Relationship between Vulvovaginitis in Women with the *Candida* Species

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Abstract:

Background: Vulvovaginal candidiasis refers to the vaginal infection that is caused most commonly by candida species, especially *candida albicans,* women in their lifetime at risk to infect by *candida species.*

The objectives of this study were to determine the relationship of Vulvovaginal infection with Candidiasis and its association with some risk factors and the incidence of different species of *Candida* among patients with vulvovaginitis.

Methods: 300 samples from high vaginal swabs obtained from females attending maternity teaching hospital in Erbil City complained from signs and symptoms of valvovaginitis from the period September 2019 to February 2020. A questionnaire form prepared for each patient, who includes: age, clinical presentation and history of pregnancy, diabetes, and history of using contraceptives and the type of contraceptives. High vaginal swabs collected from patients and direct microscopic examination performed, cultured onto Sabouraud Dextrose Agar (SDA), Blood agar and Macconkey agar and species identification done by using an automated VITEK 2 compact system.

Results: From the total of 300 high vaginal swabs collected at Maternity hospital in Erbil city and prevalence of vulvovaginal candidiasis was (57%). The highest prevalence of positive cases were in the age range (30-34) years and the positive culture among pregnant women was 73(63.4%) and also the incidence of vulvovaginal candidiasis was higher among diabetic females 49(80.3%), The incidence of positive cultures of Candida spp. in the antibiotic users group were 61(64.4%) and also the positive culture for *candida spp* was higher among the contraceptive pills 40(60.6%) however for IUCD user the positive culture was 31(68.9%). The species of *Candida* species which identified by using Vitek system were distribute as follow: *Candida albicans* was 147(86%) *Candida glabrata*16 (9.4%)*,CandidaParapsilosis* 6(3.5%)and, *Candida krusei* 2(1.2%).

Conclusion: In the 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 Intrauterine contraceptive device. *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, *Candida*spp,Vulvovaginitis.

Introduction:

Candidiasis is a fungal infection caused by a yeast called *Candida.* Some species of *Candida* can cause infection in people. *Candida species* are associated with the fundamental opportunistic yeast infection in the world, called candidiasis, among the types of the class, *Candida albicans* keeps on being the most widely recognized [(1)].

Currently*, there* are in excess of 150 known types of Candida, among these species, *C. albicans* is as yet the most

widely recognized pathogen regardless of its lessening share. There are more than 150 known species of *Candida*, among these species, *C. albicans* is still the most common pathogen inspiteofitsdwindling share. In human being, it colonizes a few areas including skin, oropharynx, lower respiratory tract, gastrointestinal tract, and genitourinary system, and the identification of species other than *C. albicans* regarding to features (age, some ailments, hospitalization ward, and so on) of the patient populace [2].

Opportunistic pathogens are these agents that cause infection in individuals with debilitated immunity but not in normal peoples.

Candidaalbicans and *Staphylococcusepi dermidis* –can cause infection in one individual but not cause infection in others, which implies that the same microorganism would be called an opportunist in one people 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 genitalmucosa caused by various species of the genus *Candida*. It is estimated to be this cond most common cause of vaginitis after Bacterial Vaginitis (BV), *Candidaalbicans* accounts for (85%) to (90%) of VVC cases [4].

Vulvovaginal candidiasis means the isolation of Candida species in culture from study groups with signs, symptoms of vaginal abnormalities. Candidiasis in the vagina is commonly called a "vaginal yeast infection." Other names for this infection are "vaginal candidiasis," "vulvovaginal candidiasis," or "candidal vaginitis" [5].

The capacity of *C. albicans* to infect such differing host is bolstered by a wide ranges of virulence factors. The main virulence factors, including themorphological transformation between yeast and hyphal forms, theexhibition of adhesionsandinvasionson the cell surface, the thigmotropism, the formation of biofilms, phenotypicswitching and these cretionofhydrolyticenzymes [6].

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

Candidal infection symptoms can be mildormoderate and include: It chingandirritation in the vagina and vulva, burning sensation, especially during inter course or while urination, erythema and swelling of the vulva, vaginal pain and soreness, vaginal rash with a thick, white, odorless vaginal discharge with a cottage cheese manifestation with watery vaginal discharge [7].

