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Value of *Candida* Polymerase Chain Reaction and Vaginal Cytokine Analysis for the Differential Diagnosis of Women with Recurrent Vulvovaginitis

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ABSTRACT

Objectives: Recurrent vulvovaginitis remains difficult to diagnose accurately and to treat. The present investigation evaluated the utility of testing vaginal specimens from women with symptomatic recurrent vulvovaginitis for *Candida* species by polymerase chain reaction (PCR) and for cytokine responses.

Methods: Sixty-one consecutive symptomatic women with pruritus, erythema, and/or a thick white discharge and a history of recurrent vulvovaginitis and 31 asymptomatic women with no such history were studied. Vaginal swabs were tested for *Candida* species by PCR, for the antiinflammatory cytokine interleukin (IL)-10, and for the proinflammatory cytokine IL-12.

Results: C. albicans was detected in 19 (31.1%) of the patients as well as in three (9.7%) controls ($P = 0.03$). Both IL-10 (31.1% vs. 0%) and IL-12 (42.6% vs. 6.5%) were also more prevalent in the recurrent vulvovaginitis patients ($P < 0.001$). However, there was no relation between the presence or absence of *Candida* and either cytokine. Detection of IL-12 in 14 women indicated the stimulation of a vaginal cell-mediated immune response possibly from an infectious agent. The presence of only IL-10 in six patients indicated a suppression of vaginal cell-mediated immunity and was consistent with a possible allergic etiology. The absence of both IL-10 and IL-12 in other patients, similar to that found in healthy controls, suggested a noninfectious, nonallergic etiology of their symptoms.

Conclusion: Many women with recurrent vulvovaginitis are not infected with *Candida*. Testing for *Candida* should be required in this population. Treatment with only anti-*Candida* medication will clearly be inadequate for the majority of women with this condition. Infect. Dis. Obstet. Gynecol. 8:244–247, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS

recurrent vulvovaginitis; interleukin-12; interleukin-10; *Candida*

Recurrent vulvovaginitis remains a difficult disorder to diagnose accurately, to treat, and for which to end the repetitive cycles. There is a widespread assumption that a *Candida* infection is always, or nearly always, involved in eliciting the associated symptoms. The sale of nonprescription drugs for self-treatment of vaginal "yeast" infections is many times greater than the number of

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Clinical Study

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infected women. Many clinicans also treat on the basis of clinical symptoms, without confirmation that *Candida* is indeed present. This may account, in part, for the ineffectiveness of antifungal treatments in many women.

There is an increasing awareness that a localized immune perturbation may lead to vulvovaginitis in some women. A localized allergic response in the vagina has been demonstrated.[1–4] Vaginal symptoms may be due to an immediate hypersensitivity response to infectious agents or to noninfectious allergens[1–3] as well as to eosinophil accumulation.[4] The subsequent production of prostaglandin E_2 and inhibition of cell-mediated immune responses[1,5] would also favor proliferation of *Candida* or other microorganisms to levels capable of eliciting clinical symptoms.

To obtain a more accurate diagnosis of women with recurrent vulvovaginitis as the basis for selective treatment, we analyzed vaginal specimens from women with this disorder for *Candida* species by polymerase chain reaction (PCR) and for vaginal concentrations of interleukin (IL)-12 and IL-10. Induction of specific immune responses is largely dependent on IL-12. Only those T lymphocytes belonging to the Th1 subset express the β chain of the IL-12 receptor. Binding of IL-12 to this receptor leads to Th1 lymphocyte activation and induction of a cell-mediated immune response.[6] This is the mechanism responsible for limiting *Candida* growth in the vagina.[1] However, in the absence of IL-12 β-receptor expression, the T lymphocytes develop along the Th2 pathway. This leads to IL-10 release, production of IgE, induction of an allergic response, and inhibition of cell-mediated immunity.[7] Under these conditions, vaginal *Candida* yeast can multiply and begin to germinate into their hyphal form.[8]

