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Identify productive species of vaginal candidiasis in women referred to health centers in Tehran

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ABSTRACT

Vaginal discharge syndrome is a clinical condition characterized by leucorrhea, which can be caused by reproductive tract infections (RTIs). The aim of this study is to identify productive species of vaginal candidiasis in women referred to health centers in Tehran. The Prerana dataset was collected as part of a prospective cohort study of 898 non-pregnant, reproductive-aged women living in and around Iran, between 2013 and 2014. The primary study objective was to evaluate the relationship between abnormal vaginal flora and the incidence of Herpes Simplex Virus-type 2 infection. Participants completed three study visits–conducted at baseline, and at three and six months-each of which involved an interviewer-administered question naire in Tehran, Iran; a pelvic examination; and collection of vaginal and blood specimens for laboratory testing for reproductive tract infections. Analyses investigating separate research aims will be conducted over three papers, as follows: Bacterial vaginosis and risk of Trichomonas vaginalisinfection,and Epidemiologic features of vulvovaginal candidiasis, and Syndromic diagnosis of vaginal infections using logic regression. The Prerana cohort dataset is well-suited to filling in multiple gaps in the research literature. The analyses are among the first to test (or re-test) specific hypotheses concerning vaginal infection using a community-based sample based in a low-income setting.

Keyword: Vaginal Candidiasis, Trichomonas Vaginalis, Tehran

INTRODUCTION

Vaginal discharge syndrome is a clinical condition characterized by leucorrhea, which can be caused by reproductive tract infections (RTIs) [1]. Negative outcomes associated with RTIs include discomfort, mental anguish and loss of economic productivity [2].Three RTIs are referred to as vaginal infections: bacterial vaginosis (BV), trichomoniasis(TV), and *vulvovaginal candidiasis* (VVC) [3].In addition to the negative outcomes associated with RTIs, these vaginal infectionsareknownto increase susceptibility tosexually transmitted infections (STI), including HIV,and tobe associated with lowbirth weight and preterm birth[4-5].The prevalence and incidence of, andrisk factors for, vaginal infections are not well-studied in many settings, precludingestablishent of effective public health programsfor their prevention.Further, diagnosis ofvaginal infections is compromised by the absence of state-of-the-art diagnostic capability in low-income settings, leading to the use of syndromic diagnosis algorithms[6].

Vulvovaginal candidiasis is caused by overgrowth of Candida yeast species in the vagina and is characteri zed by curd- like vaginal discharge, itching and erythema[7]. *Vulvovaginal candidiasis* has been associated with considerable direct and indirect economic costs [8]: enhanced susceptibility to HIV infection[8]: and an increased risk of preterm birth[9], Treatment of *vulvovaginal candidiasis* is warranted when a woman presenting with a

complaint of symptoms consistent with *vulvovaginal candidiasis* also has laboratory confirmation of the presence of Candida from a vaginal specimen. Short-course azole-based treatment regimens are considered effective and safe [10], and are accessible and affordable in most settings. Much of the epidemiologic literature concerning *vulvovaginal candidiasis* reports on studies in which women were queried on their self-reported history of *vulvovaginalcandidiasis*[11], but without laboratory-confirmation of infection by Candida. Other studies, in which investigators only measure the presence of Candida infection of the vagina [12], are not able to identify women with symptomatic *vulvovaginal candidiasis* disease; this latter study design is frequently employed for studies conducted in low-income settings. Few studies have diagnosed *vulvovaginal candidiasis* through laboratory confirmation of infection in symptomatic women, and few studies have measured the incidence of confirmed cases of *vulvovaginal candidiasis*. The lack of representative data on the epidemiologic features of laboratory-confirmed *vulvovaginal candidiasis* has been evident throughout the time in which vulvovaginal candidiasis has evolved from being considered a "nuisance infection" to a clinically relevant condition [13]. In India, only two studies have been conducted in which laboratory-confirmed *vulvovaginal candidiasis* was diagnosed in a community-based sample: Bang et al diagnosed *vulvovaginal candidiasis* in 35% of 650 adult women living in rural Maharashtra state, and Prasad et al diagnosed *vulvovaginal candidiasis* in 10% of 451 married, 16-22 year old women in rural Tamil Nadu state [14]. However, neither study assessed the incidence of or identified risk factors for *vulvovaginal candidiasis*. As reduction of HIV transmission and of adverse birth outcomes remain public policy priorities in Iran [15], and studies have shown gynecological morbidity is extremely common [16], additional investigation of the epidemiologic features of *vulvovaginal candidiasis* is warranted.