The diagnosis of VVC needs pelvic examination and laboratory investigations. The combination of erythema, edemaofvulvarandvaginalmucosa,and thick white vaginal discharge support the diagnosis of VVC, pruritus is the most ideal manifestation of VVC, culture and wet mount or saline preparation formicroscopic examination of vaginal can support the diagnosis of VVC [8].

Aim and Objective:

Aims of this study are to determine the incidence of VVC among women attending the Maternity hospital and find the relationship between this vulvovaginal candidiasis 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 September 2019 to February 2020 at the maternity hospital in Erbil city, in which 300 females include with signs and symptoms of VVC as described by obstetric gynecology specialist with the age range between (15-65) 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

Vaginal swabs were collected from study patients complaining of signs and symptoms of vulvovaginitis.

The vaginal swab were 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. So as to distinguish Candida yeast cells, mycelium, as well as pseudo-mycelium and to exclude differentcauses, for example, bacterial vaginosis, and trichomoniasis.

After culture, theswabwasplacedin 1 ml of sterilesa line and shake nuntilthesa line turned cloudy. Then the drop of the suspension takes on to the sterile glass slide and coverslip and examine under the microscope.

The swabs were immediately transferred to petridishes of blood agar containing (5%) sheep blood, Macconkey and Sabouraud's Dextrose broth (PH5.6) The culture incubated at 37Cº for 24-48 hours as mentioned by [9].

Automated VITEK 2 compact system used for *Candida* identification.

Statistical analysis:

The data obtained in this study 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 utilizing the SPSS programming (version 23) to determine the relation between the risk factors on the incidence of VVC, and P-value of < 0.05 was regard as thesignificance level while the value <0.001 regarding highly significant (HS).

Results:

A total of 300 high vaginal swabs were collected form patients attending maternity teaching hospital with sign and symptoms of vulvovaginal infection which were itching (83%), redness (82.5%), suprapubic pain (66.1%) and vaginal discharge (67.3%).

Among them 171(57%) Swabs were positive for *Candida*spp growth on culture, and 1 The highest frequency of positive cases were found to be in the age range (30-34) years (21.6%) followed by age group (25-29) years which was 29(18.7%) while the lowest number of patients with candidiasis was in the age group (15-19) years which was 4(2.3%) as shown in table and figure (2).

29(43%) were negative for Candida growth as shown in table (1) and figure (1).

Table (3) revealed that the positive culture was among pregnant women 73(61.3%) as compared to those detected among non-pregnant patients

98(54.1-%), and also the incidence of vulvovaginal candidiasis was higher among diabetic women 49(80.3%) when compared to positive result among the non-diabetic women which was 122(51%), in this study the frequency of culture positive for *Candida* spp. in those on antibiotic treatment was 61(64.9%) whereas in those was not taking antibiotics was 110(53.4%) and regarding the using of contraceptive the result of this study showed that the using of contraceptive either tablets or intrauterine contraceptive device (IUCD), the positive culture for *candida*spp was higher among the contraceptive pills 40(60.6%) in comparison to the non- user 131(56%) and for IUCD user the positive culture was 31(68.9%) with negative result among them was 140(54.9%) while those they using cleanser the positive culture was 66(55.%) which was less than negative result 105(58.3%).

The recognized *Candida* were identified using Viteksystem 2. Table (4) and figure (3) showed frequency and percentage of different *Candida* species: the highest percentage of isolated was as follow: *Candidaalbicans* which was 147(86%) followed by *Candida glabrata* which was 16(9.4%), *Candida parapsilosis* 6(3.5%) and the lowest percentage belonged to *Candida kruis* 2(1.2%).

In the table (5) the result of culture showed a strong correlation with symptoms of the patients, especially the itching and swelling.

Table (1): Positive and Negative results of *Candida*

Result of culture		Frequency	Percentage
	positive	171	57.0
	negative	129	43.0
	Total	300	100.0

