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Prevalence of *Candida spp.* from in women with vulvovaginal infection in Maternity Teaching Hospital in Erbil, Iraq

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Abstract

Objectives This study aimed to determine the prevalence of vulvovaginal candidiasis and its association with some risk factors and the incidence of different species of *Candida* attending the Maternity Hospital in Erbil City.

Methods A cross-sectional study was carried out at Maternity Teaching Hospital, over a period from July 2019 to January 2020 on female patients who complained of itching and erythema, vaginal discharge, swelling. A questionnaire form was prepared for each patient, which includes: age, clinical presentation and history of pregnancy, diabetes, and history of using contraceptives and the type of contraceptives. High vaginal swabs were collected from patients and subjected to direct microscopy, cultured onto Sabouraud Dextrose Agar (SDA) and Blood agar. The results, determined by standard microbiological methods, and species identification was done by using an automated VITEK 2 compact system.

Results From the total of 246 high vaginal swabs collected at Maternity Hospital in Erbil city, the prevalence of vulvovaginal candidiasis was 54.66%. The highest number of positive cases was found to be in the age group of 30–34 years and the positive culture among pregnant women was 71 (63.4%). Also, the prevalence of vulvovaginal candidiasis was higher among diabetic women 44(67.7%), frequency of culture positive for *Candida* spp. in the antibiotic users group was 85 (64.4%) and also the positive culture for ***Candida*** spp was higher among the contraceptive pills 31(57.4%); however, for intrauterine contraceptive device (IUCD) user, the positive culture was 22 (66.7%).

Conclusion In this present study, vulvovaginal candidiasis is more in the young age group and more frequent in the pregnant ladies and the diabetic patients and those using antibiotic and contraceptives, either the pills or IUCD. *Candida albicans* had the highest percentage among other positive isolated from high vaginal swab and there was a significant relation between clinical presentation of the patient with the result of culture.

Keywords Candidiasis, vulvovaginal, anti-bacterial agents, diabetics, Vitek

Introduction

Candidiasis is a fungal infection caused by a yeast called *Candida*. Some species of *Candida* can cause infection in people. *Candida* species are involved in the main opportunistic yeast infection in the world, called candidiasis. Among the species of the genus, *Candida albicans* continues to be the most common.[1]

Currently, there are more than 150 known species of *Candida*. Among these species, *C. albicans* is still the most common pathogen in spite of its dwindling share. In humans, it generally colonizes some regions including skin, oropharynx, lower respiratory tract, gastrointestinal tract, and genitourinary system, and the isolation rates of species other than *C. albicans* vary according to the features (age, underlying diseases, hospitalization ward, etc) of the patient population.[2]

Opportunistic pathogens are those microbes that cause disease in people with impaired immunity but not in normal individuals. *Candida albicans* and *Staphylococcus epidermidis* can cause disease in one individual but live harmlessly in others, which means that the same microbe would be called an opportunist in one individual and a commensal in another. So from there, opportunistic pathogen means that microbes live harmlessly but when the environment becomes favorable, they cause disease.[3]

Vulvovaginal candidiasis (VVC) is an infection of the genital mucosa caused by different species of the genus *Candida*. It is estimated to be the second most common cause of vaginitis after bacterial vaginitis (BV). *Candida albicans* accounts for 85%–90% of VVC cases.[4]

VVC is defined as isolation of *Candida* species in culture from study participants with signs and symptoms of vaginal abnormalities. Candidiasis in the vagina is commonly called a "vaginal yeast infection." Other names for this infection are "vaginal candidiasis," "VVC," or "candidal vaginitis."[5]

The ability of *C. albicans* to infect such diverse host niches is supported by a wide range of virulence factors and fitness attributes. A number of attributes are considered virulence factors, including the morphological transition between yeast and hyphal forms, the expression of adhesions and invasions on the cell surface, the thigmotropism, the formation of biofilms, phenotypic switching and the secretion of hydrolytic enzymes.[6]

The risk factors of VVC are pregnancy, contraceptives, diabetes mellitus, use of antibiotics, age, and some behavioral factors.

