

## PREVALENCE AND RISK FACTORS ASSOCIATED WITH VULVOVAGINAL CANDIDIASIS DURING PREGNANCY IN SANA'A, YEMEN

## ABSTRACT

**Background and objective:** Pregnant women are more susceptible to vaginal colonization and yeast infection. The role of *Candida* colonization in the occurrence of preterm birth is well established. Knowing local epidemiology and identifying risk factors for preterm birth are important for prevention and management strategies. The purpose of the study was to determine the prevalence of *Candida* species in vaginal swabs for pregnant women and determine associated risk factors of vulvovaginal candidiasis (VVC).

**Methods:** Pregnant women attending routine antenatal visits in Al-Olify –family Center in Sana'a city were enrolled into a cross-sectional study carried out from June 2018 to March 2019. The laboratory works were carried out in the National Center of Public Laboratories (NCPHL). Vaginal swabs samples were taken from participants after obtaining oral consent. The swabs were inoculated into Sabouraud's glucose agar supplemented with chloramphenicol and incubated at 37 °C for 24 to 48 hours under aerobic conditions in order to perform fungal culture. The identification of the *Candida* species was done by culture on HiCrome *Candida* Differential Agar at 35 °C for 48 h for production of species-specific colors. Data of demographic, clinical and risk factors were collected in a pre-designed questionnaire.

**Results:** A total of 190 pregnant women are included. The prevalence of VVC was 51.6%. *Candida albicans* accounted for 39.5% and non-*Candida albicans* accounted for 12.1% of isolates, mainly *C. glabrata* (4.7%), *C. lipolytica* (3.2%), and *C. famata* (2.1%). When VVC risk factors were considered, there were significant risk factors with the age group 20-24 years (61%, OR = 1.8), first trimester of pregnancy (61.1%, OR = 1.7), Multipara of parity (61.8%, OR = 1.9), low socioeconomic level (60.1%, OR = 2.4), and illiteracy (68%, OR = 2.2). When clinical symptoms were considered, only 86.2% of affected females had clinical signs of VVC.

**Conclusions:** There is a high rate of VVC among pregnant women in Yemen, and this highlights the need for health authorities to develop strategies for diagnosing VVC, including vaginal swabs for candidiasis as a routine procedure for all pregnant women. This study also revealed a steady increase in time with a non-*C. albicans* species prevalence rate. VVC syndrome management guidelines in Yemen should be revised to include a special protocol for pregnant women.

**Keywords:** *Candida*, Vulvovaginal candidiasis, VVC, Pregnancy, Epidemiology, Sana'a, Yemen

## INTRODUCTION

VVC is caused by overgrowth of yeasts, especially *Candida albicans*, which is an essential part of vaginal flora<sup>1</sup>. VVC symptoms include vaginal discharge, pain, itching and swelling of vulvar. In addition, vulgar erythematous and edema with obstruction are common results. Typical vaginal secretions in VVC are described as cottage cheese-like in character<sup>2</sup>. It is possible that 75.0% of women may experience VVC during their lifetime<sup>3</sup>. Epidemiological and microbiological studies indicate that intrauterine infection accompanies 25 to 40% of preterm births<sup>4</sup>. The role of *Candida* colonization was confirmed in the occurrence of preterm birth<sup>5</sup>, as the *Candida* were isolated from amniotic fluid in women with spontaneous preterm birth<sup>5,6</sup>. *Candida* has been found to increase metalloproteinase 9 production by chorioamniotic membranes<sup>6</sup>. Metalloproteinase 9 is a protein that remodels connective tissue and has an important role in the origin of premature labor and early rupture of membranes<sup>7</sup>. Also, the mother's vulvovaginal candidiasis is a major risk factor for neonatal *Candida* colonization<sup>8</sup>. There is evidence that eliminating *Candida* species during pregnancy may reduce the risk of spontaneous premature birth<sup>9</sup>.

In epidemiology, vulvovaginal candidiasis (VVC) is the second most common cause of vaginosis worldwide, after a bacterial infection. The risk of VVC for non-pregnant women is about 20%, but it increases by 30% during pregnancy<sup>10</sup>. Usually (90%) of infections due to *Candida albicans* are harmless and do not cause any symptoms. However, vaginal or vulvar infection may be associated with symptoms and signs such as severe itching, pruritis, pain, irritation, bad odor, dyspareunia, and dysuria and burning in urination<sup>10</sup>. In the Arabian Peninsula, the epidemic of VVC infection varies in different countries, even from regions within the same country<sup>11-13</sup>. Thus, local studies are important to obtain relevant epidemiological data and features of *Candida* sensitivity to antifungal drugs in order to help manage and treat patients with *Candida* infection.

