

Cutaneous hypersensitivity to *Candida albicans* in idiopathic vulvodynia

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We have observed that the majority of our vulvodynia patients give a previous history of vaginal candidiasis that was treated but was followed by symptoms of chronic vulvodynia. 27 vulvodynia patients were patch-tested to a standard series of contact allergens, a customized vulvar series and commensal organisms including ultraviolet-killed *Candida albicans*. Comparison tests for the commensal organism were made to a group of 13 female atopic dermatitis patients and to 19 female dermatitis patients without a history of childhood flexural dermatitis who were undergoing patch test evaluation in our clinic. Patients reporting vulvodynia were significantly ($P < 0.05$) more likely to react to *C. albicans* than the dermatitis comparison group. Interestingly, lower concentrations of *C. albicans* caused more positive patch tests than higher concentrations. Our findings suggest that previous *C. albicans* infection may predispose patients to a subsequent hypersensitivity response to *C. albicans* that is expressed only in areas of high cutaneous peripheral fibre density. Low levels of *C. albicans* may also be required to elicit this response as high levels of *C. albicans* may actually result in decreased cutaneous inflammation and decreased intensity of *C. albicans* patch test responses.

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Vulvodynia is defined by the International Society for the Study of Vulvovaginal Disease as 'vulvar discomfort, most often described as burning pain, occurring in the absence of a relevant specific infectious, inflammatory, neoplastic or neurologic disorder' (1). Patients with this disorder often describe pain that interferes with the ability to concentrate, sleep or even sit. The pain is disproportionate based on a clinical exam that usually shows only mild vulvar erythema with tenderness to the application of a cotton swab touched to the vestibule (2). Vulvodynia has been considered a chronic pain syndrome, although women with vulvodynia are psychologically similar to control women and differ in psychological attributes compared with women with chronic pelvic pain who are more likely to have a history of sexual abuse, depression and multiple somatic complaints (3).

Primary afferent fibres release substance P which produces vasodilation and transmits pain. Vulvodynia patients have a lower pain threshold than controls after application of acetic acid, which triggers release of substance P from type C sensory fibres (4). Vulvodynia patients have been shown to

have increased numbers and density of nerve fibres in the vestibule compared with controls (5). Botulinum toxin (Botox[®]) has recently been reported, in conjunction with surgery, to alleviate the symptoms of vulvodynia in a recalcitrant case (6). Botulinum toxin may effect this improvement by inhibiting release of neurotransmitters such as substance P. Chemically induced allergic contact dermatitis causes depletion of substance P in dermatitic murine skin suggesting that these neurotransmitters are released as an early event in the initiation of allergic contact dermatitis (7).

Patients with vulvodynia often give a history of vaginal candidiasis and prior treatment for this condition (8). However, in a previous study, Marinoff et al. (8) examined 13 patients and demonstrated negative patch test results to a series of vehicles, preservatives and antifungals used in topical treatment of vaginal candidiasis. In the same study, 10 patients were tested with intradermal *Candida albicans* antigen; 1 dermatographic patient had an immediate wheal response, and 2 patients showed a delayed type hypersensitivity response at 48 hr. No controls were reported.

We propose that a previous candidal infection may alter the interaction between subsequent *C. albicans* antigen exposure, the cytokine milieu and peripheral nerve fibres resulting in a hypersensitivity response localized to areas with a low (non-immunosuppressive) concentration of *C. albicans* and a high density of peripheral nerve fibres. The culmination of these events may result in a condition manifesting as vulvodynia. To obtain preliminary data relevant to this hypothesis, we patch tested women with idiopathic vulvodynia, using women with atopic dermatitis (AD) and dermatitis patients in our patch test clinic as control groups.

Methods

Subjects

27 patients with vulvodynia were recruited within a university-based clinical practice and/or through campus-wide advertising. All patients had been previously examined by their gynaecologist, and no pathologic basis for symptoms was determined prior to referral. All patients were examined prior to enrolment to rule out active inflammatory disease or infection.

Each participant completed a questionnaire, which asked the following: previous and present medical therapy and history of vulvar contactants such as soaps, toilet paper and pantliners. Patients were evaluated for AD by Hanifin–Rajka criteria (5 patients met criteria for AD) and for personal ($n = 2$) or family ($n = 3$) history of respiratory atopy.

Patients discontinued all systemic antihistamine and corticosteroid treatments as well as topical treatments on the test areas of the back 2 weeks prior to testing.

The 32 female dermatitis patients who consented to serve as controls either had AD that met Hanifin–Rajka criteria ($n = 13$) or were undergoing patch testing for allergic contact dermatitis ($n = 19$).

