Original Article

CLINICAL STUDY OF *CANDIDA* VAGINITIS IN AHVAZ, IRAN AND SUSCEPTIBILITY OF AGENTS TO TOPICAL ANTIFUNGAL

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ABSTRACT

***Objective*:** To evaluate etiological agents of vaginal candidiasis in Ahvaz, Iran. In addition, susceptibility isolates were also considered against topical anti fungal agents.

Methodology: Cotton swabs were used for sampling from vaginal lesions and inoculated on CHROMagar Candida. Cultured media were incubated at 37°C for 2-4 days aerobically. Colonies producing a green coloration were presumptively identified as *Candida. albicans*. *C. glabrata* produced pink colonies on CHROMagar Candida. One hundred vaginal isolates of *Candida* were sub-cultured on SDA and incubated at 37°C. A suspension of isolates containing 1×10^6 - 5×10^6 cfu/ml was used for susceptibility tests. Clotrimazole, miconazole and nystatin disks were used for determine of susceptibility.

Results: Prevalence of *Candida* among the 300 women enrolled was found to be 49%. Recurrent and acute vaginal candidiasis were 48.3% and 51.7% respectively. *C. albicans* was the most common species among the isolates followed by *C. glabrata*, *C. dubliniensis* and *Candida* species. Antifungal susceptibility testing in our study revealed that none of the *Candida* isolates tested were resistant to tested antifungal. However, isolates were susceptible to clotrimazole followed by miconazole and nystatin.

Conclusion: *Candida* vaginitis is more prevalent among women in Ahvaz and the most common agent is *C. albicans*. In addition our isolates were sensitive to clotrimazole followed by miconazole and nystatin.

KEY WORDS: Vulvovaginal Candidiasis, *Candida* vaginitis, *Candida albicans*, Clotrimazole, Miconazole, Nystatin.

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INTRODUCTION

Vaginal candidiasis (vulvovaginal candidiasis, *Candida* vaginitis) is a common fungal infection in women genital system in the world. Disease is usually associated with considerable morbidity, healthcare cost, discomfort and pain, days lost from work & sexual functioning; however it is seldom life threatening.[1] The symptoms of vulvovaginal candidiasis are eczematoid dermatitis lesions that sometimes show vesicular and grey-white pseudomembrane, vulval pruritus, burning, erythema and curd-like discharge. Several terms are used for vul-

vovaginal candidiasis based on clinical presentation. Recurrent vulvovaginal candidiasis is commonly defined as four or more cases in one year.[2] Disease most commonly occurs in women during their reproductive years. An episode of vulvovaginal candidiasis, which does not respond to conventional antifungal therapy after two weeks is named as chronic form of disease. Chronic or persistent vaginal candidiasis may be associated with HIV infection.

Several factors affect on recurrent vaginal candidiasis such as, stress, diabetes, pregnancy, long-term treatment with corticosteroids or antibiotics, oral contraceptives, use of intrauterine device, several sexual partners, and oral sex.[3-7] The uses of steroids (cortisones), contraceptive pills, anti-acid and oral antibiotic (tetracycline) medications are very important factors for the proliferation of *Candida*.[8] In addition, immune defects have also roles in vulvovaginal candidiasis.[9] Diagnosis of vaginal candidiasis is based on a combination of laboratory and clinical criteria. Several authors have shown that chromogenic agar (CHROMagar Candida ®) is suitable and easy for the identification of *Candida* species and mixed species infection for complicated vulvovaginal candidiasis.[10]

Several records shows that *C. albicans* is the most common cause of vaginal candidiasis, accounting for 75–90% of all cases.[11-14] The next species are *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. parapsilosis*.[11,12,15] *C. dubliniensis* is a new species that has been recently reported from vaginal disease in the world. The incidence of *C. dubliniensis* varies between different studies from 0.17 to 29.52%.[16,17]

The epidemiological data on the distribution of vaginal candidiasis and fungal species involved in disease are incomplete in Iran. As such, the aim of the present study was to evaluate etiological agents of vaginal candidiasis in Ahvaz, Iran. In addition, susceptibility isolates were also considered against topical anti fungal agents.