Regarding the causative species of VVC, some researchers report an increased prevalence of non-*Candida albicans* species, especially *C. glabrata*, *C. krusei*, and *C. parapsilosis*<sup>14</sup>. Some researchers have also found that the incidence of *C. albicans* infection decreases and other *Candida* types including *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. kefyr*, *C. africana*, *C. dubliniensis*, *C. famata*, *C. guilliermondii* and *C.*

*lusitaniae* associated mainly with vulvovaginitis from patients with immunodeficiency or in pregnant women<sup>10</sup>.

In Yemen, vaginitis is one of the most common conditions for seeking medical care. In the city of Sana'a, vaginal infections have spread in Yemen among 37.6% of women of childbearing age, with VVC associated significantly with less than 25 years of age and use of intrauterine contraceptives<sup>15</sup>, also in Hadramaut three hundred and seventy two (39.2%) of the 950 pregnant women suffered from abnormal vaginal discharge and were positive for bacterial vaginosis<sup>16</sup>.

Pregnant women are more likely to have vaginal colonization and yeast infections. The role of *Candida* colonization in the occurrence of preterm birth is well established. Knowing local epidemiology and identifying risk factors for preterm birth are important for prevention and management strategies, so the purpose of the current study was to determine the prevalence of *Candida* species in the vaginal swabs of pregnant women and to identify the risk factors associated with VVC.

## **SUBJECTS AND LABORATORY METHODS**

Pregnant women attending routine antenatal visits in Al-Olify –family Center in Sana'a city were enrolled into a cross-sectional study carried out from June 2018 to March 2019. Inclusion criteria for subject selection were healthy individuals with no systemic disease. In addition, pregnant women who currently taking antifungal, steroids, antibiotics, or immunosuppressive drugs in the past 6 months were excluded. The sample included 190 pregnant mothers. All pregnant women was examined clinically by specialist and vaginal swabs were taken. The vaginal swabs were sent to the National Center of Public Laboratories (NCPHL) where the laboratory works were carried out. Vaginal swabs samples were taken from participants after obtaining oral consent. The swabs were inoculated into Sabouraud's glucose agar supplemented with chloramphenicol and incubated at 37 °C for 24 to 48 hours under aerobic conditions in order to perform fungal culture. The identification of the *Candida* species was done by culture on HiCrome *Candida* Differential Agar at 35 °C for 48 h for production of species-specific colors. Data of demographic, clinical and risk factors were collected in a pre-designed questionnaire.

## **DATA ANALYSIS**

The data was statistically analyzed using EPI-Info version 6. The difference in the distribution of *Candida* types among groups was based on a comparison of repeat distributions by chi-square test. The odds ratio associated with VVC risk factors was performed by 2x2 tables to obtain an *OR*, 95% *CI*, Chi squared and *p* value by uncorrected static tests where the value of *p* <0.05 was considered significant.

## **ETHICAL APPROVAL**

We obtained written consent in all cases. Approval was obtained from the participants prior to collection of samples. The study proposal was evaluated and approved by the Ethics Committee, Faculty of Medicine and Health Sciences, University of Sana'a.