The study was approved by the University Hospitals of Cleveland (UHC) Institutional Review Board.

Allergens

Allergens were prepared in the same manner for all patients. These patients underwent patch tests for *Malassezia sympodialis*, *C. albicans* and *Dermatophagoides farinae/pteronysinus* at the concentrations and times indicated below.

M. sympodialis. *M. sympodialis* was obtained from the American Type Culture Collection 42132.

Organisms were grown on pityrosporum agar medium II containing neopeptone (10 g/l), bacto-agar (18 g/l), glucose (40 g/l), yeast extract (0.1 g/l), glycerol monostearate (2.5 g/l), Tween 80 (0.2%) (Difco, Sparks, MD, USA) and olive oil (2%). The pH was adjusted to 5.6 prior to sterilization by autoclave. *M. sympodialis* was grown at 37°C for 3–5 days before colony harvesting. Colonies were washed three times in phosphate-buffered saline. The yeasts were rendered non-viable by exposure to ultraviolet (UV) light in a Stratagene UV-cross linker (Spectronics Corporation, Westbury, NY, USA) at 120 000 mJ/cm² for 20 min. These organisms were then collected and concentrated by centrifugation. The organisms were then dehydrated by Speed Vac vacuum centrifugation (Labconco, Kansas City, MO, USA). Viability was assessed by replating an aliquot of the UV-treated yeast onto Sabouraud dextrose agar plates (Difco Laboratories, Detroit, MI, USA) followed by incubation at 37°C for 3 days. Dry weights were determined, and suspensions were made in petrolatum for the following concentrations: 0.4%, 4%, 40% and 80%. Vulvodynia and AD patients were tested to all concentrations; dermatitis patients in the patch test clinic were tested only to 40% *M. sympodialis*.

C. albicans. *C. albicans* (clinical isolate strain SC5314) was obtained from the Center for Medical Mycology in the Department of Dermatology at UHC. *C. albicans* was grown in Sabouraud dextrose broth media. Whole cells were obtained from 3-day cultures. The yeast was rendered non-viable by treating the cell suspension with UV light as described for *M. sympodialis*. Dried yeast was resuspended in white pet. at 0.4%, 4% and 40%. Vulvodynia and AD patients were tested at each of these concentrations; dermatitis patients in the patch test clinic were tested only to 4% *C. albicans*.

The commercial *C. albicans* antigen Candin® (*C. albicans* skin test antigen for cellular hypersensitivity, Allermed Laboratories Inc., San Diego, CA, USA) was also patch tested at undiluted concentration in the vulvodynia and AD control patient groups.

Dust mite. 20% *D. farinae/pteronysinus* was obtained from Chemotechnique (Malmo, Sweden) as a *D. farinae/pteronysinus* mix. *D. farinae/pteronysinus* mix was diluted in white pet. at 0.1%, 1% and 10%. These concentrations were based on prior reports demonstrating reactivity to *D. farinae/pteronysinus* at similar concentrations. Vulvodynia and AD patients were tested to each of these concentrations; dermatitis patients in the patch test clinic were tested only to 10% *D. farinae/pteronysinus*.

Atopy patch tests

The antigen mixtures described above, as well as 3% sodium lauryl sulfate (SLS) in saline as an irritant control, were placed onto 5-mm Finn chambers[®] and applied to intact skin on the upper back of participants. Application sites were examined for erythema and papule formation at 6 hr and 48 hr as well as 7 days post-application. Tested individuals were assessed using the atopy patch test (APT) Reaction Grading Key.

Standard patch tests

For vulvodynia patients, the Chemotechnique North American Standard Series as well as a customized vulvar series of allergens were applied on 5-mm Finn chambers[®] to the back for 48 hr and read at 48 hr and 7 days. The vulvar series contained medicaments such as econazole, miconazole, clioquinol and lidocaine as well as acrylates that may be found in sanitary pads and nail products and textile dyes often present in panty hose (Table 1).

Results

All patients had an unremarkable examination of the vulva at the time of enrolment. Subjective symptom severity did not correlate with the relatively mild findings upon clinical examination such as mild vulvar erythema, oedema or discharge. All enrolled patients completed the study protocol. Of 27 patients enrolled in this study, 10 patients (37%) were APT positive to at least one concentration of *C. albicans* compared with 0 of 13 women (0%) in a cohort of women with AD alone (χ^2 value = 4.81, P = 0.01). Table 2 compares only the 4% concentration of *C. albicans* which was tested in the vulvodynia and both control groups. Women with vulvodynia again

Table 1. Vulvar series

Caine mix IV
Cocamidopropyl betaine
Disperse yellow-3
Disperse orange 3
Disperse red 1
Disperse blue 106
Disperse blue 1224
Triethyleneglycol dimethacrylate
Clobetasol-17-propionate
Miconazole
Econazole nitrate
2-hydroxyethyl methacrylate
Propolis
Ethyleneglycol dimethacrylate

were significantly more likely to have a positive patch test to *C. albicans* 4% than were women with atopic or contact dermatitis.