MATERIALS AND METHODS

We examined the incidence, prevalence, and risk factors for *vulvovaginal candidiasis* among a cohort of women recruited for a study of the presence of bacterial vaginosis and incident Herpes simplex virus-type 2 infection[17]. The recruitment and baseline descriptive features of the cohort of 898 sexually active, non-pregnant women between 16 and 30 years of age fromcommunities around Tehran, Iran have been previously described [18]. Briefly, between 2013 and 20014, women in the cohort completed three study visits (at baseline and at three and six months), comprised of a structured interview, a clinical examination, and collection of cervico- vaginal specimens for laboratory testing. Trained interviewers used a structured interview to collect sociodemographic and behavioral information, as well as reports of symptoms associated with gynecologic morbidity.

Laboratory and clinical methods: Study physicians performed a pelvic examination of each participant and recorded signs of vaginal abnormalities. During the examination, swabs of the posterior fornix of the vagina and blood specimens were collected. Women diagnosed with *vulvovaginal candidiasis* were treated with a single 150 mg dose of oral fluconazole. *Trichomonas vaginalis* infection was treated with a single two-gram dose of oral metronidazole, and participants were given the option of receiving treatment to give to their sex partner. Women were clinically diagnosed as having bacterial vaginosis using criteria developed by Amsel et al [19]. those with bacterial vaginosis were treated with 400 mg oral metronidazole administered twice daily for one week. Women were diagnosed as having *Trichomonas vaginalis* infection if found to be positive on either wet mount microscopy or culture (InPouch, BioMed Diagnostics, White City, OR, USA). A Gram stain was used for laboratory diagnosis of bacterial vaginosis using the criteria developed by Nugent et al, whereby bacterial vaginosis was diagnosed with a score of 7-10, while a score of 4-6 was considered intermediate vaginal flora, and a score of 0-3 was considered negative for bacterial vaginosis [20]. As part of the Nugent-criteria scoring, the average number of Lactobacillus-like cells (morphotypes) detected over several visual fields in light microscopy was enumerated, and given a score corresponding to averages of 0, <1, 1-4, 5-29 and ≥30. Candida infection was assessed from culture (InTrayColorex Yeast, BioMed Diagnostics, White City, OR, USA). Participants infected with Candida were diagnosed as having vulvovaginal candidiasis if they reported vaginal itching or discharge and had vaginal erythema or discharge observed on clinical examination. Herpes simplex virus-type 2 infection was assessed by an index > 1.09 from type-specific ELISA testing of serum (Focus Technologies, Cypress, CA,USA) (Table 4).

Statistical methods: Our outcome measure was laboratory confirmed diagnosis of vulvovaginal candidiasis. First, we describe prevalence and incidence of vulvovaginal candidiasis using frequencies and percentages. We calculated the visit-specific prevalence of vulvovaginal candidiasis and assessed whether this prevalence changed over the course of the study. Next, we tabulated the number of women's clinic visits with various vaginal symptoms reported or vaginal signs observed. Among observations in which these vaginal signs or symptoms were recorded, we calculated the proportion in which a diagnosis of vulvovaginal candidiasis was made. Finally, we examined the relationship between the prevalence of vulvovaginal candidiasis and sociodemographic characteristics and possible risk factors. We used a separate univariable regression model for each characteristic and risk factor. While counts (e.g.the number of diagnoses of vulvovaginal candidiasis) are reported as observed, percentages and prevalences were estimated using generalized estimating equations (GEE) regression models. GEE allows for parameter estimation

when observations are correlated, in this case, due to multiple observations per participant over the course of the study. The GEE models were specified with binary family, identity link, and exchangeable correlation. The GEE models were bootstrapped with 500 repetitions to estimate robust standard errors. Statistical analysis was conducted using Stata 11.2 (StataCorp, College Station, TX, USA). The study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley, and the Asha Kiran Institutional Review Board of Mysore, India; all participating women provided written informed consent.

