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Original Research

The Prevalence of Non-*albicans Candida* and *Candida* Mixed-species in Vulvovaginal Candidiasis in Northeast Iran

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Abstract

Background: Vulvovaginal candidiasis (VVC) is a prevalent infectious disease that affects the majority of women. While *Candida albicans* is the most common cause of VVC, the prevalence of non-*albicans* species is increasing, and mixed infections have made treatment more challenging. This study aimed to identify *Candida* species and detect mixed infections in women with VVC in a tropical region of northeastern Iran, employing the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. **Methods**: In the prospective study, a total of 270 specimens of vaginal discharge were collected using sterile swabs from patients suspected of having VVC. After extracting DNA from *Candida* colonies, the genome was amplified using PCR with specific primers. Species identification was conducted through PCR-RFLP analysis of the ribosomal DNA internal transcribed spacer (ITS) regions, using the MspI restriction enzyme. **Results**: Out of the 270 specimens suspected of VVC, 167 tested positive for *Candida* cultures. Among these positive *Candida* cultures, *Candida* species were identified in 150 specimens, with 44 (29.3%) showing evidence of mixed *Candida* infections. The species of *Candida* in 17 specimens were not identified. Out of the 190 identified *Candida* isolates included, the distribution was as follows: 85 (44.8%) *C. albicans*, 39 (20.5%) *C. glabrata*, 28 (14.7%) *C. guilliermondii*, 11 (5.8%) *C. kefyr*, 11 (5.8%) *C. parapsilosis*, 8 (4.2%) *C. tropicalis*, and 8 (4.2%) *C. krusei*. The mixed *Candida* species combinations observed were as follows: *C. albicans*/*C. guilliermondii* 26 (65%), *C. albicans*/*C. kefyr* 4 (10%), *C. parapsilosis*/*C. glabrata* 4 (10%), *C. parapsilosis*/*C. tropicalis* 4 (10%), *C. krusei*/*C. tropicalis* 2 (5%), *C. albicans*/*C. parapsilosis* 1 (2.5%), *C. albicans*/*C. krusei* 1 (2.5%), *C. glabrata*/*C. guilliermondii* 1 (2.5%), and *C. kefyr*/*C. tropicalis* 1 (2.5%). **Conclusions**: In women with VVC in the tropical region of northeastern Iran, the prevalence of clinical non-*albicans* species is higher than that of *C. albicans*. Furthermore, there is a notable high prevalence of clinical specimens containing mixed *Candida* infections.

Keywords: vulvovaginal candidiasis; non-*albicans Candida*; women; Iran

1. Introduction

Most women are likely to experience at least one episode of vulvovaginal candidiasis (VVC) in their lifetime, attributed to the overgrowth of *Candida* species [1–3]. Globally, *C. albicans* has a high prevalence of all cases of VVC, however, the incidence of cases caused by non-*albicans* species has significantly increased [4]. Although this disease does not directly lead to death, it can give rise to severe complications with significant psychological, physical, and financial implications. Despite therapeutic advances, VVC remains a global problem, and our understanding of its pathological mechanisms is still incomplete [5,6]. Various studies have indicated that *C. albicans* is the primary causative agent of VVC, followed by *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*, respectively [4,7]. The virulence, epidemiology, and susceptibility to antifungal agents vary among *Candida* species. Therefore, it is crucial to accurately identify the species. Contemporary methods, including sequencing, restriction fragment length polymorphism (RFLP) analysis, and semi-nested polymerase chain reaction (PCR), are commonly employed for the accurate identification of *Candida* species [8,9]. Recent studies have shown that antifungal resistance can emerge through either intrinsic or acquired mechanisms, with prolonged exposure to antifungal agents serving as a contributing factor to acquired resistance [10,11]. Consequently, it is essential to understand the antifungal susceptibility profiles of *Candida* species to make the informed decisions regarding the appropriate treatment for VVC. Furthermore, there is limited epidemiological data on the distribution of *Candida* species, and there has been some indica-