6 of 10 patients (60%) with positive *C. albicans* APTs showed an inverse concentration response (i.e. stronger APT reactions were observed in the lower 0.4% concentration as compared with the higher 40% concentration of *C. albicans*) (Fig. 1). No patient reacted to patch tests with the commercial preparation of Candin[®].

16 of 27 patients (59%) were APT positive to *D. farinae/pteronysinus*, but only 5 of these satisfied Hanifin–Rajka criteria for AD. An additional 5 of the 16 *D. farinae/pteronysinus* positive patients had a personal (n = 2) or family (n = 3) history of respiratory atopy, and 1 had a personal history of dyshidrotic eczema. This percentage of *D. farinae/pteronysinus* APT-positive vulvar patients was similar to that observed in AD female patients (n = 7 of 13 or 54%) but lower than that observed in the control group undergoing patch testing for allergic contact dermatitis in the clinic (n = 5 of 19 or 26%).

All vulvodynia patients had erythema and the characteristic shiny appearance at the control site for irritancy patch test site (3% SLS).

Table 2. Rates of response to allergens patch tested in three female cohorts

	Female atopic dermatitis (n = 13)	Female dermatitis patients in the patch test clinic ('controls') (n = 19*)	Vulvodynia patients (n = 27)
Patients with $\geq 1+$ reaction to <i>Dermatophagoides farinae/pteronysinus</i> 10%	7 (54%)	5 (26%)	10 (37%)
Patients positive to <i>Candida albicans</i> 4% only†	0 (0%)	1 (5.2%)	8 (30%)
Patients positive to <i>Candida albicans</i> any concentration	0 (0%)	NA (tested only to 4%)	10
Patients positive to <i>Malassezia sympodialis</i> 40%	4 (31%)	0 (0%)	2 (7.4%)

*Prospective enrolment of all female patients undergoing patch testing in our clinic except those with a history of childhood flexural dermatitis, vulvovaginitis or intertrigo as the sole reason for referral, and those who refused to give consent.

†Number of vulvodynia patients reacting to 4% *Candida* is lower than total reported in text to react to any of the concentrations; 'control' patients were tested only to 4% *Candida albicans*.

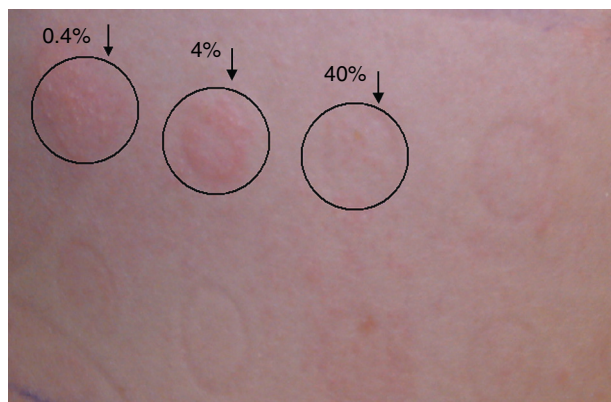


Fig. 1. Paradoxical reaction of *C. albicans* APT + site showing decreased erythema and papules with increased concentrations of *C. albicans* allergen. Day 2.

There were 26 positive patch tests to the standard and/or vulvar series in the vulvodynia group. 7 of these reactions in 7 patients had present or past relevance (Table 3). Overall, 24% of patients demonstrated relevant contact hypersensitivity reactions, mainly to neomycin, econazole, and tixocortol pivate. Patients were advised on the avoidance of products and treatments that may contain those allergens for which they were allergic on patch testing.

Discussion

Allergic contact dermatitis has been previously recognized as a potential cause of vulvar dermatoses, albeit an uncommon aetiology (9, 10). In this patient population, an expanded standard series composed of topical corticosteroids and a vulvar series including azole antifungals, lidocaine mix and textile dyes may be diagnostically relevant.

