

## A HOSPITAL BASED OBSERVATIONAL STUDY OF VULVOVAGINAL CANDIDIASIS IN PREGNANCY AT A RURAL AREA IN WESTERN REGION OF RAJASTHAN AT NEWLY ESTABLISHED TERTIARY CARE CENTER

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### ABSTRACT

**Background:** Vulvovaginal candidiasis (VVC) is the most common vaginal infection affecting women of reproductive age. Culture is particularly important for diagnosing and treating complicated vulvovaginal candidiasis because patients are more likely to have nonalbicans infection.<sup>14</sup> The aim of this study to assess the prevalence of vulvovaginal candidiasis in pregnancy at a rural area in western region of Rajasthan at newly established tertiary care center.

**Materials & Methods:** This is a hospital-based prospective observational study was conducted at the Department of Microbiology at Government Medical College, Barmer, Rajasthan, India during one year period. Suspected cases of VVC, married and sexually active women (age between 16 to 45 years) were included in the study. The clinical and demographic data such as age, parity, oral contraceptive pills (OCPs) uses, antibiotic profile and diabetic history were recorded. **Results:** A total of 50 patients were included in the study. The majority (54%) of women were in the 18-30 years of age group. Overall, 94% of women were in moderate socioeconomic status. Approximately 82% of women had vaginal discharge, whereas 14% of women had recurrent discharge. The majority of women (82%) had a moderate hygiene status. The patients with vaginal discharge had a significantly higher proportion of Candida-positive isolates than negative isolates [100% vs 77.5%; P=0.001]. Patients with recurrent discharge had significantly higher Candida-positive isolates compared to negative isolates (40% vs 7.5%; P=0.0001). The patients who had OCPs had higher chances of development of Candida-positive isolates as compared to negative isolates [10% vs 2.5%; P=0.06]. Antibiotic use was associated with an increased risk of colonization by Candida species (40%). Candida-positive isolates were significantly higher in pregnant patients. **Conclusion:** The study revealed that VVC among pregnant women in this locality was not uncommon so that continuous ante-natal screening should be an on-going exercise for all pregnant women with history of itching and vaginal discomfort. This will prevent further complications and even transmission to partners.

## INTRODUCTION

Vulvovaginal candidiasis (VVC) is a Candida infection characterized by vaginal discharge, itching, and erythema. Nearly 70–75% of women experience VVC at least once in their lifetime.<sup>[1]</sup> Over 90% of infections are caused by *Candida albicans*, followed by non-albicans species (e.g., *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis*).<sup>[2]</sup> It is one of the major causes of gynecological consultation worldwide and

is associated with considerable direct and indirect economic costs.<sup>[3]</sup> Risk factors such as previous sexually transmitted infections (STIs), vaginal douching, pre-marital sexual intercourse, diabetes mellitus, pregnancy, and STIs in a sexual partner are responsible for VVC.<sup>[4]</sup> Complicated VVC occurs in nearly 10%-20% of women necessitating appropriate diagnosis.<sup>[5]</sup> In the majority of cases, clinical diagnosis is apparent.

Infection of the estrogenized vagina and the vestibulum that can spread to the outside of the labia

minora, the labia majora, and the intercrural region is defined as vulvovaginal candidosis.<sup>[6]</sup> After bacterial vaginosis, it is considered the 2<sup>nd</sup> most common among many causes of vaginitis.<sup>[7]</sup> It is produced most often by the overabundance of an opportunistic pathogenic yeast, *Candida albicans* (approximately 90%), which is a common member of the vaginal flora.<sup>[8,9]</sup> This is a dimorphic commensal yeast usually involved in the colonization of the skin and reproductive and gastrointestinal tracts.<sup>[7]</sup> Almost 20 to 30% of healthy asymptomatic women may have this yeast within their vaginal tracts at any moment in their lifetime, if tested by culture, but more than 60%, if tested by NAAT methods.<sup>[10]</sup> *Candida* spp. can cause an infection like VVC when the balance between the host and colonizing yeast gets temporarily disturbed. However, non-*albicans* *Candida* (NAC) species such as *glabrata*, *parapsilosis*, and *tropicalis* are also emerging as identifiable causes of VVC.<sup>[8]</sup>

On the basis of episodic frequency, candida vaginitis can be either sporadic or recurrent.<sup>[11]</sup> Uncomplicated or sporadic VVC includes mild to moderate clinical signs and symptoms such as a thick cottage cheese-like discharge, pain, vaginal and vulvar pruritus, erythema, burning, and/or edema, along with external dyspareunia and dysuria.<sup>[8]</sup> Complicated or recurrent VVC may be defined as that which has recurrent episodes (4 or more episodes in a 12-month period) associated with severe symptoms.<sup>[11]</sup>