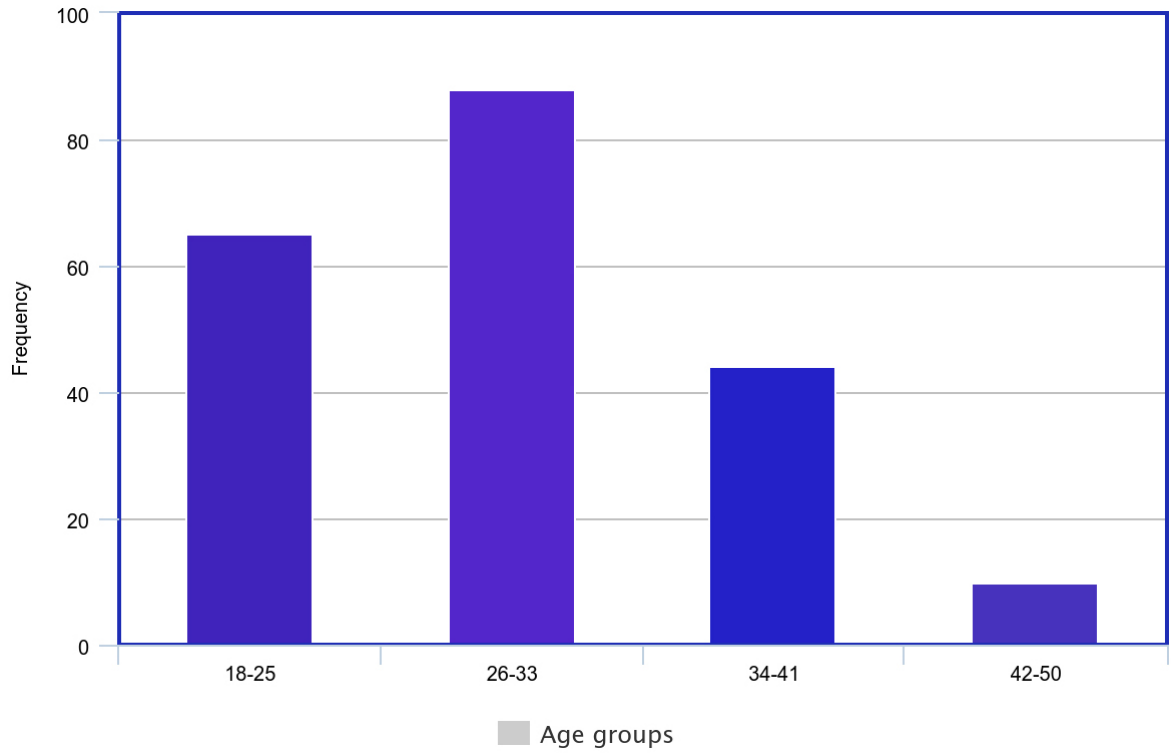

Fig. 1. Frequency of vulvovaginal candidiasis (VVC) among patients in Neishabour City according to age groups.

tion of geographic variations in the dispersion of *Candida* species. Additionally, there is limited information available regarding the epidemiology of VVC in Iran [4,5,12]. The present study aimed to investigate the identification of *Candida* species and mixed infections among women with VVC in a tropical region of northeastern Iran, employing the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for species identification.

2. Materials and Methods

This prospective study was conducted over the course of one year (2018–2019), using 167 vaginal specimens obtained from 22 Bahman Hospital in Neishabour city, northeastern Iran. The specimens were collected by a gynecologist using two sterile swabs from the posterior vaginal fornix to obtained vaginal secretions, and then transferred to the medical mycology laboratory for further analysis. One swab was used for direct microscopic examination with 15% potassium hydroxide (KOH), while the other was designated for culture. The specimens were cultured on Sabouraud dextrose agar (SDA) (1.05438.0500, Merck, Darmstad, Germany) supplemented with chloramphenicol. The genomic DNA of *Candida* colonies was extracted using a boiling lysis procedure, as previously described [7]. The common primers for the internal transcribed spacer (ITS) of fungi were used to molecularly identify *Candida* species [13,14]. These primers consisted of ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [13]. The reaction solutions consisted of 12.5 µL of premix (A180301, Amplicon, Copenhagen, Denmark), 10.5 µL of distilled water, 0.5 µL of each primer (10 pM), 1 µL of DNA template, and negative control. The amplification protocol consisted of an initial denaturation step at 96 °C for 7 minutes, followed by 35 cycles of 95 °C for 40 seconds, 52 °C for 40 seconds, and 72 °C for 40 seconds. The final extension step was carried out at 72 °C for 7 minutes. The PCR products were treated with the MspI restriction enzyme (Fermentas, ER0541, Waltham, MA, USA) in a 32 µL reaction mixture. The reaction mixture comprised 10 µL of amplified ITS product, 2 µL of MspI, 2 µL of 10× Buffer Tango, and 18 µL of distilled water. The digestion was incubated for 16 hours at 37 °C. Subsequently, the digested products were analyzed using electrophoresis on a 2.5% agarose gel. The isolates were identified by comparing the resulting band patterns with those reported in the study by Mirhendi *et al.* [15] for various *Candida* species.