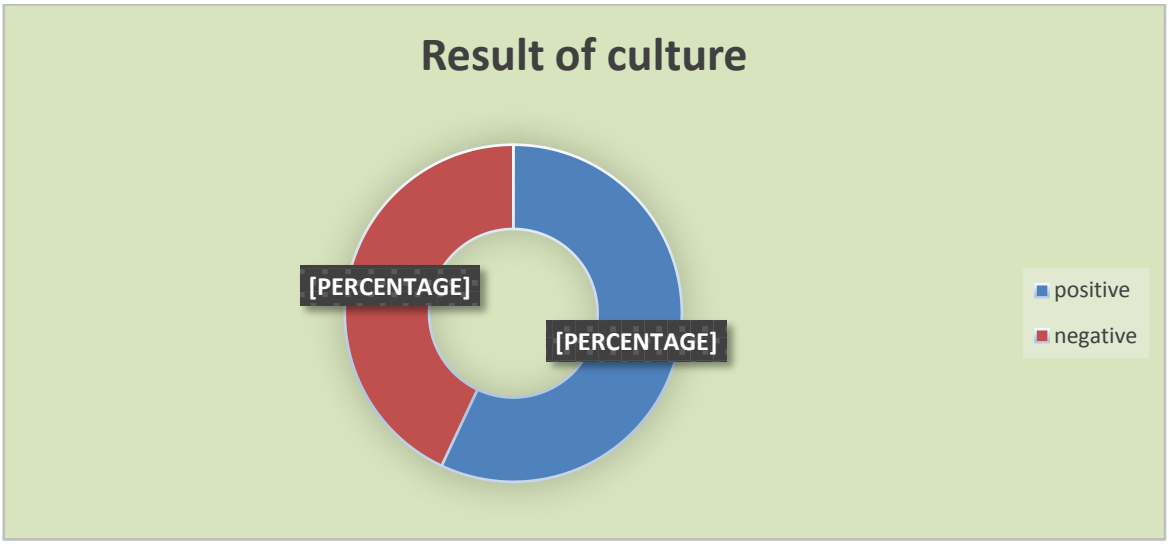

Figure (1): Compare between positive and negative culture result.

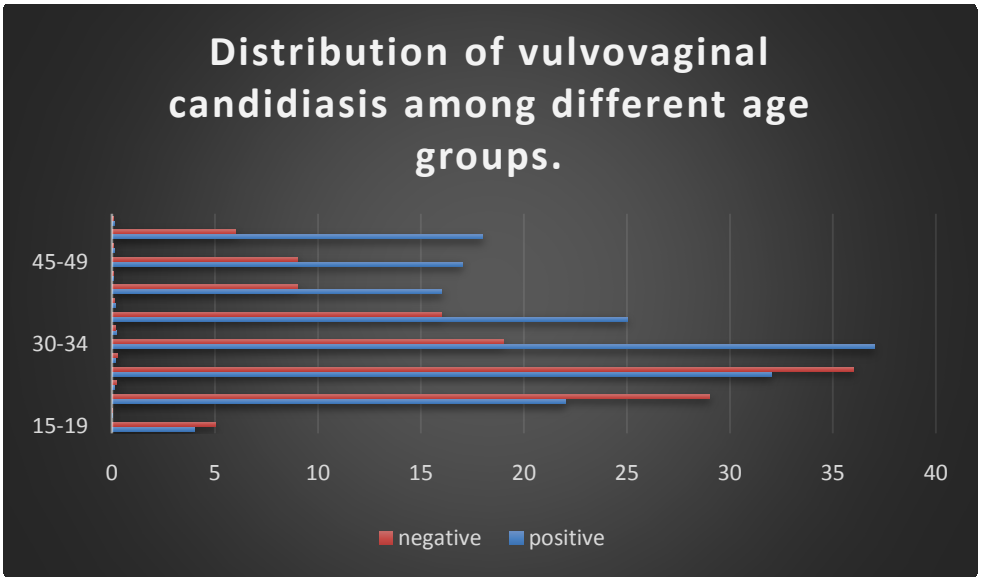

Figure (2): Distribution of vulvovaginal candidiasis among different age groups.

Table (2): Distribution of vulvovaginal candidiasis among different age groups.

Age/years		Result of culture		Total
		positive	negative	
15-19	Count	4	5	9
	%	2.3%	3.9%	3.0%
20-24	Count	22	29	51
	%	12.9%	22.5%	17.0%
25-29	Count	32	36	68
	%	18.7%	27.9%	22.7%
30-34	Count	37	19	56
	%	21.6%	14.7%	18.7%
35-39	Count	25	16	41
	%	14.6%	12.4%	13.7%
40-44	Count	16	9	25
	%	9.4%	7.0%	8.3%
45-49	Count	17	9	26
	%	9.9%	7.0%	8.7%
≥ 50	Count	18	6	24
	%	10.52	4.65	7.90%
Total	Count	171	129	300
	%	100.0%	100.0%	100.0%

Table (3): Correlation of some risk factors with vulvovaginal candidiasis.

