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Research article

Prevalence and risk factors for vaginal *Candida* colonization in women with type 1 and type 2 diabetes

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Abstract

Background: Diabetes mellitus increases the rate of vaginal colonization and infection with *Candida* species

Methods: We surveyed women with diabetes receiving care at either an urban or suburban diabetes clinic to examine the relationship between vaginal *Candida* colonization, diabetes type and duration, and HbA_{1c} level. 101 participants completed the self-administered questionnaire and self-collected a vaginal swab for *Candida* culture. *Candida* colonization was similar by age and race.

Results: Type 1 diabetics were three times as likely as type 2 diabetics to be colonized with any *Candida* species (OR = 3.4; 95% CI: 1.03, 11.41; p = 0.04); even after adjusting for abnormal HbA_{1c}, which had an independent effect (OR = 1.4; 95% CI: 1.04, 1.76; p = 0.02). Recent antibiotic use (OR = 4.5; 95% CI: 1.18, 16.79; p = 0.03), lifetime history of chlamydia (OR = 5.8; 95% CI: 1.09, 30.54; p = 0.04), and performing oral sex during the past 2 weeks (OR = 4.9; 95% CI:0.84, 28.27; p = 0.08) were also associated with *Candida* carriage after adjusting for diabetic type and abnormal HbA_{1c}. *C. albicans* was isolated from the majority of colonized type 1 participants (56%), while *C. glabrata* was the most common isolate among colonized type 2 participants (54%).

Conclusions: Improving glucose control and possibly modifying sexual behavior may reduce risk of *Candida* colonization, and potentially symptomatic infection, among women with diabetes.

Background

Diabetes mellitus predisposes individuals to bacterial and fungal infections, including those caused by *Candida* species. Many investigators have suggested that vulvovaginal candidiasis (VVC) occurs more frequently in diabetics [1–8]. Further, chronic recurring VVC may be a marker of diabetes [9]. Several studies report increased rates of asymptomatic vaginal carriage rates of *Candida* species and incidence of symptomatic infection in diabetic women, but results are inconsistent [5,10–14]. While some show no increase in carriage of vaginal *Candida*[5], those demonstrating increased *Candida* carriage in female diabetes patients do not examine the effect of glucose control or diabetes type [12,14]. However, *in vitro* studies demonstrating impaired host response directed against *Candida* in diabetics support the idea of increased rates of *Candida*

colonization among diabetics [4,6,7]. Whether diabetes leads to more symptomatic and/or more recurrent VVC episodes is a subject of controversy.

Potential risk factors for VVC include diabetes type, severity, and degree of glucose control.[26–30] In addition to diabetes mellitus, a number of VVC risk factors have been identified, including African-American heritage [15–17], previous history of VVC [15], higher education degree, intermediate age, oral contraceptive pills [3,10,16,18], use of commercially available solutions for cleansing of external genitalia or vaginal douching, frequent sexual intercourse [17,19,10], sexual behavior (age at first intercourse, frequency of oral-genital contact) [20], contraception devices (diaphragm, vaginal contraceptive sponge, intrauterine device), and antibiotics [3,15,19]. Whether these factors increase risk of*Candida* colonization or symptomatic infection following colonization, or both, is unknown, however, it should be emphasized that a prerequisite for symptomatic vaginitis is vaginal colonization.

Few studies have addressed the relationship between *Candida* colonization, VVC, and diabetes; none we are aware of have examined the role of behavioral VVC risk factors for colonization among persons with diabetes. To evaluate the relationship between *Candida* colonization and hypothesized risk factors for vulvovaginal candidiasis colonization in diabetics, we conducted a cross-sectional study among female diabetes patients receiving care at one of two diabetes clinics. We describe the associations between *Candida* colonization, sociodemographics, health behaviors, diabetes type, diabetes severity, and Hemoglobin A_{1c} level among women with type 1 and type 2 diabetes.