MATERIALS AND METHODS

Subjects

The study population consisted of 61 consecutive women with at least four previous episodes of symptomatic vulvovaginitis in the past 12 months and who were currently complaining of pruritis, erythema, and/or a vaginal discharge. Thirty-one consecutive asymptomatic women seen at the same center with no self-reported history of vulvovaginitis and no evidence of a current vaginal infection or inflammation were also studied. All subjects were white. Women with herpesvirus, human papillomavirus, and bacterial vaginosis as defined by clinical criteria were excluded. All subjects were tested for *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, syphilis, and HIV. None was receiving medication at the time of the study and for at least 4 weeks previously. Informed consent was obtained from each participant.

Specimen Collection

A vaginal specimen was obtained by instilling 1.0 ml phosphate-buffered saline (PBS) into the posterior vagina, rubbing the lateral walls with a cotton swab, and removing the specimen with a syringe. The sample was centrifuged, and the supernatant and pellet were frozen separately at –80°C until tested.

Candida Testing

The pellet fractions were thawed, treated with lyticase to disrupt the *Candida* cell wall, and tested for *C. albicans* by PCR.[9] This assay has previously been demonstrated to detect all *C. albicans* culture-positive specimens from the vagina as well as in additional culture-negative specimens.[10,11] Negative specimens were retested by another PCR that is able to detect all other *Candida* species.[12]

Cytokine Analysis

The supernatant fractions were thawed and assayed for IL-10 and IL-12 by commercial ELISA assays (BioSource, Camarillo, CA). Values were converted to picograms per milliliter by reference to a standard curve generated in parallel to the test specimens. The lower limits of sensitivity were 5 pg/ml for IL-10 and 1 pg/ml for IL-12. The positive values ranged from 10 to 144 pg/ml for IL-10 and from 31 to 164 pg/ml for IL-12. Duplicate values differing by more than 10% were reassayed.

Statistical Analysis

Differences between variables were evaluated by Fisher's exact test and the nonparametric Mann-Whitney test, as appropriate. A P value <0.05 was considered significant.

RESULTS

Demographic and Historical Variables

A comparison of the patient and control populations is shown in Table 1. There were no statisti-

TABLE 1. Demographic and historical variables in patients and controls

Variable	Patients (n = 61)	Controls (n = 31)
Mean age (S.D.) in years	31.6 (7.1)	34.4 (6.2)
Mean no. pregnancies (S.D.)	1.1 (1.2)	1.1 (1.0)
Allergic history[a] (%)	43.7	32.3
Current smoker (%)	16.3	25.8
Oral contraceptive user (%)	26.3	35.4

[a]Self-reported history of seasonal or food allergies or allergic responses to medications.

cally significant differences in any of the variables examined. Except for detection of *M. hominis* and/or *U. urealyticum* in seven of the patients, none of the patients or controls was currently positive for any of the non-*Candida* microorganisms examined. The presence of these mycoplasmas appeared to be unrelated to the cytokine findings.

Detection of Vaginal *Candida* Species and Vaginal Cytokines

C. albicans was detected in the vaginal specimens from only 19 (31.1%) of the women with symptomatic recurrent vulvovaginitis. Three (9.7%) of the healthy controls were also positive for this organism ($P = 0.03$). No additional *Candida* species were detected in any of the specimens.

IL-12 was present in 42.6% of the vaginal samples from symptomatic women with recurrent vulvovaginitis, and 31.1% were positive for IL-10. In contrast, only two (6.5%) control vaginal specimen contained IL-12, and none was positive for IL-10 ($P < 0.001$). There was no relation between the presence or absence of *Candida* in the recurrent vulvovaginitis patients and any of the variables examined (Table 2).

The relationships between cytokine and *Candida* detection in the recurrent vulvovaginitis patients are detailed in Table 3. In almost one-third of the symptomatic patients, neither *Candida* nor IL-12 or IL-10 was detected. A mixture of IL-12 and IL-10 was present in 14 women, 12 had only IL-12, and 6 were positive only for IL-10 in their vaginas. In nine women, *Candida* was detected in the absence of either cytokine.