RESULTS

The median age of the women at the baseline visit was 26 years (Interquartile range [IQR] 24-29 years), and participants had been with their current sex partner for a median of nine years (IQR 6-12 years). Over one- quarter of the women had no formal education (27%). Nearly one in three women identified as Muslim (29%). Few women were nulliparous (15%) , and very few women reported using oral contraception, condoms, or an intrauterine device at any point during the study. Furthermore, less than 5% reported having oral sex or having had more than one lifetime sex partner. No woman reported douching or smoking tobacco. Throughout the duration of the study, the mean prevalences of reproductive tract infections were as follows: Trichomonas vaginalis (6%), clinical diagnosis (Amsel criteria) of bacterial vaginosis (12%), laboratory diagnosis (Nugent criteria) of bacterial vaginosis (16%),and infection with Herpes simplex virus-type 2 (13%). At least 30 Lactobacillus morphotypes were detected on the majority (66%) of Gram stains of vaginal swabs, with smaller proportions of swabs with 5-29, 1-4, <1 and 0morphotypes detected (11, 5, 6 and 12%, respectively).Vaginal signs and symptoms, and diagnosis of vulvovaginal candidiasis: Including all three study visits, we found that substantial proportions of the women reported vaginal itching (29%) or vaginal discharge (31%), or had vaginal erythema (9%) or vaginal discharge (35%) on examination. The positive predictive values of these signs and symptoms for predicting *vulvovaginal candidiasis* were low: a minority of women with these symptoms or signs was subsequently diagnosed with vulvovaginal candidiasis (18, 15, 25, and 18%, respectively). Combinations of the vaginal signs and symptoms were increasingly rare, though the probability of a correct diagnosis of vulvovaginal candidiasis increased to 41% when both signs and both symptoms were present. (Table5) Diagnosis, prevalence and incidence of vulvovaginal candidiasis: Of the 885 observations in which Candida was detected in vaginal specimens, 180 (20%) satisfied the case definition for diagnosis of *vulvovaginal candidiasis*, while the remaining 705 (80%) were considered asymptomatic infection.

Table 4: Diagnostic criteria and treatment given for laboratory-and clinically-diagnosed gynecological conditions, Tehran, Iran, 2013-2014

Diagnosis	Diagnostic criteria	Treatment
Candida infection	Positive culture	None
Vulvovaginal candidiasis	Positive culture for Candida infection and one clinical sign	150 mg oral fluconazole, single dose
Trichomoniasis	Positive culture or positive saline wet mount microscopy	2 g oral metronidazole, single dose
Bacterial vaginosis, laboratory	Score of 7-10 on Nugent criteria from Gram stain	None
Intermediate flora, laboratory	Score of 4-6 on Nugent criteria from Gram stain	None
Abnormal flora, laboratory	Score of 4-10 on Nugent criteria from Gram stain	None
Bacterial vaginosis, clinical	Positive on at least three of four Amsel criteria	400 mg oralmetronidazole, bid for 7 days
Herpes simplex virus type 2	Index value > 1.1 on ELISA test of serum	Acyclovir 400mg tid for 7- 10 days

The prevalence of *vulvovaginal candidiasis* declined over the three study visits: from 77/893 (9%) at baseline to 65/840 at three months (8%) and 38/795 (5%) at six months (p-trend <0.001). Of the 1487 baseline or three month study visits in which a woman was not diagnosed with *vulvovaginal candidiasis*, 72 (5%) were positive for *vulvovaginal candidiasis* at the next study visit. Repeat diagnoses of *vulvovaginal candidiasis* were common: Of the 137 baseline or three-month study visits in which a woman was diagnosed with vulvovaginal candidiasis, 30 (28%) were again diagnosed with vulvovaginal candidiasis at the next study visit. Cross-sectional analysis of

vulvovaginalcandidiasis:nWe did not find strong evidence for associations between sociodemographic characteristics and the diagnosis of *vulvovaginal candidiasis*. The prevalence of vulvovaginal candidiasis among women who first had sex before 15 years of age (Prevalence 4.4%, 95% CI 2.6, 6.3) appeared lower than for women who first had sex between 15-18 years (Prevalence 7.5%, 95% CI 6.1, 9.0) or over 19 years of age (Prevalence 8.2%, 95% CI 5.5, 11.0). There was a large difference in prevalence of *vulvovaginalcandidias* is among those with bacterial vaginosis diagnosed by clinical (Amsel) criteria 21 (Prevalence 12.0%, 95% CI 8.2, 15.8), compared to the 6.5% prevalence of *vulvovaginal candidiasis* among women who were not clinically diagnosed with bacterial vaginosis (95% CI5.3, 7.6). We did not find evidence of differences in the prevalence of *vulvovaginal candidiasis* by other laboratory diagnoses or behavioral characteristics (Teble 6, Fig 6).