Methods

Study population

Female diabetes patients seen at an outpatient academic diabetes practice of Wayne State University Division of Endocrinology, Department of Internal Medicine, were eligible for study. The practice included an urban (University Health Center, Detroit, MI) clinic and a suburban clinic (Hechtman Clinic, Bingham Farms, MI). Patients were diagnosed with type 1 and type 2 diabetes on clinical presentation by a board certified endocrinologist. Potential participants were approached in the clinic waiting room and consent obtained. As an incentive for participation, subjects were rewarded with video rental gift certificates (value $5). Participants consented to a medical record review which included abstraction of total glycated hemoglobin levels obtained at the enrollment visit, completed a self-administered questionnaire and self-collected a vaginal swab. The questionnaire addressed the following variables: sociodemographic (age, race, marital status, insurance status, highest education completed, occupation); diabetes duration, type, severity, and abnormal glucose level; lifetime history of physician diagnosed VVC episodes; recent antibiotic use, by type; sexual history; health behaviors. For the diabetes section we used questions from the Diabetes Care Profile, an instrument developed and validated by the Michigan Diabetes Research and Training Center [21,22]. The study protocol was approved by the Institutional Review Boards at the University of Michigan and Wayne State University.

Of 155 women approached, 105 (67.7%) consented. One woman was ineligible because her vaginal sample was lost. Two women decided to stop participating during the study; one did not understand the questionnaire, while the other felt the questionnaire was too intrusive, leaving a final sample size of 101 participants.

Laboratory methods

All vaginal specimens were cultured for yeast. Specimens were placed on plates with media selective for *Candida* growth (Sabouraud's dextrose agar). Suspected mixed samples, as determined by colony type and microscopic characteristics, were placed on plates with media showing species-specific colony color change (Chromagar, CHROMagar, Paris, France). *C. albicans* and *C. glabrata* were identified by chlamydospore and germ tube tests; all other species were identified by carbohydrate assimilation tests (API-20C AUX).

Data analysis

We described the distributions of hypothesized variables among participants, using simple descriptive statistics, and the associations between hypothesized risk factors and *Candida* colonization using odds ratios (OR) and Cornfield 95% confidence intervals (CI). After a detailed stratified analysis, to assess confounding and effect modification, we fit a logistic regression model for *Candida* carriage. We calculated ORS and 95% CIs for the bivariate analyses using EpiInfo [23]; all other analyses and data management were done using SAS [24]. Several variables strongly associated with *Candida* colonization had small numbers of missing values, particularly for oral sex (13%) and douching (9%) (others did not exceed 5%). In this case, for the multivariate analyses, we inferred that a missing response was equivalent to a negative response. If we are wrong in this inference, the resulting estimates will be biased towards the null hypothesis of no effect. Excluding the missing responses from the relevant analyses only modestly changed the point estimates.

Results

Demographic characteristics by clinic

Fifty-nine participants were recruited from UHC; forty-two were recruited from Hechtman. Mean age among UHC participants was 52.3 years, while mean age among

Hechtman participants was 50.0 years (p = 0.5). The clinics varied greatly, however, in participant ethnicity. Forty of fifty-nine (67.8%) UHC participants were of African American descent. By contrast, eight of 42 (19.0%) Hechtman participants were of African American descent.

Diabetes characteristics

Most participants had type 2 diabetes (62/101 = 61%). By clinic, type 2 was more common among UHC participants (69%), but was equally distributed among Hechtman participants.

Total glycated hemoglobin levels measured at the time of the enrollment visit were abstracted from patient's charts. We assessed glycemia by transforming total glycated hemoglobin levels to hemoglobin A1c (HbA_{1c}) values using a linear regression formula [25]. For the Wayne State University patient population, a value of HbA_{1c} > 6.4% is abnormal. This cutpoint was determined by standard clinical laboratory comparison methods and verification of the reference interval [26–30]. The mean HbA_{1c} for our study population was 7.91, with a range of 4.4–15.7. Overall, only 20 of 101 participants (19.8%) had normal HbA_{1c} at time of enrollment. Type 1 subjects had a higher proportion of abnormal levels (92.3%) than type 2 subjects (72.6%) (p = 0.02).

