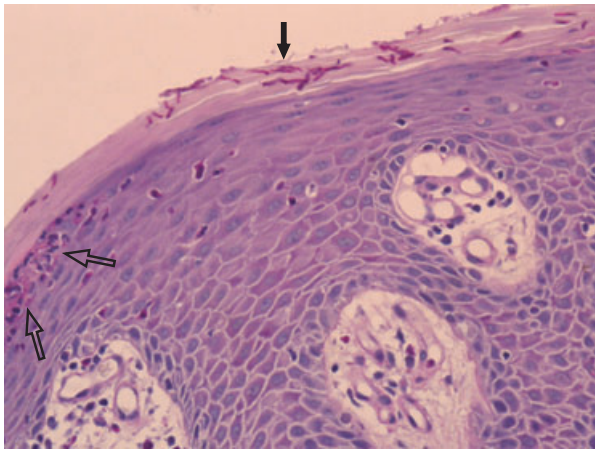

Figure 1 Arrow: Skin biopsy with pseudohyphae in the stratum corneum of the external vulva. Open arrows: Microabscess with neutrophil granulocytes just below the stratum corneum. No leucocyte penetration into the stratum corneum.

(84%, CI 0.76–0.92) remained disease free. Mycologic recurrences occured in 33 women (27%) at 6 months after cessation of treatment, leaving 89 women (73%, CI 0.64–0.82) symptom free. Mycologic recurrence rates at 9 and 12 months were 33% and 34% respectively. Overall, 81 of 122 women (66%, CI 0.57–0.76) experienced no mycologic recurrence throughout the 12-month observation phase. Side effects were rare and consisted mainly of minor gastrointestinal symptoms.

Discussion

Conceivably, the gut could be the initial source of *Candida albicans* colonisation in the vagina. However, past studies revealed that endogenous re-infections in RVVC are often associated with negative rectal cultures for yeast.[8] This finding and the observation that in most cases of RVVC, recurrences are caused by identical *Candida albicans* strains[4] suggest a site of *Candida albicans* persistence in the anogenital area rather than re-infection with *Candida albicans* from the woman's faeces. Past studies showed the capability of yeast cells to invade the non-squamous epithelium of the vagina in acute vaginal candidosis.[9] Skin biopsies performed in the course of our case series demonstrate that *Candida albicans* pseudohyphae can also invade the stratum corneum of the moist keratinised epithelium of the interlabial sulci of the external vulva (Fig. 1). In contrast to the dry skin of the labia majora, where none of the 139 women displayed clinical signs of inflammation, the moist interlabial sulci represent an ideal environment for the growth and persistence of *Candida albicans*.

Increased skin moisture leads to maceration of the stratum corneum and pH elevation. In turn, this is associated with extensive disruption of the stratum corneum's intercellular lipid lamellae,[10] weakening of its physical integrity and reduction of its antimicrobial activity.[11,12] These interactions are widely recognised as key factors in the pathophysiology of intertrigo and fungal superinfections in diaper dermatitis,[11,12] but these considerations have not yet been implemented in the pathophysiology of RVVC.

Leucocytes easily penetrate through the non-keratinised squamous epithelium of the vagina and rapidly eliminate *Candida albicans* from the vaginal lumen, but it appears that leucocytes do not enter the stratum corneum of the interlabial epidermis as demonstrated in Fig. 1. However, when the *Candida albicans* penetrates into the stratum granulosum, an inflammatory response with formation of microabscesses is induced (Fig. 1, open arrow).

Swabs from the interlabial sulci cannot differentiate between contamination and infection with *Candida albicans*. Thus, vulvar fissures, vulvar oedema and vulvar pruritus in combination with positive *Candida albicans* cultures are necessary to strongly indicate *Candida albicans* infection of the interlabial sulci rather than contamination. Pruritus and vulvar oedema are typical clinical signs of inflammation. Vulvar inflammation leads to increased hydration of the stratum corneum and thinning of the vulvar epidermis. Consequently, the interlabial skin is less resistant to friction resulting in fissure formation, e.g. after vulvar swabbing, sexual intercourse or biking. In successfully treated women, vulvar inflammation had subsided and skin conditions had normalised completely after combined treatment with 20 days fluconazole and 28 days ciclopiroxolamin.

Basing of these pathophysiological considerations and analogous to the treatment of onychomycoses in which higher cure rates can be achieved in patients treated with combined regimens,[13–15] we treated women with RVVC both systemically and topically. As described before by Sobel *et al.* [5], recurrences most frequently occur in the first months after the cessation of treatment. In our case series, almost 80% of all recurrences occurred in the first 6 months after cessation of treatment, with the risk of recurrence gradually decreasing from 16% (1–3 months after treatment) to 1% (9–12 months after treatment). Compared with other published RVVC regimens, our combined treatment showed encouraging results: 6 months after cessation of treatment, we observed a recurrence rate of 27%, whereas other studies reported recurrence rates of 45%[16] and 57%.[5]

Conceivably, although high doses of fungistatic fluconazole can be achieved in the stratum corneum of the vulva,[17] the additional topical treatment with a fungicidal cream is more effective in eliminating *Candida albicans* from the stratum corneum.

As the recurrence rates and treatment regimens in RVVC, intertrigo and diaper dermatitis illustrate, eliminating *Candida albicans* from the keratinised squamous epithelium is far from an easy task.

Clinical symptoms, colposcopic findings and the encouraging results of the combined treatment support our hypothesis that *Candida albicans* persists in the moist keratinised epithelium representing an important source of recurrences in women with RVVC. However, double blind, randomised, controlled trials are needed to prove the additional benefit of a topically applied fungicidal agent. We are currently performing a study comparing a 20-day oral treatment with 100 mg fluconazole daily

with a 20-day treatment with 100 mg fluconazole orally plus ciclopiroxolamin topically for 28 days.

Authorship

FCB: contributed to the conception, design, analysis, interpretation of data and article writing.

MTL: contributed to the conception, design, analysis, interpretation of data and revision of the manuscript.

AK and KT: contributed to data collection and revision of the manuscript.

AC: examined and treated all patients of our case series and contributed to the conception, design, interpretation of data, article writing and revision of the manuscript.

Conflict of interest

No conflict of interest to declare.

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