## **RESULTS**

A total of 190 pregnant women are included. The prevalence of vulvovaginal candidiasis (VVC) was 51.6%. *C. albicans* accounted for 39.5% and non-*Candida albicans* accounted for 12.1% of isolates, mainly *C. glabrata* (4.7%), *C. lipolytica* (3.2%), and *C. famata* (2.1%). When VVC risk factors were considered, there were significant risk factors with the age group 20-24 years (61%, *OR* = 1.8), first trimester of pregnancy (61.1%, *OR* = 1.7), Multipara of parity (61.8%, *OR* = 1.9), low socioeconomic level (60.4%, *OR* = 2.4), and illiteracy (68%, *OR* = 2.2). When clinical symptoms were considered, only 86.2% of affected females had clinical signs of VVC. Subgroup prevalence of vulvovaginal candidiasis is presented in Table 2. Younger women, <20 years had a somewhat lower prevalence (33.3%) of vulvovaginal candidiasis, while in the 20-24 years and older group, the prevalence was between 41.3% and 71.4%. The adjusted odds ratio showed that vulvovaginitis was not significantly associated with older age (*p* = 0.12). The prevalence of vulvovaginal candidiasis varied with gestational trimester, parity, education and sociodemographic level. Women who are illiterate were more affected than those patients with primary school education and above. Similarly, vulvovaginal candidiasis was higher among low level of sociodemographic. Also vulvovaginal candidiasis was higher among multipara mothers (61.8%) compared to nulliparous (38.5%) or paucipares (54.4%) study subjects (Table 2). Table 4 shows the prevalence of *Candida* species according to presence of symptoms. The *Candida* was isolated in most pregnant women who had symptoms of *Candida* VV (86.2%), while only 22.3% was isolated in pregnant women who had no symptoms of *Candida* VV. There was a higher rate of isolated *albicans Candida* in women with symptoms (73.6%) compared to 10.7% in women without symptoms. On the other hand, the percentage of non-*Candida albicans* in symptomatic women (11.7%) is almost similar to 12.6% in asymptomatic women.

## **DISCUSSION**

Information on the prevalence of vulvovaginal candidiasis in Yemen is not known. Unfortunately, vulvovaginal candidiasis is not a reportable disease and diseases are routinely diagnosed by sign and symptoms without supporting laboratory diagnosis. As a result, the spectrum of yeasts involved in causing

the disease is unknown in Yemen. The prevalence of vulvovaginal candidiasis among pregnant women is also unknown in Yemen, but it is known worldwide that it is the second most common infection in the vaginal vulva area in women with symptoms as it represents about 17% to 42.%<sup>17-19</sup>.

Even though the prevalence of infection in the current study (51.6%) was slightly higher than the reported range,<sup>17-19</sup> it was higher than the prevalence rates reported by Ahmed and others in India among pregnant and non-pregnant women<sup>18</sup> and Olowe *et al.* in Nigeria among pregnant women<sup>19</sup>, however lower prevalence was reported by Erylander *et al.* among sexually active young women and association with orogenital sex<sup>20</sup>. Differences in rates can be explained by identifying differences in the sociodemographic characteristics and immunity status of patients<sup>21</sup>, treatment of patients with extensive antibiotics, immunosuppressive drugs<sup>22</sup> and hormonal effects<sup>23</sup> as some of the factors for differences in prevalence and / or recurrent vulvovaginal candidiasis between studies.

Age, level of education, pregnancy stages, parity and socio-demographic status were investigated as potential risk factors for vulvovaginitis in the present study. There was no strong evidence for the association between level of education, gestational trimester, and prevalence of vulvovaginal candidiasis. Among the sociodemographic characteristics, age appears to be an important factor in the occurrence of vulvovaginal candidiasis in general as the rate of vulvovaginal candidiasis was 61% with  $OR = 1.8$ ,  $95\% CI = 1-3.9$ ,  $P = 0.04$  in the 20- 24 years age group (Table 2). The current result was similar with a previous study by Sobel *et al.*<sup>24</sup>, they indicated that vulvovaginal candidiasis is rare in puberty (the first occurrence of menstruation), but its frequency increases with the end of the second decade of life (10-19 years) and reaches its peak in the third (20-29 years) and the fourth decade (30-39 years old).