Some evidence in recent days shows the association of candidiasis with an elevated risk of complications during pregnancy, like premature rupture of membranes and poor pregnancy outcomes including chorioamnionitis and preterm labor whereas congenital cutaneous infections are reported since decades as rare events during pregnancy.<sup>[12,13]</sup>

Vulvovaginal candidiasis can be diagnosed by visualization of yeast hyphae on potassium hydroxide preparation a woman with typical symptoms. It can also be diagnosed using antigen or DNA probe testing, with sensitivities of 77 to 97% and specificities of 77 to 99%, compared with culture as the diagnostic standard. Acidic vaginal pt. recurrent (four or more episodes in one year) or severe infections, or infections that occur in a patient who is Women with vulvovaginal candidiasis have a normal complicated vulvovaginal candidiasis is defined as immunocompromised such as someone with AIDS or poorly controlled diabetes mellitus. Culture is particularly important for diagnosing and treating complicated vulvovaginal candidiasis because patients are more likely to have nonalbicans infection.<sup>[14]</sup> The aim of this study to assess the prevalence of vulvovaginal candidiasis in pregnancy at a rural area in western region of Rajasthan at newly established tertiary care center.

## MATERIALS AND METHODS

This is a hospital-based prospective observational study was conducted at the Department of

Microbiology at Government Medical College, Barmer, Rajasthan, India during one year period. Suspected cases of VVC, married and sexually active women (age between 16 to 45 years) were included in the study. The unmarried and postmenopausal women were excluded from the study.

**Data collection:** Patient's detailed history and local examination were done. The clinical and demographic data such as age, parity, oral contraceptive pills (OCPs) uses, antibiotic profile and diabetic history were recorded.

**Sample collection and processing:** The first swab was cultured on blood agar, MacConkey agar, and Sabouraud's dextrose agar (SDA), and incubated at 37°C and 25°C for candida species isolation. The second swab was used for 10% potassium hydroxide (KOH) mount, wet mount, gram stain, and microscopic examination for budding yeast like cells with pseudohyphae and to distinguish from other sexually transmitted infections (STIs).

**Identifications of isolates:** The identification of isolates was made by gram staining and lacto phenol cotton blue staining.

**Identification procedure for yeast:** Germ tube formation test, sugar fermentation, sugar assimilation, and chromID agar tests were used for identification of *Candida* and its species.

**Germ tube formation test:** The three drops of fresh pooled human serum were dispensed into tubes using a Pasteur pipette. The yeast was suspended in the serum and the tube was incubated at 35°C. The suspension was examined using low power objectives, and the high power objective was used to confirm the presence or absence of germ tubes. This test was validated with the corn meal agar (CMA) test. CMA plate was prepared with 1% Tween 80 and divided into four quadrants. Four streaks of yeast colony were made on the agar plate. A flame sterilized and cooled cover glass was placed over the control part of the streak and incubated at 25°C. The morphological features were examined.

**Sugar fermentation:** The liquid fermentation media was prepared, and sterilized by autoclave at 120°C for 15 min. About 2% of sterilized sugar was added in to the sterilized tube. The inoculum preparation was done by suspending heavy inoculum of yeast grown on sugar free medium and incubated at 25°C for 1 week. The tubes were examined at every 48 to 72 h of interval for the production of acid and gas. The production of gas in the tube indicates fermentation positive.

**Sugar assimilation test:** A yeast suspension was prepared by combining a 24- 48 h old culture with 2ml of yeast nitrogen base (YNB). The suspension was added to 18mL of molted agar and poured into a petri plate. Kept the petri plate at room temperature and carbohydrate-impregnated discs were placed on the agar surface. The plates were then incubated at 37°C for 3-4 days. Presence of growth around each disc indicates yeast assimilation of sugar.

**chromID agar candida medium:** The yeast colony was streak inoculated on the surface of CHROM agar

plate in a fish-tailing manner. The plate was then incubated at 37°C in ambient air for 48 hours followed by observation of colony colour.