Correlates of Candida colonization

29 of 101 (28.7%) participants were colonized with *Candida* species. 2 of the 29 (6.9%) were colonized with two *Candida* species. The most common species were *C. albicans* (12/29 = 41.4%) and *C. glabrata* (11/29 = 37.9%). *C. albicans* predominated among women with type 1 (9/16 colonized or 56%) and *C. glabrata* among women with Type 2 (7/13 colonized or 54%). We identified one isolate of *C. parapsilosis*, *C. guilliermondii*, and *Trichosporon beigelli*.

Type 1 patients were more likely to be colonized with *Candida* than type 2 patients (OR = 2.6; 95% CI: 0.99, 6.98; p = 0.03) (Table 1). Women with abnormal HbA_{1c} were almost three times more likely to be colonized with *Candida* than those with normal HBA_{1c}, but this association was not statistically significant (OR = 2.7; 95% CI: 0.68, 15.39; p = 0.13). No statistically significant association was detected between *Candida* colonization and duration of diabetes, although those who were colonized in each diabetic type tended to have longer duration of diabetes (mean duration diabetes, colonized v. not: type 1: 15.7 v. 20.2 y, p = 0.34; type 2: 9.8 v. 16.1 y, p = 0.28). Average duration of diabetes among colonized women did not differ by type (type 1: 20.2 v. type 2: 16.1; p = 0.53).

Age, race, marital status, education, and employment were not significantly associated with *Candida* colonization (Table 1). Antibiotic use in the past 2 weeks, lifetime history of chlamydia, douching, and report of both performing and receiving oral sex in the last two weeks were strongly associated with *Candida* colonization, whereas vaginal-penile sex had only a modest and not statistically significant assocation with *Candida* colonization. When stratified by oral sex, there was no association between vaginal sex and colonization status. However, the effect of oral sex remained after stratification by vaginal sex.

Multivariate Analyses

To simultaneously adjust for variables independently associated with *Candida* colonization in the crude analyses, we fit a logistic regression model predicting *Candida* carriage (Table 2). Because of the small sample size, we created a base model including age, diabetes type, abnormal HbA_{1c}, recent antibiotic use, and performing oral sex. We assumed for this analysis that those who did not respond to questions about oral sex did not engage in oral sex. Receiving oral sex was not associated after adjustment for performing oral sex (OR = 1.07; 95 % CI: 0.12, 9.42, p = 0.95) and thus was excluded from the base model. Other variables associated in the bivariate analyses were added one at a time to the base model. After adjustment, older age, having type 1 diabetes, an abnormal HbA_{1c} level, and report of antibiotic use during the previous 2 weeks were statistically significantly associated with *Candida* colonization. Performing oral sex was associated with an almost fivefold increase in colonization, but the association was not statistically significant (p = 0.08). When added individually to the base model, lifetime history of chlamydia (OR = 5.8; 95% CI: 10.9, 30.54) but not douching (OR = 2.2; 95 % CI: 0.68, 7.00) remained strongly associated with colonization. Four participants positive for *Candida* also had a previous chlamydia diagnosis and reported douching. When entered simultaneously into the model, the estimates were unstable, thus, they were entered separately. Douching and history of chlamydia were also strongly associated with performing oral sex; adding each of these variables reduced the parameter estimate and statistical significance of the association with performing oral sex. Pregnancy history had no association with colonization after adjustment (OR = 0.97; 95% CI:0.26, 3.66).

Discussion

Among women with diabetes, *Candida* carriage increased with older age, type 1 diabetes, abnormal HbA_{1c} level, oral antibiotic use in the previous two weeks, and ever history of chlamydia. Other VVC risk factors, including African American descent, lifetime history of VVC, higher education, oral contraceptive use, and frequency of vaginal intercourse were not significantly associated with *Candida* colonization after adjustment for other variables. These data suggest that behavioral factors, as well as HbA_{1c}, are important determinants of *Candida* colonization among women with diabetes.