Although in the current study, the *odds ratio* detected for the 20-24 age group was significant; the association of older age groups and vaginal candidiasis was not statistically significant as far as the adjusted odds ratio was considered (Table 2); this may point toward that the association was affected by other variables. The incidence was more in pregnant women who are illiterate (68%) than in patients with university education (39.7%). The improvement in personal hygiene and / or the economic situation resulting from education may explain the difference in the incidence rate among the illiterate and the people who got better education. Our results were consistent with those of Rathod *et al.* in India<sup>25</sup>, but in disagreement to the conclusion of Vadav and Prakash<sup>26</sup>. Among the six *Candida* species isolated in this study, the recovery rate for *C. albicans* was 39.5% , 9 (4.7%) for *C.globrata*, 2 (1.1%) for *C.dubliniensis*, 1 (0.52%) for *C.rugosa*, 6 (3.2%) for *C.lipolytica* and for *C.famata* 4 (2.1%) (Table 3). The results of the current study of *C. albicans* as the predominant species were reliable with similar previous studies<sup>24, 27</sup>. Although many studies on the prevalence of different types of *Candida* have led to the general concurrence that *C. albicans* are the most common isolated species in patients with vulvovaginal candidiasis, there has been an increasing trend to recover non-*albicans Candida* species. This is evidenced by the current study in which the isolation rate of non-*albicans Candida* species was 12.1%. By comparison, more recovery rates for non-*albicans Candida* species were reported at 41.4% in India<sup>28</sup> 31.7% in Belgium<sup>29</sup> and 19.8% in the United States<sup>27</sup>. Also more higher recovery rates of 53.1%, 65.0% and 57.5% of non-*albicans Candida* species have been reported in studies conducted in India<sup>18</sup>, Egypt<sup>30</sup> and Iran<sup>31</sup>, respectively.

Differences in the cure rate were observed between types of non-*albicans Candida* species between the current study and many previous studies. The recovery rate is 4.7% for *C.globrata*, 1.1% for *C. dubliniensis*, 0.52% for *C.rugosa* and 3.2% for *C.lipolytica* and *C.famata* 2.1% similar to what Trama *et al.*<sup>27</sup>. Studies of Sobel *et al.*<sup>24</sup>, Nergessie<sup>32</sup>, Sobel *et al.*<sup>33</sup>, they were revealed that *C. glabrata* was the predominant yeast among non-*albicans Candida* species. Bauters *et al.* research<sup>29</sup> showed that *C. glabrata* was the most common non-*albicans Candida* species (16.3%), followed by *C. parapsilosis* (8.9%), *C. humicola* (1.6%), *C. krusei* (0.8%), and *C. lusitaniae* (0.8 %). Hassanvand *et al.*<sup>34</sup> *C. albicans* proved to be the most common isolated species followed by *C. glabrata*, *C. tropicalis*, and *C. parapsilosis*. The significance of this result can be explained with caution that *C.globrata* may replace *C. albicans* under selective pressure of fluconazole, leading to inflammatory resistance to current fluconazole-based treatment in Yemen.

## CONCLUSIONS

There is a high rate of VVC among pregnant women in Yemen, and this highlights the need for health authorities to develop strategies to diagnose VVC, including vaginal swabs for candidiasis as a routine procedure for all pregnant women. This study also revealed a steady increase in time with a non-*C. albicans* species prevalence rate. The guidelines for managing VVC syndrome in Yemen should be revised to include a special protocol for pregnant women.

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## CONFLICT OF INTEREST

"No conflict of interest associated with this work".

## AUTHOR'S CONTRIBUTION

This research work is part of the National Center for Public Health Laboratory (NCPHL) project. The authors did clinical and laboratory work. The second author (SMMA) and the corresponding author (HAA) supervised the work of the lab, revised and edited the research.

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Table 1: The distribution of pregnant women according to their age

| Age groups   | Case n =190 pregnant women |      |
|--------------|----------------------------|------|
|              | NO.                        | %    |
| < 20 years   | 18                         | 9.5  |
| 20 -24 years | 72                         | 37.9 |
| 25-29 years  | 58                         | 30.5 |
| 30-34 years  | 28                         | 14.7 |
| ≥ 35         | 14                         | 7.4  |
| Mean age     | 25.4 years                 |      |
| S. D         | 4.9 years                  |      |
| Mode         | 25 years                   |      |
| Median       | 25 years                   |      |
| Max          | 39 years                   |      |
| Min          | 17 years                   |      |
| Total        | 95 %                       |      |