3. Results

Among the 270 specimens suspected of VVC, 167 tested positive for *Candida* cultures. According to the analysis of relationship between age and the presence of infection, the youngest patient identified was 18 years old, while the oldest was 50 years old. Most participants fell within the age range of 26–33, while the fewest participants were in the 42–50 age group (Fig. 1). Among the affected patients, 131 (78.4%) had a previous history of vaginal infection, 13

Table 1. Prevalence of *Candida* species and its recurrence in different age groups in Neishabur city, northeastern Iran.

Candida species	Age (years)					Recurrent *Candida* (%)
	18–25	26–33	34–41	42–50	Total (%)	
C. albicans	16	22	18	1	57 (31.7)	41 (32.2)
C. glabrata	10	14	8	2	34 (20.4)	26 (19.7)
C. albicans/C. guilliermondii	8	12	4	2	26 (15.60)	22 (16.7)
C. kefyr	1	3	2	0	6 (3.6)	4 (3)
C. krusei	1	2	1	1	5 (3)	4 (3)
C. tropicalis	2	3	0	0	5 (3)	3 (2.3)
C. parapsilosis	0	0	2	0	2 (1.2)	2 (1.4)
C. guilliermondii	1	0	0	0	1 (0.6)	-
C. albicans/C. kefyr	1	3	0	0	4 (2.4)	4 (3)
C. glabrata/C. parapsilosis	2	1	1	0	4 (2.4)	3 (2.3)
C. tropicalis/parapsilosis	1	1	2	0	4 (2.4)	3 (2.3)
C. krusei/C. tropicalis	2	0	0	0	2 (1.2)	1 (0.7)
C. albicans/C. krusei	0	1	0	0	1 (0.6)	-
C. albicans/C. parapsilosis	1	0	0	0	1 (0.6)	1 (0.7)
C. glabrata/C. guilliermondii	1	0	0	0	1 (0.6)	1 (0.7)
C. kefyr/C. tropicalis	0	0	1	0	1 (0.6)	1 (0.7)
Not identified	4	8	3	2	17 (10.1)	15 (11.3)
Total	51	70	38	8	167 (100)	131 (100)

(7.8%) had underlying diseases or immunosuppressive diseases, 11 (6.6%) were pregnant, and 7 (4.2%) were recipients of antifungal drugs. However, Pearson's chi-squared test was conducted, revealing no significant correlation between these factors and *Candida* species. The prevalence of *Candida* species and its recurrence in different age groups is mentioned in Table 1.

4. Discussion

VVC stands as the most prevalent disease attributed by the *Candida* genus among women, with approximately 75% of women experiencing this infection at least once in their lifetime [16,17]. Fast and accurate diagnosis of various *Candida* species plays a crucial role in effectively managing diseases caused by this microorganism [5,9]. Although *C. albicans* remains the most common causative agent of VVC, the prevalence of non-albicans species is on the rise, and mixed infections have contributed to treatment challenging. According to the study by Jahanshiri *et al*. [18], the molecular methos is reported as the most accurate method for the detection of *Candida*.

In studies conducted in various regions, *C. albicans* is recognized as the most prevalent cause of VVC [4,5,7]. The high prevalence of *C. albicans* can also be attributed to its affinity for vaginal mucosal cells. Furthermore, *C. albicans* is one of the *Candida* species known for its ability to form true hyphae [19,20]. In the present study, out of 270 women suspected of VVC in Neishabour City, 167 were definitively diagnosed with VVC, and the *Candida* species were identified using the PCR-RFLP technique. Among *Candida* species, *C. albicans* (44.8%) was the most prevalent species responsible for causing VVC in this region. Among non-*albicans* species, *C. glabrata* exhibited a prevalence of 20.5%. In the study conducted by Gharaei *et al*. [21] in Chabahar, *C. albicans* exhibited a frequency of 80.87%, *C. glabrata* 6%, and other species 13.1%, respectively. In the study by Moreira *et al*. [22], out of 160 cases, the most commonly isolated species were *C. albicans* (50.1%), followed by *C. parapsilosis* (13.7%), *C. glabrata* (12.5%), and *C. tropicalis* (6.2%), respectively. In the study by Ghajari *et al*. [23], conducted in Damavand, Iran, *C. albicans* (67.7%) was identified as the most prevalent species, followed by *C. glabrata* (25.8%) and *C. kefyr* (3.2%), respectively.