Candidal infection symptoms can range from mild to moderate and include: itching and irritation in the vagina and vulva, burning sensation, especially during intercourse or while urinating, redness and swelling of the vulva, vaginal pain and soreness, vaginal rash with a thick, white, odor-free vaginal discharge with a cottage cheese appearance with watery vaginal discharge.[7]

The diagnosis of VVC requires pelvic examination and laboratory tests. The combination of erythema, edema of vulvar, and vaginal tissues, and thick white vaginal discharge suggests the diagnosis of VVC. Diagnosis of VVC is from

history-taking, physical examination, and some additional examinations. From history-taking, pruritus is the most typical symptoms of VVC. From the appearance of vaginal discharge usually minimal, serous-mucous inconsistency, homogeny, cottage cheese-like, with minimal odor. Culture and wet mount or saline preparation for microscopic examination of vaginal can support the diagnosis of VVC.[8]

The study aimed to determine the prevalence of VVC among women attending the Maternity Hospital and find the relationship between this VVC with some risk factors and to determine the *Candida* spp. distribution among infected women with VVC

Materials and methods

Study population

A cross-sectional study was conducted from July 2019 to February 2020 at the Maternity Hospital in Erbil city, in which 246 females include with signs and symptoms of VVC as described by obstetric gynecology specialist with the age range between 15 and 65 years.

Questionnaire form prepared for each patient, which included some information like name, age, history of pregnancy, history of diabetes, history of antibiotic, and contraceptive using either oral or intrauterine contraceptive device (IUCD) and using of vaginal cleanser with the sign and symptoms at presentation.

Sample collection

Patients with genital tract problems were clinically investigated by gynecologists on duty, with signs and symptoms of vaginal abnormalities were recorded.

Vaginal swabs were collected from study participants having different vaginal abnormalities such as vaginal discharge, itching, vulvar pruritus, irritation, pain during intercourse.

The patient will be in the lithotomy position and put a speculum lubricated by saline into the vagina, swabbed a bit of vaginal secretion by dry cotton, and put the cotton swab into a clean tube with little saline. Then, vaginal secretion scraped by another dry cotton and put the cotton swab into a clean, dry tube.

The vaginal swab was obtained from the patient by using the thin cotton swab attached to the cap of plastic tube to collect the sample under sterile conditions for light microscopic examination and the other for fungal culture. In order to identify *Candida* yeast cells, mycelium, and/or pseudomycelium and to rule out other diagnoses, such as bacterial vaginosis, aerobic vaginitis, and trichomoniasis.

After culture, the swab was placed in 1 ml of sterile saline and shaken until the saline turned cloudy. Then the drop of the suspension takes on to the sterile glass slide and coverslip and examine under the microscope.

Sterile cotton swabs were prepared, gently smeared over the in the vaginal region and the swabs were immediately transferred to petri dishes of blood agar containing 5% sheep blood, Macconkey and Sabouraud's Dextrose broth (pH,5.6) containing dextrose (4%), peptone (1%) in double-distilled water and incorporated with chloramphenicol (5.0 mg). The culture incubated at 37C° for 24-48 hours as described by Viviano et al.[9]

For yeast identification and determination of antifungal susceptibility: the quality control yeast and pure cultures of yeast isolates were suspended in 3 ml of sterile saline (aqueous 0.45–0.50% NaCl, pH 4.5–7.0) in a 12×75 mm clear plastic (polystyrene) test tube to achieve turbidity equivalent to that of a McFarland 0.50 standard (range, 1.80–2.20), as measured by the DensiChek (bioMe'rieux) turbidity meter. These suspensions were used for the inoculation of YST-21343 identification cards, while AST-YS01 cards were inoculated after yeast suspensions were further diluted following the instruction of the manufacture.

Statistical analysis

The data obtained in this study were analyzed by using Microsoft Windows 10 and Excel program and SPSS (Statistical Package for the Social Sciences) version 23.

