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Vulvovaginal Candidiasis in Sexually Active Population: Prevalence and Distribution of Different *Candida* species

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The purpose of this study was to determine the prevalence of vaginal candidiasis by *Candida* species and the distribution of its species by using different detection methods. A total number of 250 women aged between 15 to 45 years having the symptoms of vaginitis were investigated. Age, parity and socio-economic conditions matched 50 healthy married and unmarried women attending the OPD of the hospital were taken as control groups. Samples were checked for the presence of yeast and vaginal pH. Data obtained by gynecologic examination were also recorded. High vaginal swabs were collected from the selected women at the outpatient clinic of the department of Obstetrics and Gynaecology for the detection of vaginal Candidiasis by different conventional diagnostic techniques and a comparison was made among them. Out of 250 high vaginal swabs, *Candida* species were found 134 (53.6%) cases and from healthy control, it was 17 (20.1%) cases ($P < 0.05$). Within 134 *Candida* species, *Candida albicans* and non-*Candida albicans* were 93.3% and 6.7% respectively. Next to Gram staining, wet mount microscopy with 10% Potassium hydroxide was the most sensitive detection technique for vaginal candidiasis in where culture method was taken as control. In conclusion, the most prevalent *Candida* species isolated from vaginal candidiasis was *Candida albicans* and the most sensitive detection technique for vaginal candidiasis was culture method and the wet mount microscopy with 10% KOH.

Key words: Vulvovaginal candidiasis, Non-*Candida albicans*, *Candida albicans*.

Vulvovaginal candidiasis is a major public health problem in developing country like Bangladesh[1]. It is one of the most common and frequent infections of the female genital tract with a high incidence[2]. It is the most common cause in Europe and the second most common cause of vaginitis in the United States[3]. At least one episode of vulvovaginal candidiasis is reported in up to 75% of all sexually active women during their life time[4]. It is less common in postmenopausal women[5]. It is often associated with the use of broad spectrum antibiotics, low vaginal p^H, oral contraceptives, frequent sexual activity and diabetes mellitus. Without any specific predisposing factor, 15-20% women will experience repeated infections within 3 months of treatment[6]. The majority of these infections are reported to be caused by *Candida* species[7]. *Candida albicans* is the most frequent species and the rate of isolation is usually in 85 to 90% from the vaginal mycoses[8]. Its prevalence increases during the third trimester of pregnancy[9] and it facilitates infection with HIV and other Sex Transmitted Infections (STIs)[10]. Vulvovaginal candidiasis (VVC) is an extremely common

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infection in women of childbearing age of all strata of society.

Since it has now been excluded from the ranks of sexually transmitted diseases and is also not a notifiable disease[11], not much information regarding its incidence and epidemiology is available. An accurate diagnosis of vaginitis is contingent upon patient information, clinical findings and interpretation of vaginal specimen in laboratory analyses. However, patient symptoms and clinical findings alone are often unreliable for determining the aetiology of a woman's complaint[12,13,14]. In particular, vulvovaginal candidiasis is more commonly misdiagnosed than are vaginal trichomoniasis and bacterial vaginosis[12, 14]. Thus, inaccurate diagnosis of vulvovaginal candidiasis may delay the proper treatment.

In Bangladesh, we know very little about the prevalence and incidence of vaginal candidiasis. The diagnosis of vaginal candidiasis in Bangladesh is mainly based on clinical presentations. Laboratories and health centres at peripheral laboratories carry out only the microscopic diagnosis from the vaginal fluid. It was necessary to update the prevalence of associated microorganisms in order to review the laboratory support and adjust prevention and control guidelines.

Objectives

The goal of this study was to examine the prevalence of fungal infection and the patterns of yeast among the Bangladeshi women of reproductive age group with vaginitis from a single geographic area. The study emphasised to know the frequency of yeast in vulvovaginal secretions. Moreover, the conventional methods of yeast diagnosis from vaginal samples used in Bangladesh and yeast culture methods were also compared.

Research Approach

The overall approach of the study can be seen in Fig. 1. Details are discussed below.