Table 1: Association of *Candida* colonization with host characteristics among 101 women with diabetes recruited from urban and sub-urban clinics (2000)

Characteristic	**Not Colonized** *Number*	**Colonized** *Number*	*OR*	*95% CI*	*p-value*
Diabetes Type					
Type 1	23	16	2.62	(0.99, 6.98)	0.03
Type 2	49	13			
Hemoglobin A_{1c}[+]					
Normal (4–6.4%)	17	3			
Abnormal (> 6.4%)	55	26	2.68	(0.68, 15.39)	0.13
Duration (miss = 5)					
< 5 years	24	9			
6–14 years	26	4	0.41	(0.08,1.74)	0.17
> 14 years	19	14	1.96	(0.63, 6.32)	0.20
Age					
18–44	24	7			
45–59	21	15	2.45	(0.75, 8.45)	0.10
60+	27	7	0.89	(0.23, 3.46)	0.85
Race (miss = 1)					
White	38	13			
Black	32	15	1.37	(0.52, 3.64)	0.48
Asian	1	1	2.92	(0.03, 234.35)	0.44
Marital Status (miss = 6)					
Never married	11	6			
Married, living with spouse	30	12	0.73	(0.19,3.00)	0.61
Married, not living with spouse; and/or separated	5	2	0.73	(0.05, 6.55)	0.75
Widowed or divorced	22	7	0.58	(0.13,2.69)	0.42
Education (miss = 4)					
High school or less	25	6			
Vocational/Associate/ Some college	26	15	2.40	(0.72, 8.72)	0.11
Bachelor's degree and higher	19	6	1.32	(0.30, 5.78)	0.67
Employed (miss = 8)					
Not employed	30	15			
Part-time	14	3	0.43	(0.07., 1.91)	0.22
Full-time	22	9	0.82	(0.26, 2.44)	0.69
Insurance (miss = 10)					
No	0	3			
Yes	64	24	--	(0.0, 0.98)	0.02[*]
Antibiotics (oral only)					
None	65	20			
Taken in last two weeks	7	9	4.18	(1.19, 14.84)	0.01[*]
Lifetime history of Chlamydia (miss = 5)					
No	65	23			
Yes	3	5	4.71	(0.83,32.12)	0.04[*]
Douche (miss = 9)					
No	48	15			
Yes	17	12	2.26	(0.79, 6.37)	0.09
Oral sex in past 2 weeks (miss = 13)					
None	58	17			
Perform only	1	1	3.41	(0.04,272.31)	0.42[*]
Receive only	3	1	1.14	(0.02, 15.23)	1.00[*]
Perform and Receive	2	5	8.53	(1.22,93.93)	0.02[*]
Vaginal-penile sex in past 2 weeks (miss = 8)					
None	45	22			
Yes	15	11	1.50	(0.53,4.17)	0.39

[+]Note: HbA_{1c} calculated from a linear regression formula using total glycated hemoglobin values. Cut points based on local laboratory values. [*]2-tailed Fisher's Exact Test

Table 2: Logistic regression models predicting *Candida* colonization among 101 women with type 1 or type 2 diabetes (2000)

Variable Name	*Parameter Estimate*	*p-value*	*Odds Ratio*	*(95% CI)*
Age 45 and older	1.92	0.008	6.8	(1.64, 28.37)
Diabetes type 1	-1.23	0.04	3.4	(1.03, 11.41)
Glycemia (% HbA_{1c})*	0.30	0.02	1.36	(1.04,1.76)
Antibiotics in past 2 weeks (oral only)	1.49	0.03	4.5	(1.18, 16.79)
Perform oral sex in past 2 weeks	1.59	0.08	4.9	(0.84, 28.27)
Douche	0.78	0.19	2.2	(0.68, 7.00)
Lifetime history of chlamydia	1.75	0.04	5.8	(1.09,30.54)

* HbA_{1c} (%) > 6.4. HbA_{1c} calculated from a linear regression formula using total glycated hemoglobin values. Cut points based on local laboratory values. NOTE: All models included age, diabetes type, HbA_{1c}, antibiotic use, and oral sex.

Women with type 1 diabetes had higher *Candida* colonization rates than those with type 2, even after adjusting for age, behavioral factors and HbA_{1c}. One possible explanation is difference in duration of *Candida* carriage: women with type 1 and type 2 diabetes may be equally likely to acquire *Candida*, but those with type 1 may be less able to clear it. Similar to previous reports, *C. albicans* was isolated from the majority of colonized type 1 participants, while *C. glabrata* was the most common isolate in type 2 participants, but our numbers are small [1]. Whether this is due to diabetes type or reflects different distributions of *Candida* species by age or both is uncertain.