#### Antifungal testing methods

**Disk diffusion test:** This test for fluconazole and voriconazole was performed in accordance with the Clinical & Laboratory Standards Institute (CLSI) document MAA-A3. Agar plates (90 mm) containing MullerHilton agar were used. Inoculum was prepared by picking five distinct colonies from 24-h old growth on sabouraud dextrose agar in saline solution. The resulting suspension was vortex and turbidity adjusted to yield  $1 \times 10^6 - 5 \times 10^6$  cells/mL (0.5 McFarland standard). The agar surface was then inoculated with the suspension using a swab. Fluconazole and voriconazole discs were placed on the plates. The plates were incubated at 37°C for 18-24 h, and the zone diameter of growth reduction was measured. Pinpoint micro colonies and large colonies within the zone were ignored.

**Micro broth dilution method for yeast:** The antifungal susceptibility testing for all isolates was

performed in accordance with CLSI M27-A3. This test was performed by using sterile disposable multiwell micro dilution plates (96 U-shaped well). The stock solutions of voriconazole were prepared in distilled water whereas fluconazole, itraconazole, and amphotericin B were prepared in dimethylsulfoxide (DMSO) and stored at -70°C. RPMI 1640 medium was used as the broth medium. Inoculum was prepared from Candida species colonies grown on SDA. Vortex was suspended and the resulting suspension's turbidity was adjusted to yield  $1 \times 10^6 - 5 \times 10^6$  cells/mL (0.5 McFarland standard). Minimal inhibitory concentration (MIC) was determined at 48 h.

**Statistical Analysis:** Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 21.0. Descriptive statistics were used to describe categorical variables (frequency and percentages). Comparison of quantitative between the groups was done using chi-square test.

**Table 1: Demographic profile**

| PARAMETERS                  | Number of patients (N=50) |
|-----------------------------|---------------------------|
| <b>AGE GROUPS (YEARS)</b>   |                           |
| 18-30                       | 27 (54%)                  |
| 31-40                       | 19 (38%)                  |
| >40                         | 4 (8%)                    |
| <b>SOCIOECONOMIC STATUS</b> |                           |
| Low                         | 3 (6%)                    |
| Moderate                    | 47 (94%)                  |
| <b>HYGIENE STATUS</b>       |                           |
| Moderate                    | 41 (82%)                  |
| Poor                        | 9 (18%)                   |
| <b>VAGINAL DISCHARGE</b>    | 41 (82%)                  |
| <b>RECURRENT DISCHARGE</b>  | 7 (14%)                   |
| <b>CANDIDA ISOLATES</b>     | 10 (20%)                  |

**Table 2: Association of Candida status with demographic and associated risk factor**

| PARAMETERS                   | Candida                       |                               | P value |
|------------------------------|-------------------------------|-------------------------------|---------|
|                              | Positive isolates<br>[n = 10] | Negative isolates<br>[n = 40] |         |
| <b>AGE IN YEARS, (N=130)</b> |                               |                               | 0.0001  |
| 18-30                        | 6 (60%)                       | 21 (52.5%)                    |         |
| 31-40                        | 3 (30%)                       | 16 (40%)                      |         |
| >40                          | 1 (10%)                       | 3 (7.5%)                      |         |
| <b>SOCIOECONOMIC STATUS</b>  |                               |                               | -       |
| Middle                       | 8 (80%)                       | 39 (97.5%)                    |         |
| Poor                         | 2 (20%)                       | 1 (2.5%)                      |         |
| <b>HYGIENE STATUS</b>        |                               |                               | -       |
| Moderate                     | 4 (40%)                       | 37 (92.5%)                    |         |
| Poor                         | 6 (60%)                       | 3 (7.5%)                      |         |
| <b>VAGINAL DISCHARGE</b>     | 10 (100%)                     | 31 (77.5%)                    | 0.001   |
| <b>RECURRENT DISCHARGE</b>   | 4 (40%)                       | 3 (7.5%)                      | 0.0001  |
| <b>RISK FACTORS</b>          |                               |                               |         |
| Oral contraceptives pills    | 1 (10%)                       | 1 (2.5%)                      | 0.06    |
| Antibiotics                  | 4 (40%)                       | 3 (7.5%)                      | 0.0001  |
| Parity $\geq 3$              | 1 (10%)                       | 18 (45%)                      | 0.0001  |
| Diabetes                     | 2 (20%)                       | 1 (2.5%)                      | 0.0001  |

## RESULTS

A total of 50 patients were included in the study. The majority (54%) of women were in the 18-30 years of age group. Overall, 94% of women were in moderate

socioeconomic status. Approximately 82% of women had vaginal discharge, whereas 14% of women had recurrent discharge. The majority of women (82%) had a moderate hygiene status (Table 1).