The chi-squared test was applied using the SPSS software (version 23) to determine the relation between the risk factors on the occurrence of VVC. And p-value of <0.05 was considered as the significance level while the value <0.001 consider being highly significant (HS).

Results

A total of 246 high vaginal swabs were collected and reported in the present study and analyzed for patients attending Maternity Teaching Hospital with sign and symptoms of vulvovaginal infection which were itching (77.2%), redness (72.4%), suprapubic pain (63%), and vaginal discharge (63.4%).

Among them, 135 (54.9%) swabs were positive for *Candida* spp growth on culture, and 111 (44.94%) were negative for Candida growth as shown in Table 1 and Fig 1.

Culture and Vitek-2 positive patients' clinical details were analyzed. The highest number of positive cases were found to be in the age group of 30–34 years (**23.0**%) followed by age group of 26–29 years which was 29 (21.5%), while the lowest number of patients with candidiasis belonged to the age group of 15–19 years which was 4 (3%) as shown in Table 2.

As shown in Table 3, the positive culture was more among pregnant women 71 (63.4%) as compared to those detected among non-pregnant patients 64 (47.8%), and also the prevalence of VVC was higher among diabetic women 44 (67.7%) when compared to positive result among the non-diabetic women which was 91 (50.3%). Likewise in this study, 132 women previously had been taking antibiotics and 114 women did not. The frequency of culture positive for *Candida* spp. in the former group was 85 (64.4%), whereas in the later group, it was 50 (43.9). Regarding the using of contraceptive, the result of this study showed that the using of contraceptive either tablets or IUCD, the positive culture for candida spp was higher among the contraceptive pills 31(57.4%) in comparison to the non-users 104 (45.2%). For IUCD user, the positive culture was 22 (66.7%) with negative result among 11 (33.3%) while for those using cleanser, the

Table 1. **Compariosn between positive and negative culture result.**

Result of culture	Frequency	Percent
Positive	135	54.9
Negative	111	45.1
Total	246	100.0

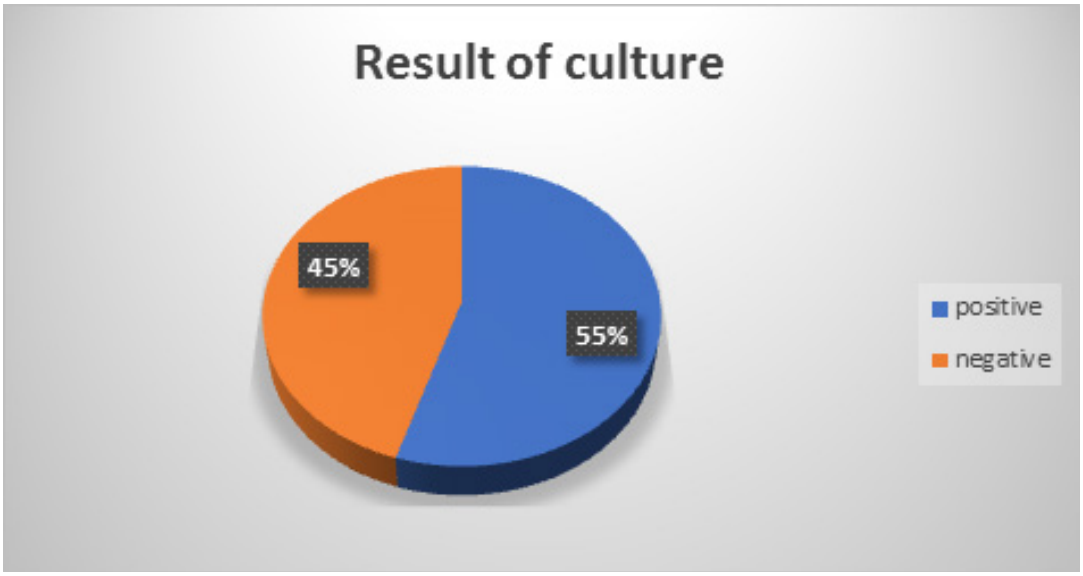

Fig 1. **The result of culture.**

Table 2. **Distribution of vulvovaginal candidiasis among different age groups.**