Table 5: Prevalence of observed clinical signs, reported symptoms, and diagnosis (positive predictive value) of vulvovaginal candidiasis, Tehran, Iran 2013-2014

Vaginal sign observed or symptom reported	Prevalence (n=2528 clinical visits)	% prevalence (95% CI)**	Diagnosed with vulvovaginal candidiasis	% diagnosed with vulvovaginal candidiasis (95% CI)**
Pruritis reported	835	27	158	12.8
Discharge reported	823	28.7	115	10.8
Erythema observed	526	35.9	56	26.8
Discharge observed	258	25.7	64	147
None	887	32.7	0	0
Any one	956	37.4	0	0
Any two	325	18.6	85	13.8
Any three	128	9.7	75	41.9
All four	56	6	62	58

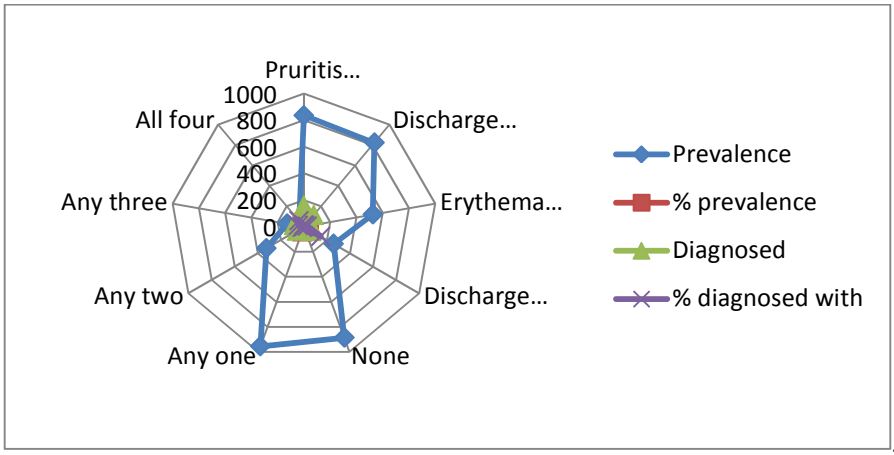

Fig 6.Prevalence of observed clinical signs, reported symptoms, and diagnosis (positive predictive value) of vulvovaginal candidiasis, Tehran, Iran 2013-2014

DISCUSSION

Among this cohort of reproductive-age women in India, we found evidence that a presumptive diagnosis of vulvovaginal candidiasis based only on presence of signs or symptoms, in absence of laboratory confirmation, would be mostly incorrect. Consistent with previous research, we could not identify behavioral risk factors for vulvovaginal candidiasis [21], which provides impetus for additional investigation into intrinsic factors such as the composition of vaginal flora, the presence or absence of genetic factors, and the features of the host and local immune response. Vaginal discharge, itching and erythema, while quite common, were insufficient to diagnose *vulvovaginal candidiasis* in the absence of laboratory confirmation. Had syndromic diagnosis been used to diagnose vulvovaginal candidiasis in this cohort, the positive predictive values would have been very low (15-41%). Our results are consistent with other studies detailing the overtreatment that results from the use of syndromic diagnosis based on vaginal discharge to diagnose vaginal conditions [22]. Previous findings also demonstrate that a minority of women with vaginal discharge have vulvovaginal candidiasis [17], Thus, the diagnosis of *vulvovaginal candidiasis* based solely on signs or symptoms leads to over-estimation of the prevalence of *vulvovaginal candidiasis* its over-treatment, while leaving the actual cause of the vaginal symptoms untreated. This finding of misdiagnosis based on symptoms is also relevant for women who self-diagnose *vulvovaginal candidiasis.*

The prevalence of laboratory-confirmed vulvovaginal candidiasis we observed is consistent with the results of two other community-based studies in Iran [23]. Given that reproductive tract conditions account for nearly half of the days lost due to illness among women in this region of Iran [24], it is critical to understand the incidence and prevalence of individual conditions; to our knowledge, this is the first study from Iran to describe the incidence of and possible risk factors for *vulvovaginal candidiasis*.

The study visit-specific point prevalence of vulvovaginal candidiasis in this cohort ranged between 5 and 9%. Only 20% of those infected with Giulan were diagnosed as having *vulvovaginal candidiasis*, much lower than the 53% found in a community-based study in Tamil Tehran, Iran [25]. We were not able to determine whether the 28% of women with a diagnosis of *vulvovaginal candidiasis* on two consecutive visits were cases in which, despite treatment, *vulvovaginal candidiasis* had cleared and then recurred. More likely, the repeat diagnoses at consecutive visits represent instances in which the vulvovaginal candidiasis was caused by Candida species not susceptible to fluconazole treatment. Previous research in India has found a high proportion of women are infected by non – albicans Candida species [26], which are more resistant to treatment with azoles [27-28].