The observation that hyperglycemia in type 1 diabetes increases risk for *Candida* colonization is consistent with previous reports [1,5,6,31]. A New Zealand study of 124 women with type 1 diabetes found 7 of 7 women with elevated glycated hemoglobin reported vaginal symptoms for the previous year, compared with 60% overall [10]. Hyperglycemia limits neutrophil function among persons with type 1 diabetes, including neutrophils' ability to phagocytose and kill *Candida* organisms. With the oxidative killing ability of neutrophils hindered, diabetics may not be able to clear pathogens as well as non-diabetics [1,6,7,31]. Hyperglycemic individuals may also have increased risk for *Candida* colonization because their secretions contain glucose, which can serve as nutrients for *Candida* organisms. Sobel and colleagues reported a fucose (6-deoxy-galactose) vaginal epithelial cell receptor that aids in adhesion of *Candida* to vaginal epithelial cells [32]. Since fucose contains an isomer of glucose and acts as one form of receptor site for *Candida* adhesion, it is possible that increased *Candida* colonization is proportional to glucose level. Receptor avidity may be a reflection of increased glucose levels in the blood and tissues. Glycemia, however, does not fully explain the observed increased risk of *Candida* colonization.

We found a borderline significant association with performing oral sex associated with an almost five-fold increase in vaginal colonization with *Candida*. Receiving but not performing oral sex has been previously reported as a risk factor for VVC [15]. Oral sex may facilitate transmission of yeast, as *Candida* often colonizes the oral cavity [15,33]. Diabetics may be more likely to carry Candida in the oral cavity than their sex partner; thus, performing oral sex on their sex partner prior to vaginal intercourse may serve to inoculate the vaginal cavity. Douching has also been previously associated with *Candida* colonization [17,19,20]. The practice is hypothesized to disrupt vaginal flora; vaginal symptoms may also lead a women to douche so the direction of effect is unclear. The association we observed with chlamydia is probably a marker of sexual behavior rather than a causal factor; for example, women who reported a history of chlamydia were more likely to douche and to report engaging in oral sex, although it is possible that chlamydia carriage adversely affects the vaginal flora increasing the chance of *Candida* colonization. Recent antibiotic use is a well-described correlate of symptomatic *Candida* vaginitis [3,15–18,34]. While asymptomatic infection is not equivalent to disease, antibiotic use may act to alter the vaginal microenvironment to facilitate *Candida* colonization in the same way it is hypothesized to promote VVC.

Women aged 45 years and older in our study were more likely to be colonized with *Candida* species. We are unaware of colonization studies in women of comparable age; most have emphasized women of childbearing age [8,35,36]. Whether our observation is a real finding or a reflection of our age distribution (18–84) or unknown selection biases which led to inclusion in our study requires further investigation. However, incidence of symptomatic infection is higher in younger (18–44) women [15].

This study is small and was intended to be hypothesis generating. The study population is in no way representative of all females with diabetes, although the age and racial distribution of participants reflected that of the participating clinics. Although it is possible that women with a history of symptomatic infection might have been more likely to participate, we have no reason to believe that women differentially participated by both symptomatic history and other variables under study which would have biased our results. Further, as women were unaware of whether they carried *Candida* at the time of completing the questionnaire, it seems unlikely that a differential recall of behaviors among colonized and non-colonized might have occurred. A final concern is that *Candida* colonization, while the first step toward VVC, is not equivalent to symptomatic infection. Larger, more definitive studies, which include a non-diabetic control group and prospective follow-up, are planned.

Conclusions

We found strong, statistically significant associations between vaginal *Candida* colonization and antibiotic use, lifetime history of chlamydia, diabetes type and HbA_{1c} level and a borderline significant association with performing oral sex. More *non-albicans* species were observed among women with type 2 diabetes. Improving glucose control and possibly modifying sexual behavior may reduce risk of *Candida* colonization, and potentially symptomatic infection, among women with diabetes.

Competing interests

None declared.

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