TABLE 2. Relation between *C. albicans* and various parameters in women with symptomatic recurrent vulvovaginitis

Variable	*Candida* present (n = 19)	*Candida* absent (n = 42)
Vaginal interleukin-12 (%)	47.4	38.6
Vaginal interleukin-10 (%)	31.6	31.8
Allergic history (%)	31.6	36.4
Current smoker (%)	10.5	9.1
Mean No. pregnancies (S.D.)	1.4 (1.2)	1.1 (1.3)
Mean age (S.D.) in years	33.2 (7.2)	31.0 (7.4)
Mean no. episodes of vaginitis per year (S.D.)	8.7 (2.5)	8.2 (7.4)
Oral contraceptive user (%)	31.5	55.8

TABLE 3. Detection of IL-10, IL-12, and *C. albicans* in vaginal specimens from women during an episode of recurrent vulvovaginitis

Cytokines	*Candida*	No. patients (%)
Only IL-10	Present	1 (1.6)
Only IL-10	Absent	5 (8.2)
Only IL-12	Present	4 (6.6)
Only IL-12	Absent	8 (13.1)
IL-12, IL-10	Present	5 (8.2)
IL-12, IL-10	Absent	9 (14.8)
None	Present	9 (14.8)
None	Absent	20 (32.8)

DISCUSSION

By utilizing the very sensitive PCR, *C. albicans* was identified in only 31.1% of symptomatic women with recurrent vulvovaginitis; none was positive for other *Candida* species. Therefore, the vaginal symptoms in the majority of these patients were not due to the presence of *Candida* at this anatomical site. In addition, there was no consistent pattern of vaginal cytokine production in these women.

The heterogeneity of cytokine responses indicates multiple etiologies of the clinical symptoms. The presence of IL-12 in 26 of the patients indicated a localized cell-mediated immune activation, consistent with a possible infectious etiology for their symptoms. The finding of IL-10 in some of the IL-12-positive samples probably indicates activation of the immune regulatory mechanism necessary to limit the extent of the proinflammatory response. IL-12 has been shown to induce IL-10 production.[13] *Candida* proliferation might have stimulated production of IL-12 and IL-10 in the women positive for this organism, but this was clearly not the case for the majority of patients examined. Although women with bacterial vaginosis were excluded from this study, quantitative mi-

crobial cultures for aerobic and anaerobic microorganisms were not available for the present subjects, so the possible role of other microbes in eliciting the vaginal symptoms remains undetermined.

The absence of vaginal IL-12 in 10 of 19 subjects who were positive for *C. albicans* strongly suggests a deficiency in the ability to mount a vaginal cell-mediated immune reaction in response to this microorganism. This lack of an appropriate immune response may contribute to susceptibility to recurrent vaginal candidiasis in these women. Similarly, detection of IL-10 in the absence of IL-12 in six women is consistent with induction of an allergic Th2-mediated immune response being involved in the symptomatology of vulvovaginitis.

The absence of IL-10, IL-12, and *Candida* in 32.8% of the symptomatic patients indicates the probable absence of an infectious or allergic etiology in these women. The cause(s) of their vaginal symptoms remains unknown and is currently under investigation. More detailed microbiological, immunological, and genetic testing may be necessary to resolve this question.

The absence of *Candida* species in most of the study subjects with symptomatic recurrent vulvoginitis points out that treatment with an antiyeast medication will be both ineffective and inappropriate for many women with recurrent vulvovaginitis. Clearly, there are multiple etiologies contributing to similar symptoms in women with vulvovaginitis and this problem is not as simple to diagnose accurately as many clinicians and lay people believe. An improved capacity to make individualized diagnoses, perhaps by incorporating testing for IL-12, IL-10, and/or other cytokines or mediators indicative of infection, allergy, or other immune processes, and subsequent appropriate treatment, may improve our capabilities to treat more effectively vulvovaginitis in these women. Further studies are needed to validate cytokine analyses for determining the etiology of vulvovaginal symptoms.

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