Age/years		Result of culture		Total
		positive	negative	
15-19	Count	4	5	9
	%	3.0%	4.5%	3.7%
20-24	Count	19	23	42
	%	14.1%	20.7%	17.1%
25-29	Count	29	27	56
	%	21.5%	24.3%	22.8%
30-34	Count	31	19	50
	%	23.0%	17.1%	20.3%
35-39	Count	16	13	29
	%	11.9%	11.7%	11.8%
40-44	Count	13	9	22
	%	9.6%	8.1%	8.9%
45-49	Count	11	9	20
	%	8.1%	8.1%	8.1%
>50	Count	12	6	18
	%	8.9%	5.4%	7.3%
Total	Count	135	111	246
	%	100.0%	100.0%	100.0%

positive culture was 51 (53.1%) which was less than negative result 84(56%).

The isolated *Candida* was processed for species identification by using Vitek system 2. The various *Candida* species isolated in the current study were shown in Table 4 and Fig 2 as follows: the highest percentage of isolated belonged to *Candida albicans* which was 117 (86.6%) followed by *Candida glabrata* which was 12 (8.8%), *Candida parapsilosis* 4 (2.9%), and the lowest percentage belonged to *Candida kruis* 2 (1.7%).

In Table 5, the result of culture showed a strong correlation with symptoms of presentation of the patients, especially the itching and swelling.

Discussion

Vaginitis is a general problem affecting millions of women around the world. VVC can be defined as signs and symptoms of inflammation of the vulva and vagina with the presence of *Candida* spp.[10]

The prevalence of VVC is difficult to determine because the clinical diagnosis is often based on symptoms and not confirmed by microscopic examination or culture.[11]

In the present study, out of 246 symptomatic patients, candidiasis was detected in 54.9% (135/246) cases on the culture of the high vaginal swab, and these data are similar to reports by Rad et al[12] which were (66%) and Kalia et al[13] (47%), and studies done by Ugwa[14] who reported (84.5%) in North-West Nigeria.

Our result is higher than reported by Krishnasamy et al,[15] which was done on 160 women, and 56 high vaginal swabs were 35% positive culture and developed *Candida* spp. and higher than reported by Kumari et al[10] which was (30.6%).

This study found the highest numbers of VVC were in 30–34 years age group (23%) followed by 25–29 years with (21.5%), while the lowest incidence was in the age group of 15–19 years (4%). This indicates that this disease is a common problem of active reproductive life and is almost similar to the study reported by Ugwa[14] and Yadav and Prakash[16]. Ugwa reported the highest prevalence in 26–35 years age group (53%), while Yadav et al, who found the highest numbers of VVC were in 21–25 years age group (40.44%) followed by 26–30 years with 32.58%.

The age group 25–35 years contains women who are younger and are sexually active have low vaginal defense mechanisms against *Candida* species.[17]

A young age group 26–35 years of women are mostly multiparous and use contraception, which also favors candidiasis. In the present study, women above 40 years of age group had the least infection (14%) and the progression in age, on the

Table 3. **Correlation of some risk factors with vulvovaginal candidiasis.**

Risk factors			Result of culture		Total	p-value
			Positive	negative		
Pregnancy	Pregnant	Count	71	41	112	0.007
		% within pregnancy	63.4	36.6	100.0	
	Non pregnant	Count	64	70	134	
		% within pregnancy	47.8	52.2	100.0	
History of diabetes	Yes	Count	44	21	65	0.004
		% within history of diabetes	67.7	32.3	100.0	
	No	Count	91	90	181	
		% within history of diabetes	50.3	49.7	100.0	
Antibiotic using	Yes	Count	85	47	132	0.653
		% within antibiotic using	64.4	35.6	100.0	
	No	Count	50	64	114	
		% within antibiotic using	43.9	56.1	100.0	
Contraceptive pills	Yes	Count	31	23	54	0.672
		% within contraceptive pills	57.4	42.6	100.0	
	No	Count	104	88	192	
		% within contraceptive pills	54.2	45.8	100.0	
IUCD	Yes	Count	22	11	33	0.144
		% within IUCD	66.7	33.3	100.	
	No	Count	113	100	213	
		% within IUCD	53.1%	46.9	100	
Cleaner user	Yes	Count	51	45	93	0.992
		% within cleaner user	53.1	46.9	100	
	No	Count	84	66	153	
		% within cleaner user	56	44	100	