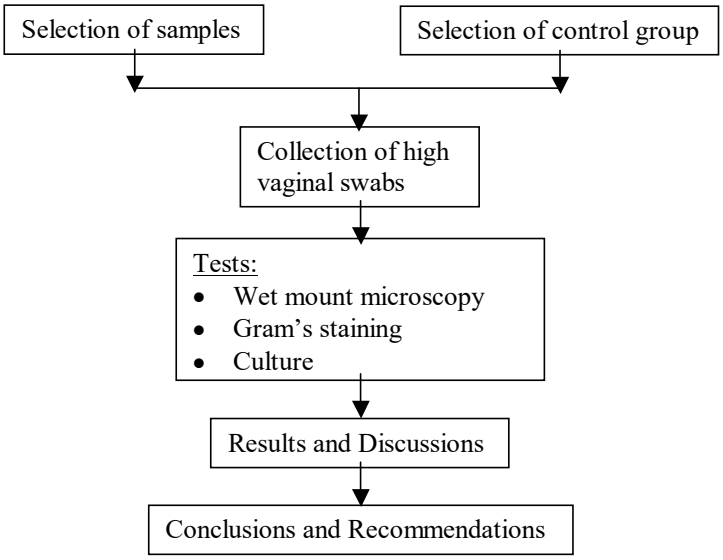

Fig. 1. Overall approach of the study

MATERIALS AND METHODS

A total of two hundred and fifty (250) women with abnormal vaginal discharge aged between 15 to 45 years attending at the outpatient department of Sir Salimullah Medical College (SSMC), Dhaka; Dhaka Medical College, Dhaka and Bangabandhu Sheik Mujib Medical University, Dhaka were included in this study. Fifty healthy women with no abnormal vaginal discharge and no symptoms of vaginitis of comparable age, parity and socioeconomic condition were studied as control group. Vaginitis is characterized by excessive foul smelling vaginal discharge with or without itching and burning. All women had regular menstrual cycle. Pregnant

women, menstruating women at the time of examination, on antimycotic agent or vaginal medication within the last one month were excluded from the study. Three high vaginal swabs from each woman were collected from the posterior fornix with the Cusco's vaginal speculum. The first swab was used for wet mount microscopy, the second swab was for Gram's staining and the third swab was for culture. All laboratory works were performed at the microbiology department of SSMC, Dhaka.

Laboratory methods

Wet mount preparation with and without KOH: A drop of normal saline was put on a clean glass slide and was mixed with one high vaginal swab. On another glass slide, a drop of 10% KOH solution was mixed with specimen and kept for 15 minutes. Then both slides were covered with a cover-slip. The slides were examined under microscope for the presence of yeast cell and hyphae.

Gram's Staining

Smears prepared by the specimen was fixed by flaming and was stained by Gram's Method[15]. The slide was examined under microscope at 40× for the detection of strongly gram positive budding yeast cell, hyphae and pseudohyphae.

Culture

The third swab was inoculated onto Sabouraud Dextrose Agar medium and incubated at 37^0 C for 24 hours and 48 hours. A cream colour pasty round smooth moist colony was produced by *Candida* species. The colonies have a distinctive yeast smell. A wet mount direct microscopy was done to see the oval, budding cells with some pseudomycelium which were *Candida* species.

Species identification

Then the species identification was based on germ tube test[15], sugar assimilation test[16], and sugar fermentation test[16]. The different microscopic tests with the culture chosen as gold standard were compared. This is at present considered as the most sensitive method[11].

RESULTS

It was mentioned earlier that a total of 250 women with abnormal vaginal discharge aged between 15-45 years were the subject of the study. Age, parity and socioeconomic matched 50 healthy women attending the hospital OPD for other purposes were taken as control groups.

Table 1. *Candida* species isolated from the patient and control group

Name of isolates	Patients group (n=250)		Control group (n=50)	
	Number	Percentage	Number	Percentage
Candida species	134	53.6	17	20.1
Culture–ve cases	116	46.4	33	79.9
Total	250	100	50	100

$P < 0.001$ (highly significant)

Table 2. The Rate of isolation of Different *Candida species* isolated from the patient and control group

Name of isolates	Patients group (n=134)		Control group (n=17)	
	Number	Percentage	Number	Percentage
*Candida albicans**	125	93.3	11	64.7
Candida glabrata	06	4.5	05	29.4
Candida tropicalis	02	1.5	01	5.9
Candida kruei	01	0.7	00	00
Total	134	100	17	100

*$P < 0.001$ (highly significant)

Frequency of candidiasis

Yeast belonging to the genus *Candida* was isolated in 134 (53.6%) out of 250 women in patient group, while no trace of yeast was found in 116 (46.4%) women who were either culture negative (or were cases of bacterial or trichomonas vaginosis). In the control group, the isolation rate of *Candida* species was 17 (20.1%) cases and rest of the 33 (79.9%) cases were culture negative. The rate of isolation of *Candida* species among the patient and control group of the study is shown in Table 1.