Patch test reactions to *C. albicans* occurred at a higher frequency (37%) in this study than reactions to standard chemical allergens of present or past relevance (26%). Our results show that patch tests can be used to detect hypersensitivity to proteins such as *C. albicans* even on intact skin. Although proteins are large molecules, penetration of the epidermis may occur due to the presence of smaller peptides in the antigen mixture or possibly because

of the breakdown of the larger peptides in the skin either occurring spontaneously or as a result of breakdown associated with active proteases. The inner vulva is non-keratinized and highly hydrated, which, together with friction, may enhance penetration of irritants and some allergens (11). The skin of the back, while not equivalent to the vulvar skin (11), is utilized as the standard test site to evaluate elicitation of chemical allergies. Langerhans' cell densities are similar on the skin and vulvar mucosa (12). The vulvar mucosa is a highly innervated locale where Langerhans' cells are often in close proximity to epidermal nerves (13). Location, along with other potential interactions of Langerhans' cells with nerve products, may predispose this site of mucosal tissue to sensitivity towards neuronal-mediated response. Potentially pathogenic micro-organisms may also stimulate the innate immune system, and this may affect the protein patch test response. An example of this is shown by Netea et al. (14) who demonstrated that *C. albicans* can stimulate toll-like receptor 2 which upregulates interleukin (IL)-10 and CD4⁺ CD25⁺ T-regulatory cells resulting in suppression of immune response to *C. albicans*. Toll-like receptor 2-deficient mice can still mount a humoral response to *Candida* and vaccine-induced resistance (15). Fungal pathogens have also been proposed to stimulate toll-like receptors disproportionately depending on the organism and the route of infection (16). Therefore, patch testing with micro-organisms may elicit both hypersensitivity and immunosuppressive responses simultaneously.

Several of our patients had more intense patch test reactions to the lower *C. albicans* concentrations with weaker or no reaction to higher concentrations. Higher *C. albicans* concentrations may result in toll-like receptor 2 signalling that leads to local immunosuppression to *C. albicans*. Thus, lower concentrations of *C. albicans* elicit a positive patch test response while higher concentrations invoke immunosuppression. In addition to the possible role of toll-like receptor 2 in suppressing a patch test response, *C. albicans* itself has been demonstrated to produce a soluble factor that suppresses IL-12 and thus prevents Th1-like inflammation that would help patients overcome infection (17). This factor, dubbed secretory IL-12 inhibitory factor (SIIF) is released only by live and virulent organisms (17). If SIIF is the inhibitor in our higher concentrations of UV-killed *C. albicans* APTs, this may relate to secretion from the *Candida* during preparation of our extract. To date, our preparation of *C. albicans* has not been examined for the presence of SIIF; this work is ongoing.

Table 3. Reaction to standard and vulvar series thought to be current or past relevance

Allergen	Number of reactions
Neomycin sulfate	3
Econazole nitrate	2
Fragrance mix	1
Tixocortol-21-pivate	1

The lack of response to commercial Candidal antigen (Candin[®]) raises intriguing questions about the allergenic epitope that appears to be present in our crude *C. albicans* extract but not in the purified commercial preparation. *C. albicans* may contain several antigenic epitopes, and it is possible that previous infection results in enzymes in the cutaneous microenvironment that cleave *C. albicans* resulting in exposure and sensitization to additional allergenic epitopes. It is possible that these antigenic epitopes are not represented in the commercial Candin[®] preparation as this is not a whole organism product but rather a defined component of *C. albicans*. Alternatively, it is possible that high concentrations of the Candidal antigen could decrease percutaneous absorption.

Identification of the potential allergenic *C. albicans* epitopes, determination of ideal test concentrations, conditions that best mimic the environment of the vulva and improved understanding of the relationship between *Candida* hypersensitivity and symptoms of vulvodynia remain as challenges for further study.

Conclusion

Women with idiopathic vulvodynia occasionally have a relevant patch test to a chemical contained in a personal care product or medication but more frequently react to patch tests for *C. albicans*. The immunological mechanism that leads to symptoms from *C. albicans* exposure is speculative but may involve neurotransmitters that have been shown to influence contact hypersensitivity and are present in abundance in the vulvar vestibule. Patch tests to *C. albicans* may only be effective at low concentrations in order to avoid potential immunosuppressive stimulation observed with higher concentrations of *C. albicans*. A crude preparation of *C. albicans* resulted in positive patch test reactions that were significantly more common in women with idiopathic vulvodynia than in atopic and contact dermatitis patients; commercial *Candida* skin test allergen used off-label for patch testing did not result in any such reactions. Epitope recognition of intact organisms or breakdown products from whole organisms may mediate the positive patch test results observed in a vulvodynia patient population.

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