Table 4. **Number and percentage of *Candida* species in the positive culture.**

***Candida* species**	**Number**	**Percentage**
Candida albican	117	86.6
Candida glabrata	12	8.8
Candida parapsilosis	4	2.9
Candida krusei	2	1.7

other hand, the aging decreases the effect of estrogen hormone in females, which could lead to lower infection rates as women advance in age. Most women aged over 40 years are less or not sexually active.[18]

VVC is an estrogen-dependent disorder: estrogens enhance both candida adherence to the vaginal epithelium and yeast mycelium formation.[19]

The prevalence of VVC increases with age up to menopause, and the disorder is uncommon in postmenopausal women unless they are taking estrogen therapy, and it is also uncommon in prepubertal girls.[11]

Among the 135 women with positive cultures, 112 were non-pregnant.

The incidence of *Candida* spp among pregnant and non-pregnant was 63.4% and 47.8%, respectively

This result is higher than the Rasti et al[20] who found that out of 12 pregnant women, 4 women (33.3%) showed positive results of *C. albicans* infection and also higher than Sutaria et al[21] who reported 61 (45.18%) pregnant patients were shown positive fungal infection of *Candida* species.

In our study, there was a strong association of positive cultures with pregnancy which could be attributed to high levels of reproductive hormones inducing higher glycogen content in vaginal epithelial cells favoring the growth of *Candida,* and some studies say that estrogens have a direct

Table 5. **Correlation between different *Candida* species with the symptoms.**

Complain		*Candida spp*			
		Candida albican	***Candida glabrata***	***Candida parapsilosis***	***Candida krusei***
Itching	Count	98	8	4	1
	Percentage	83.8	66.7	100	50
Vaginal discharge	Count	99	9	3	2
	Percentage	84.6	75	75	100
Suprapubic pain	Count	77	7	3	2
	Percentage	65.8	58.3	75	100
Redness	Count	70	8	2	2
	Percentage	59.8	66.7	50	100