Table 2: The risk factors of VV Candidiasis among pregnant women in Sana'a city

| characters                  | Positive VVC<br>n=98 |      | OR   | CI        | X <sup>2</sup> | P       |
|-----------------------------|----------------------|------|------|-----------|----------------|---------|
|                             | No                   | %    |      |           |                |         |
| Age group                   |                      |      |      |           |                |         |
| < 20 years n=18             | 8                    | 33.3 | 0.7  | 0.27-1.9  | 0.4            | 0.52    |
| 20-24years n=72             | 44                   | 61   | 1.8  | 1.0-3.4   | 4.2            | 0.04    |
| 25-29 years n=58            | 24                   | 41.3 | 0.5  | 0.29-1.0  | 3.5            | 0.06    |
| 30-34 years n=28            | 12                   | 42.8 | 0.66 | 0.2-1.4   | 1.0            | 0.3     |
| > 35 years n=14             | 10                   | 71.4 | 2.5  | 0.7-8     | 2.3            | 0.12    |
| Gestational trimester n=190 |                      |      |      |           |                |         |
| First n= 54                 | 33                   | 61.1 | 1.7  | 0.9-3.2   | 2.7            | 0.09    |
| Second n=78                 | 36                   | 46.2 | 0.7  | 0.3-1.2   | 1.5            | 0.21    |
| Third n=58                  | 29                   | 50   | 0.91 | 0.4-1.6   | 0.08           | 0.77    |
| Parity                      |                      |      |      |           |                |         |
| Nulliparous (0 birth) n=65  | 25                   | 38.5 | 0.4  | 0.21-0.8  | 6.8            | 0.009   |
| Paucipares (1-2 birth) n=57 | 31                   | 54.4 | 1.2  | 0.6-2.1   | 0.25           | 0.6     |
| Multipara (>2 birth) n= 68  | 42                   | 61.8 | 1.9  | 1.03-3.5  | 4.4            | 0.03    |
| Sociodemographic            |                      |      |      |           |                |         |
| Low n= 111                  | 67                   | 60.4 | 2.4  | 1.3-4.2   | 8.2            | 0.004   |
| Intermediate n=79           | 31                   | 34.4 | 0.4  | 0.2-0.76  | 8.2            | 0.004   |
| Educational status          |                      |      |      |           |                |         |
| Illiterate n=25             | 17                   | 68   | 2.2  | 1-5.3     | 3.1            | 0.07    |
| Primary school n=32         | 19                   | 59.4 | 1.5  | 0.9-3.1   | 0.9            | 0.33    |
| Secondary school n=65       | 35                   | 53.8 | 1.1  | 0.6-8     | 0.2            | 0.65    |
| University n=68             | 27                   | 39.7 | 0.47 | 0.25-0.8  | 5.9            | 0.01    |
| VVC clinical signs          |                      |      |      |           |                |         |
| Yes n=87                    | 75                   | 86.2 | 21.7 | 10-46     | 77             | <0.0001 |
| No n= 103                   | 23                   | 22.3 | 0.04 | 0.02-0.09 | 77             | <0.0001 |

OR=Odds ratio, CI= confidence interval, X<sup>2</sup>=Chi square, p= p value.

Table 3: The frequency of different species of *Candida* isolated from pregnant women

| Micro-organism species           | Pregnant women |      |
|----------------------------------|----------------|------|
|                                  | No.            | %    |
| <i>Candida</i> spp               | 98             | 51.6 |
| <i>Candida albicans</i>          | 75             | 39.5 |
| Non- <i>candida albicans</i> spp | 23             | 12.1 |
| <i>C.globrata</i>                | 9              | 4.7  |
| <i>C.dublinsiensis</i>           | 2              | 1.1  |
| <i>C.rugosa</i>                  | 1              | 0.52 |
| <i>C.lipolytica</i>              | 6              | 3.2  |
| <i>C.famata</i>                  | 4              | 2.1  |

Table 4: The prevalence of *Candida* species according to presence of symptoms

| Micro-organism species       | Asymptomatic n=103 |      | Symptomatic n=87 |      |
|------------------------------|--------------------|------|------------------|------|
|                              | No.                | %    | No               | %    |
| <i>Candida</i> spp           | 23                 | 22.3 | 75               | 86.2 |
| <i>C.albicans</i>            | 11                 | 10.7 | 64               | 73.6 |
| Non- <i>candida albicans</i> | 12                 | 11.7 | 11               | 12.6 |
| <i>C.globrata</i>            | 6                  | 5.8  | 3                | 3.4  |
| <i>C.dublinsiensis</i>       | 2                  | 1.9  | 0                | 0    |
| <i>C.rugosa</i>              | 1                  | 0.97 | 0                | 0    |
| <i>C.lipolytica</i>          | 1                  | 0.97 | 5                | 5.7  |
| <i>C.famata</i>              | 2                  | 1.9  | 2                | 2.3  |
| Total                        | 23                 | 22.3 | 75               | 86.2 |