METHODOLOGY

Three hundred patients between the ages of 17–53 were enrolled in the present study. The patients presented with several symptoms, such as vulvovaginal pruritis, irritation, unpleasant odor, and burning at micturition. They had signs such as edema, erythema, fissures, and caseous discharge. Cotton-tipped swabs were used for sampling from vaginal lesions and inoculated on chromogenic agar (CHROMagar Candida®, Paris, France). Inoculated media were incubated at 37°C for 2-4 days aerobically. Identification was based on the criteria described by Pfaller *et al*. and company instruction.[18] Colonies producing a green coloration were presumptively identified as *C. albicans*. Germ tube test and production of chlamydoconidia also confirmed the isolates. *C. glabrata* produced pink colonies on CHROMagar Candida and microscopy on corn meal agar confirmed it.[12]

Differentiation C. dubliniensis from C. albicans: All the 141 germ tube and chlamydoconidia positive isolates were subcultured on Sabouraud's Dextrose Agar (SDA) plates (Merck, Germany) and incubated at 45°C for 48 h. Dark green colony on CHROMagar Candida, inability to grow at 45°C and producing abundant chlamydoconidia and pseudohyphae after 48 h are characterized *C. dubliniensis* from *C. albicans*.[16,19] The isolates were kept in sterile distilled water at room temperature for future study.

Susceptibility tests: A total of 100 vaginal isolates of *Candida* were sub-cultured on SDA and incubated at 37°C. A suspension of overnight cultures of *C. albicans* (94), *C. glabrata* (3) and *Candida* species (3) was prepared in sterile PBS. Turbidity was adjusted to 0.5 McFarland density standard resulting in an inoculum containing 1×10^6 to 5×10^6 cfu/ml. Paper disks containing clotrimazole at 50 µg/disk, miconazole at 10 µg/disk and nystatin 100 U/disk were obtained from Liofilchem Bacterriology Products (Italy). Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium.[20] The inoculated plates were dried for 15 minutes at room temperature in laminar hood. Then clotrimazole, miconazole, and nystatin disks were applied to the inoculated agar with a forceps. The plates were incubated at

Table-I: Basic characteristics of the patients (n = 300)

Age range	*No*	*%*
17-22	88	29.3
23-28	57	19
29-34	61	20.3
35-41	41	13.7
42-46	24	8
47-52	28	9.3
53	1	0.4
Total	300	100

37°C for 24 h, and then zone diameters were read manually.

RESULTS

Clinical results: A total of 300 women participated in the present study during May to November, 2008. Table-I shows the details of age range of patients. Most of the patients (29.3%) were between 17-21 years. Among the 300 women enrolled, 20% had one child, 22% two children, 21.7% three children, 9.7% four children, 4% five children, 4.7% six children, 0.6% seven children, 2.6% eight children, 1% nine children and 13.7% of them had no child. One hundred fifty two of the patients (50.6%) had a history of treatment by antifungal drugs. Prevalence of *Candida* in investigated community was found to be 49% (147 of 300). Recurrent and acute vaginal candidiasis were respectively 71 (48.3%) and 76 (51.7%). In addition, 78 cases (48.3%) have a history of treatment with antifungal drugs. In the present study, several species of *Candida* were isolated from patients.

Susceptibility results: Susceptibility testing to clotrimazole, miconazole, and nystatin was carried out on 100 representative isolates (*C. albicans*, 94 isolates, *C. glabrata* three isolates, and *Candida* spp., three isolates). Table-II shows the details of susceptibility of 94 isolates of *C. albicans* to antifungal. As shown zone diameter for 65.9% of isolates of *C. albicans* was between 25-32mm for clotrimazole, whereas these zone diameters for 45.7% and 51.1% of isolates was respectively between 17-20mm and 21-24mm for miconazole and nystatin. Totally minimum zone diameter for nystatin was 13mm compared to 17mm and 18mm for miconazole and clotrimazole. Zone diameters for *C. glabrata* and *Candida* species were shown in Table-III.