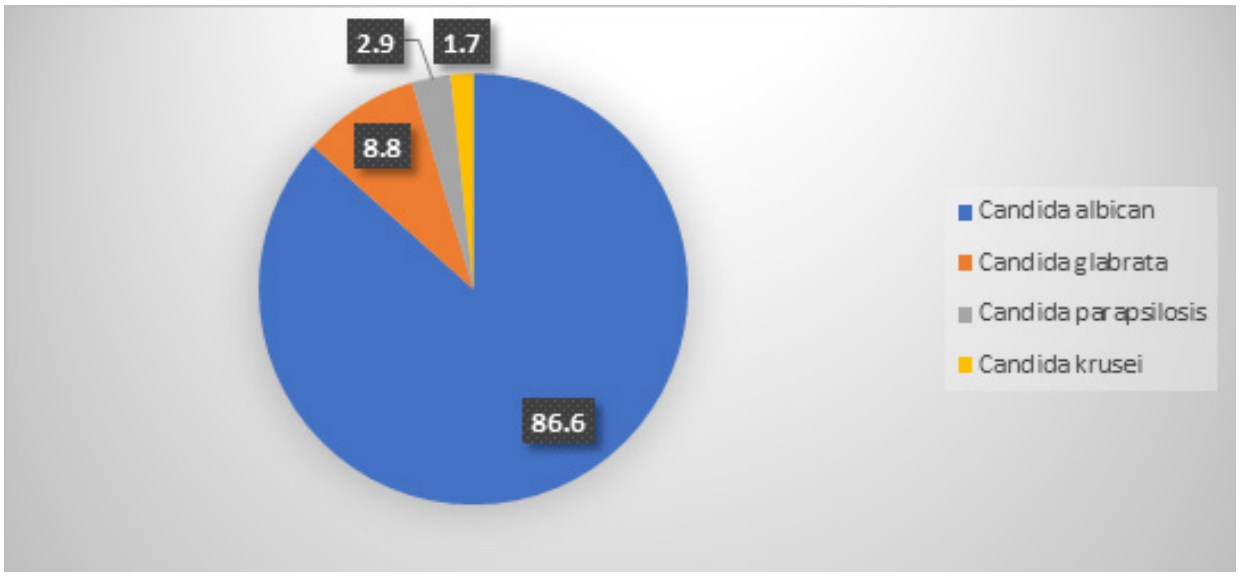

Fig 2. **Percentage-wise distribution of various Candida species causing vulvovaginal candidiasis**

effect on the growth of *Candida* and its adherence to the vaginal epithelium.[22]

Vaginal candidiasis in pregnant women is a common and frequently distressing infection in women of childbearing age; approximately. 75% of all women experience at least one episode of *Candida* infections during their lifetime.[23]

During pregnancy, which is listed as a risk factor, the vagina is more sensitive, and the infections occur significantly more often. The high incidence of vaginitis in pregnant women is related to levels of estrogens, which is, in turn, considered the primary factor for the observed VVC was more prevalent in pregnant women.[24]

In our study, from 65 patients with diabetes, the incidence of *Candida spp* was 44 (67.7%), and this is similar to a study done by Salvi[11] and Al-akee et al[25], which was 56.5% and 53.06%, respectively.

It has been reported that increased glucose levels in genital tissues enhance yeast adhesion and growth and that vaginal epithelial cells bind to *Candida* spp. cells with a greater propensity in diabetic women than in non-diabetic ones.[26]

The current study showed that among 132 antibiotic users, the incidence of positive culture was 85 (64.4%).

It is concluded that most commonly the growth-promoting effect of antibiotics is obtained by way of an eradication of the bacterial flora harbored in the vagina. The increased incidence of clinical candidiasis after the advent of broad-spectrum antibiotics has been commented upon by many authors. It has been suggested that antibiotics may suppress phagocytosis, or directly stimulate the growth of the yeast.[27]

Some studies have shown that VVC commonly follows antibiotic, antibiotics decreasing the protective normal bacterial flora in the vagina, i.e., lactobacillus, thus allowing overgrowth of yeast. It's been reported that 28–33% of women put on antibiotic therapy develop symptomatic genital candidiasis. A recent study among non-pregnant women receiving antibiotics for non-gynecological conditions and observed that short courses of oral antibiotics seem to increase the prevalence and incidence of symptomatic VVC.[28]

The current study showed that from a total of 54 women of oral contraceptive users, *Candida* spp was isolated in 31 (57.4%) cases.

A study done by Mishra et al[29] revealed that among the women using contraceptives, the highest prevalence of 69.4% was observed in oral pill users coinciding with our result and

Oriel et al[30] reported a higher rate of prevalence of vaginal candidiasis in oral contraceptives users than non-contraceptives users. This could be attributed to the presence of estrogen and progesterone hormones in the contraceptives that increased glycogen in the vagina, thus exposing it to the activities of lactobacilli. The lactobacilli are widely believed to play a role in the conversion of glycogen to lactic acid, thus decreasing the pH of the vagina.[31]

The decreased pH reduces the activities of the bacterial biota while it favors the growth of yeasts, including *Candida* species.[32]

Our study showed that from the total of 246 women, 33 IUCD users, 22 (66.7%) of the users shows positive *Candida* spp. This result is similar to Lubis et al[33] which found that out of the 36 specimens taken from the VVC patients using the IUD, 20 specimens were found to be yeast positive (56%).