The incidence of Candida-positive isolates was significantly higher in 18-30 years of age group (60%) of patients compared to the 31-40 and >40 years of age group (30% and 10%, respectively). The patients with vaginal discharge had a significantly higher proportion of Candida-positive isolates than negative isolates [100% vs 77.5%;  $P=0.001$ ]. Patients with recurrent discharge had significantly higher Candida-positive isolates compared to negative isolates (40% vs 7.5%;  $P=0.0001$ ) (Table 2).

The patients who had OCPs had higher chances of development of Candida-positive isolates as compared to negative isolates [10% vs 2.5%;  $P=0.06$ ]. Antibiotic use was associated with an increased risk of colonization by Candida species (40%). Candida-positive isolates were significantly higher in pregnant patients (Table 2).

## DISCUSSION

A high rate of VVC was reported in women in the age range of 20-29 years in many studies,<sup>[15,16]</sup> which was consistent with the findings of the present study (18-30 years), probably due to drug usage and or contraceptives. Among the previous studies that evaluated VVC prevalence, previous retrospective studies from Germany involved a large population ( $n=954,186$ ), out of which 50,279 of these women were diagnosed with VVC. The highest prevalence rates were found in the age groups of 18-25 years ( $n = 10,063$ ), 26-30 years ( $n = 7,631$ ), and 31-35 years ( $n = 7,384$ ) as compared to the older adult population (>60 years;  $n = 4,877$ ).<sup>[17]</sup> The present study findings align with previous research conducted by Samal et al., demonstrating a decreased occurrence of VVC in women over the age of 40. This is consistent with the results reported in their study.<sup>[18]</sup> Similarly, Sen's research also revealed that the age range of 21-30 years had the highest prevalence of VVC, which supports the present study observations. This suggests that women of reproductive age are more susceptible to developing VVC.<sup>[19]</sup>

The likelihood of developing VVC is higher in women who take oral contraceptives or undergo hormone replacement therapy after menopause than other women.<sup>[20]</sup> Using contraceptives elevates vaginal glycogen levels, creating a more favorable environment for Candida growth. Several studies indicate a higher incidence of Candida colonization and VVC among women who use OCPs.<sup>[21]</sup> Some studies suggest that estrogens directly influence Candida growth and its adherence to the vaginal epithelium, providing an environment for the increased incidence of VVC in women using OCPs.<sup>[22]</sup> In contrast to previous findings, the present study has shown a smaller number of (12.0%) patients experiencing VVC in patients taking OCPs. The predisposing factors observed in this study, such as the use of multiple antibiotics (44.0%) and pregnancy (48.0%), were consistent with those previously reported.<sup>[23]</sup> Interestingly, multiple studies

have established a connection between the trimester of pregnancy and the susceptibility of pregnant women to VVC infection. The vulnerability to infection among pregnant mothers increases as pregnancy progresses, resulting in the highest prevalence of VVC in the third trimester.<sup>[24]</sup> A previous prospective study evaluating the identification of Candida Species in women with VVC noted that out of 56 women with positive cultures, 20 were pregnant, six had diabetes, four had a history of antibiotic use, and 4 were using OCPs. Notably, there was a significant association between positive cultures and pregnancy, which may be attributed to elevated levels of reproductive hormones inducing a higher glycogen content in vaginal epithelial cells, creating a favorable environment for Candida growth.<sup>[25]</sup> However, Yadav and Prakash found no correlation between VVC and the gestational period.<sup>[26]</sup> Therefore, the role of the gestational period as a risk factor for VVC during pregnancy remains controversial.

Al-Rukeimi et al. (2020)<sup>[27]</sup> and Edrees et al. (2020)<sup>[28]</sup> had found the highest prevalence of VVC among pregnant women from low socio economic level (60.4%) and from rural area (65%) respectively. Women in their reproductive age experience at least one episode of candidiasis. The rate of Candida colonization has been found to increase during pregnancy particularly in the 3rd trimester. It has become a matter of concern due to the emerging evidences on the association of VVC with increased risk of pregnancy related complications such as low birthweight and premature delivery.

## CONCLUSION

The current research demonstrated that most of the patients with VVC belonged to the reproductive age category. The diagnosis of VVC was linked to the use of antibiotics as well as the presence of diabetes and pregnancy. The study revealed that VVC among pregnant women in this locality was not uncommon so that continuous ante-natal screening should be an on-going exercise for all pregnant women with history of itching and vaginal discomfort. This will prevent further complications and even transmission to partners.

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