Candida albicans was the most frequently isolated species accounting for 125 (93.3%) cases in the patient group and therefore, non-*Candida albicans* species were 9 (6.7%) cases. The most prevalent among the non-*Candida albicans* was *Candida glabrata* which was isolated from 6 (4.5%) cases followed by *Candida tropicalis* 2 (1.5%) cases. *Candida krusei* was found in only one case (0.7%). In the healthy control group, the isolation rate of *Candida albicans* was 16 (94.1%) cases in out of 17 cases and only one (5.9%) *Candida glabrata* was detected. The rate of isolation of different *Candida* species isolated from the patient and control group is shown in Table 2.

DISCUSSIONS

Prevalence of different *Candida* species: In the present study, the isolation rate of *Candida* species from vaginal discharge was 53.6% of women and from the control group, it was 20.1% which is statistically significant ($P<0.05$). This finding is in agreement with Bartolomeo *et al.,*[18] and Abu-Elteen[19] who isolated *Candida* species in 53.5% and 51.5% respectively. The prevalence of vulvovaginal candidiasis is more or less similar with our findings in Sweden[20], Italy[21] and India[22]. In another study, Azzam *et al.,*[23] found a very high prevalence of *Candida* species (84.2%) in vaginal discharge in Venezuela which was contradict with our result because the study populations were those who were possessed the high risk factors. In a local study, Nahar *et al.,*[24] found a lower prevalence rate of *Candida* species from vaginal discharge which was 31.1%. The reason behind this was the case selection which was dissimilar in this study. In relation to the species, this research showed a predominance of *Candida albicans*, accounting for 93.3% of the total of the yeasts isolated from vaginal discharge and 20.1% from healthy control. This difference was statistically significant ($P<0.05$). The study findings were correlated with Otero *et al.,*[25] and Azzam *et al.,*[23] which were 89.3% and 87% respectively.

In Bangladesh, Nahar *et al.,*[24] found a 79.7% isolation rate. Highest isolation rate of *Candida albicans* from vaginal discharges similar to this study was also found by Jindal *et al.,*[26] in India, Phuong[27] in Vietnam, Abu-Elteen[19] in Jordan, Buscemi *et al.,*[28] in Argentina, Neto *et al.,*[29] in Nicaragua, and Consolaro *et al.,*[30] in Brazil. *Candida glabrata* was the second most frequently isolated yeast (2%), coinciding with the results of other authors like Otero *et al.,*[25] and Finn *et al.,*[31]. This species is represented in 11%, 15.6% and 10.42% of the cases in the studies of Jindal *et al.,*[26] in India, Buscemi *et al.,*[28] in Argentina and Azzam *et al.,*[23] in Venezuela respectively. Higher isolation rate was because the study women were suffering from recurrent vulvovaginal candidiasis in which the mycological shift was occurred from *C. albicans* to the non-*albicans Candida* species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*[32]. *Candida krusei* was found in only 1% cases which were similar with Buscemi *et al.,*[28] and Nahar *et al.,*[24]. *Candida stellatoidea* which was isolated by Nahar *et al.,*[24] in 3% cases, but now, "The Yeasts: A Taxonomic Study", written by Kreger-Van Rij[33], indicates that *Candida stellatoidea* is no longer a valid species and has been combined with *Candida albicans*[15].

CONCLUSIONS

The study shows that vaginal candidiasis is still in high prevalence among the sexually active women in Bangladesh and *Candida albicans* is the highest rate of isolated *Candida* species. The study suggests that for the diagnosis of vulvovaginal candidiasis, the wet mount microscopic examination with KOH test and at least Gram staining should be done; but culture should be done in those cases where vaginal discharges of symptomatic women are microscopically negative.

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