The use of the IUD is a highly effective, cost-efficient method of preventing pregnancy, and it is one of the most popular methods of contraception in the world today.[34]

Chassot et al. who carried out *in vitro* tests have observed that yeast cells can adhere strongly to the parts of the IUD and form biofilms and the data in this study suggested that the presence of biofilm on the patients' IUDs served as a reservoir of yeasts and contributed to recurrent infection by *Candida albicans.*[35]

A study by Lubis et al, reported 24 specimens to yield positive yeast results out of 30 specimens taken from IUD users (80%), and this is higher than the result of our study (33).

Our result was concurrent to a study by Anindita[36] where she found that contraceptive device usage is a prominent risk factor for VVC, and *Candida* spp, may grow on vaginal areas under unsanitary and humid conditions.

Our research showed that using cleanser decreasing the incidence of VVC as shown in Table 3 that the incidence of VVC among the cleanser user was 51 (53.1%) compared to the non-user which was 84(56%).

At the same time, this study showed that frequency of cleaning the vulva exerted no effect on the occurrence of VVC, while cleaning the vulva before or after sexual life was an advantageous factor for preventing against VVC.[37]

Some studies demonstrated that particular attention should be paid to vaginal cleanser, as women carrying out vaginal cleanser were more likely to develop vaginal candidiasis than no.-users. The vaginal cleanser increased the case rate by 1–4% because the females lacked professional knowledge about cleanser and used the wrong methods for using it. On the other hand, cleanser caused vaginal dysbacteriosis, especially the *Lactobacillus,* which competed with *Candida* spp. for nutrients.[19] Also, the cleanser destroys epithelial cells; as a result, *Candida* spp. could easily penetrate and invade vaginal surface cells.[24]

In our study, the identification of *Candida* spp was done by the culture method and confirmed by Vitek 2 compact.

There were various Candida species detected in our study, with highest being *C. albicans* (86.6%), and *non-albicans Candida* (NAC)(13.4%) of cases.

The prevalence of non-albicans species in our study was as follows *Candida glabrata* which was 12 (8.8%), *Candida parapsilosis* 4 (2.9%) and the lowest percentage belonged to *Candida kruis* 2(1.7%)

Our study showed results similar to two studies by Krishnasamy and Salvi by which they found that the highest percentage of *Candida* spp isolated was *Candida albicans* and also regarding the NAC which was similar to our study: *Candida glabrata* 11%,*Candida parapsilosis*11%and *Candida kruis* 2%.[15,11]

A study done by Jayalakshmi et al. found that the predominant non-albicans species was *C. glabrata* and the other species *C. parapsilosis, C. krusei* have been reported less frequently in patients with vulvovaginitis which is similar to our study.[38]

Candida albicans was found to be the single most prevalent species in VVC. This finding of the present study was similar to the studies conducted in Nigeria, USA, Kenya, India, UAE, Iran; however, this is in contrast to a single study reported in India where *C. tropicalis* was found to be more prevalent than *C. albicans.*[13]

The result of culture showed a strong correlation with symptoms of presentation of the patients, especially the itching and vaginal discharge.

Vaginal discharge, itching, and erythema, while quite common, were insufficient to diagnose VVC in the absence of laboratory confirmation. The authors found a positive association between having clinically diagnosed and laboratory diagnosed VVC. The positive predictive value was high 64.7%. In a study done by Kanagal et al[39] reported the highest number of VVC was observed in those of the respondents who had symptoms of vaginal candidiasis and a similar study was conducted by Kalia et al reported 82% of candida positive women were symptomatic, and the remaining 18% were asymptomatic.[13]

Conclusions

The incidence of Candida species in high vaginal swab was 54.9%; however, the higher incidence Candida species were in the age group of 30–34 years while the lowest percentage was in the age group of 15–19, which was 3%. There is an association between the risk factors and positive Candida species culture: pregnancy, diabetes, previous history of taking antibiotics, using of contraceptives either oral or IUCD and history of using the vaginal cleanser. *Candida albican* is the most prevalent species 86.6% among other *Candida* species and the culture and Vitek-2 is reliable method to isolate and diagnosis the species of Candida.

Ethics approval

The study was conducted after the Ethical form reviewed and approved by the ethic form Review committee of the College of Health Sciences and verbal acceptance were also obtained from participants.

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