In a study by Fan *et al*. [24], only 0.2% of the 1070 patients with VVC had more than one *Candida* species as the causative agent of the disease. In the study by Richter *et al*. [25], 4.8% of the specimens were diagnosed with a mixed infection involving several types of yeast, which is lower than the results obtained in our study. Among the 167 positive samples in this study, 44 cases (26.34%) of VVC were caused by more than one *Candida* species [26]. The study revealed a high frequency of *C. albicans*/*C. glabrata* among clinical specimens, indicating the need for accurate identification of the *Candida* species involved in mixed infections to ensure successful treatments [26]. *Candida* mixed infections can occur due to the development of resistance to antifungal drugs as a result of the empirical use. On the other hand, mixed *Candida* infections could also arise due to secondary developing when primary *Candida* infections are not promptly treated. However, the current study revealed that *C. albicans*/*C. guilliermondii* had the highest prevalence among mixed *Candida* infections, accounting for 65%. In the current study, we investigated the relationship between yeast frequency and the age of the patient. At

the age of 42–50, the lowest infection rate was 8 cases, and no yeast was isolated in the group aged over 50. Between the ages of 26 and 33, there were 70 cases of infection, representing the highest frequency of vaginal candidiasis observed. In the study by Edrees *et al*. [27], 12 (8.95%) individuals in the 18–27 age group, 73 (54.48%) in the 28–37 age group, and 49 (36.57%) in the 38–47 age group were diagnosed with VVC. In the study by Cetin *et al*. [28], positive cases of VVC were observed in different age groups as follows: 37 (15.7%) individuals in the 21–25 age group, 57 (23.7%) individuals in the 26–30 age group, 36 (15.0%) individuals in the 31–35 age group, 42 (17.5%) individuals in the 36–40 age group, 34 (14.5%) individuals in the 41–45 age group, 18 (7.6%) individuals in the 46–50 age group, 10 (4.6%) individuals in the 51–55 age group, and 6 (2.6%) individuals in the 56 and older age group. This study and the present research both report similar findings regarding the highest VVC infection rate, which is observed in individuals aged between 25 and 35 years [28]. In the study by Tafazoli *et al*. [29], the average age of 68 patients with VVC was 35.55 $\pm$ 5.47 years. The participants' ages ranged from 15 to 49 years.

In the present study, 11 (6.6%) individuals were pregnant, while 156 (94.3%) were not. In the study by Krishnasamy *et al*. [30], 20 out of 56 women with VVC (35%) were pregnant. In contrast to the previous two studies, the study conducted by Al-akeel *et al*. [31] found that the percentage of pregnant patients (76.43%) was higher than that of the non-pregnant group (23.56%). This variation could be attributed to a larger number of individuals examined in the pregnant group (70.01%) compared to the non-pregnant group (29.98%).

Among the patients examined in this study, 13 (7.8%) had a previous history of diseases such as immunodeficiency and diabetes, among others, while 154 (92.2%) had no history of such diseases. In the study by Gandhi *et al*. [32], it was found that 13.93% of the participants had diabetes, and 5.73% of them had human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). The limitations of the present study include, firstly, that examining more specimens over a longer period could provide more detailed information on the distribution of various fungal species in this area. Secondly, although molecular methods are preferred in the study, employing other identification methods could improve the reproducibility of the data and enhance the quality of publication. Thirdly, determining the antifungal susceptibility results of the detected strains could serve as a geographical reference point to monitor the development and spread of resistance.

5. Conclusions

The prevalence of non-*albicans* species in clinical cases is higher than that of *C. albicans* among women with VVC in the tropical area of northeastern Iran. However, *C. albicans* was the most commonly identified species in patients with VVC, followed by *C. glabrata*. Furthermore, the prevalence of clinical specimens containing mixed *Candida* is notably high. The highest rate of VVC infection was found in the age range of 26 to 33 years, while the lowest rate was found among individuals aged 42 to 50 years.

Availability of Data and Materials

Datasets are available from the corresponding author upon reasonable request after permission from the local authorities.

Author Contributions

BJ performed the research. AP and AI participate in acquisition data, and wrote the manuscript. HZ designed the research study. MJN and AF participated in the data analyses. All authors contributed to editorial changes, and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects.

Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran (approval number: IR.MUMS.REC.1393.702).

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Conflict of Interest

The authors declare no conflict of interest.

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