Of the sociodemographic and behavioral characteristics we examined, only age at initiation of sexual activity appeared to be associated with the prevalence of *vulvovaginal candidiasis*, such that those with later initiation of sexual activity had a higher prevalence of *vulvovaginal candidiasis*. As the number of years women had been with their sex partners was not associated with *vulvovaginal candidiasis*, these two sociodemographic results appear discrepant and warrant additional investigation. We found a positive association between having clinically diagnosed bacterial vaginosis and *vulvovaginal candidiasis*. As both diagnoses include vaginal discharge as a component of their respective diagnostic criteria, it is very likely there is misclassification between *vulvovaginal candidiasis* and clinically-defined bacterial vaginosis. For example, women infected with Candida may have discharge caused by bacterial vaginosis and could thus be misdiagnosed with *vulvovaginal candidiasis*[29]. Our findings emphasize the problems inherent in making diagnoses of vaginal conditions based on clinical examination alone [30]. We found some evidence that the prevalence of vulvovaginal candidiasis varied with the presence of *Lactobacillus morphotypes. The evidence for a relationship between the prevalence of vulvovaginal* candidiasis and the presence of Lactobacillus in the vagina is conflicting, including studies in which the H2O2-production status of Lactobacillus was considered [31]. Recently, a prospective cohort study of female sex workers in Kenya found the presence of Lactobacillus, regardless of H2O2-production status, was positively associated with prevalent *vulvovaginal candidiasis* (adjusted odds ratio [aOR] 2.3, 95% CI 0.8, 6.4), a relationship that was strengthened after restricting the analysis to women without a diagnosis of bacterial vaginosis (aOR 3.8, 95% CI 1.3, 10.8) [32]. he loss of vaginal Lactobacilli is the hypothesized mediator for the relationship between the receipt of antibiotics and the risk of vulvovaginal candidiasis [33]. The mediation hypothesis also underpins the long-standing interest in use of probiotic interventions to reduce the risk of developing vulvovagin al candidiasis [34]. the results here do not provide strong support for this hypothesis. Strengths of this study include a large effective sample size derived from the use of participants' repeated observations, which allows for measurements of prevalence and incidence. Additionally, other studies of vulvovaginal candidiasis in India used samples of symptomatic women recruited from clinics or used syndromic diagnosis, and as a result were not able to estimate the community-level prevalence of *vulvovaginal candidiasis*. Our study is one of the few to examine the prevalence of vulvovaginal candidiasis across a range of number of Lactobacillus morphotypes detected in the vagina ; a dose-response effect, if present,provides better evidence of a causal relationship. There are also important limitations of our study to consider. First, the women in this cohort were recruited by non-random sampling; unmeasured sampling bias can limit the generalizability of these results. Second, because of the cross-sectional nature of our analysis, we cannot make causal interpretations for the variations in the prevalence of *vulvovaginal candidiasis* observed here. Third, we did not speciate the Candida organisms detected. The associations between sociodemographic characteristics and potential risk factors, and the prevalence of vulvovaginal candidiasis may differ by the Candida species which infect women, which are known to vary considerably by geographical location [35]. Fourth, we could not verify whether participants were self-medicating between visits with antibiotics or antifungals, which would influence the incidence and prevalence measurements of vulvovaginal candidiasis. Finally, given the limited duration of the study, we could not identify a subset of women with re current *vulvovaginal candidiasis*-an important condition with epidemiologic features distinct from acute *vulvovaginal candidiasis*[35]. We found that syndromic diagnosis will result in substantial overdiagnosis and overtreatment of *vulvovaginal candidiasis*-negative women. For correct diagnosis of *vulvovaginal candidiasis*, aboratory confirmation of vaginal infection with Candida is necessary, as is a means of assessing whether the discharge has been caused by bacterial vaginosis. Absent accurate means of di agnosingvulvovaginalcandidiasis , women remain at risk for vulvovaginal candidiasis-associated negative birth outcomes and acquisition of sexually transmitted infections. Follow-up studies are needed of women infected with Candida yeast species to determi ne the risk factors for the yeast's overgrowth, as it appears that the examination of behavioral risk factors does not appear to be a fruitful avenue for further inquiry.

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