Table II: Susceptibility of 94 isolates of *C. albicans* to clotrimazole, miconazole, and nystatin

Zone diameters	*Nystatin*		*Miconazole*		*Clotrimazole*	
13-16mm	5	5.3%	0	0	0	0
17-20mm	33	35.1%	43	45.7%	4	4.2%
21-24mm	48	51.1%	37	39.4%	11	11.7%
25-28mm	8	8.5%	13	13.8%	30	31.9%
29-32mm	0	0	1	1.1%	32	34%
33-36mm	0	0	0	0	15	16%
37-40mm	0	0	0	0	2	2.1%
Min	13mm		17mm		18mm	
Max	28mm		28mm		37mm	

DISCUSSION

Although many of the vaginitis literatures are on the clearly identified conditions of vulvovaginal candidiasis, few studies have discussed disease in Iran especially in Khuzestan SW of Iran. Our study provides a detailed look at a group of women referred for vulvovaginal candidiasis symptoms. The prevalence of vulvovaginal candidiasis in Iran was 19.8% in Kerman,[14] 46% in Qazvin[7] and 40% in Arak.[17] Our result shows that the prevalence of disease is 49% in Ahvaz. Vaginal candidiasis is a common disorder in women during lifetimes. Approximately 75% of women will experience an episode of vulvovaginal candidiasis in their lifetimes. In addition, 5-10% of women of childbearing age suffer from recurrent vulvovaginal candidiasis, having four or more episodes per year.

Table-III: Susceptibility of 3 isolates of *C. glabrata* and 3 isolates of *Candida* species to clotrimazole, miconazole, and nystatin

Species	*Zone diameter (mm)*		
	Clotrimazole	*Miconazole*	*Nystatin*
C. glabrata	30	25	22
C. glabrata	26	29	25
C. glabrata	25	24	22
Mean	27	26	23
Candida sp.	23	27	22
Candida sp.	49	27	19
Candida sp.	27	18	21
Mean	30	24	20.7

Candida albicans was the most common species among the isolates (138, 93.9%) followed by *C. glabrata* (3, 2%), *C. dubliniensis* (3, 2%), and *Candida* species (3, 2%). Thus, the overall prevalence of non- *C. albicans* species was 6%. Only three isolates of 138 isolates that initially identified as *C. albicans* did not grow at 45°C and produce more chlamydoconidia on cornmeal agar after 1-2 days incubation at ambient temperature. *C. albicans* was reported as the most common etiologic agent of *Candida* vaginitis.[7,14,17] Several reports show Non-*albicans* species, especially *C. glabrata* are isolated in 5-10% of vulvovaginal candidiasis but cannot be identified from *C. albicans* on clinical criteria.[10] However, in Mohanty et al. report *C. glabrata* (50.4%) was main agent of vaginitis followed by *C. albicans* (35.1%).[21] *C. dubliniensis* is a new species that is usually recovered from vaginal and oral samples. Jamilian et al. have reported the highest frequency of *C. dubliniensis* in Iranian women (29.5%).[17] In the present study, only three isolates of *C. dubliniensis* were detected.

Antifungal susceptibility testing in our study revealed that none of the *Candida* isolates tested were resistant to clotrimazole, miconazole and nystatin. Nystatin is a polyene macrolide antibiotic that inhibits sterol synthesis in the cytoplasmic membrane. Many fungi are sensitive to drug, including *Candida* species. A major advantage of nystatin is the fact that it is not absorbed across intact skin.

CONCLUSIONS

In the present study the prevalence of vulvovaginal candidiasis in Ahvaz was 49% which is higher than we had expected. *C. albicans* was also the predominant *Candida* species isolated from this series of patients with vulvovaginal candidiasis. In addition our isolates were sensitive to clotrimazole followed by miconazole and nystatin.

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