Risk factors			Result of culture		Total	p-value
			Positive	negative		
Pregnancy	pregnant	Count	73	46	119	0.007
		% within pregnancy	61.3	38.7	100.0	
	non pregnant	Count	98	83	181	
		% within pregnancy	54.1	45.9	100.0	
History of diabetes	Yes	Count	49	12	61	0.0003
		% within history of diabetes	80.3	19.7	100.0	
	No	Count	122	117	239	
		% within history of diabetes	51.0	49.0	100.0	
Antibiotic using	yes	Count	61	33	94	0.062
		% within antibiotic using	64.9	35.1	100.0	
	no	Count	110	96	206	
		% within antibiotic using	53.4	46.6	100.0	
Contraceptive pills	Yes	Count	40	26	66	0.672
		% within contraceptive	60.6	39.4	100.0	
	No	Count	131	103	234	
		% within contraceptive	56.0	44.0	100.0	
IUCD	Yes	Count	31	14	45	0.144
		% within IUCD	68.9	31.1	100.0	
	No	Count	140	115	255	
		% within IUCD	54.9	45.1	100.0	
Cleaner user	Yes	Count	66	54	120	0.568
		% within cleaner user	55.0	45.0	100.0	
	No	Count	105	75	180	
		% within cleaner user	58.3	41.7	100.0	

Table (4): Frequency and percentage of *candida* species in the positive culture.

Candida species	Frequency	Percentage
Candida albican	147	86.0
Candida glabrata	16	9.4
Candida parapsilosis	6	3.5
Candida krusei	2	1.2

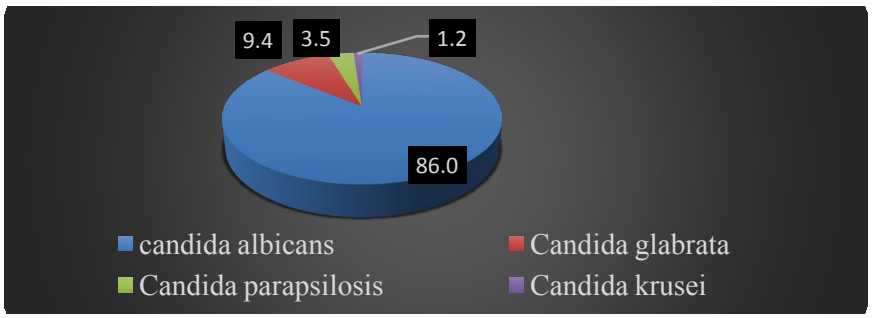

Figure (3): Percentage of various Candida species causing vulvovaginal candidiasis.

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

Complain		*Candida spp*				
		candida albican	*Candida glabrata*	*Candida parapsilosis*	*Candida krusei*	
Itching	Count	124	11	6	1	142
	Percentage	84.4%	68.8%	100.0%	50.0%	83.0%
Vaginal discharge	Count	98	11	4	2	115
	Percentage	66.7%	68.8%	66.7%	100.0%	67.3%
Suprapubic pain	Count	97	11	3	2	113
	Percentage	66.0%	68.8%	50.0%	100.0%	66.1%
Redness	Count	122	12	5	2	141
	Percentage	83.0%	75.0%	83.3%	100.0%	82.5%

Discussion:

Vulvovaginitis is a general problem attacking large numbers of females around the globe, vulvovaginal candidiasis (VVC) can becharacterized as signs and symptoms of inflammation of the vulva and vagina with the existence of *Candida* spp [10].

Incidence of vulvovaginal candidiasis is hard to decide because the clinical diagnosis is usually dependingon symptoms and was not determined by microscopic examination or culture [11].

In the present study, out of 300 symptomatic patients, candidiasis was detected in (57%) (171/300) cases on the culture of the high vaginal swab, and this data is similar to reports by (Rad, *etal*) [12] which were (66%) and (Kalia, *etal*) [13] (47%), and lower than that reported by (Ugwa) [14] who reported (84.5%) in North-West Nigeria.

Our result is higher than reported by (Krishnasamy*etal*) [15], which had done on 160 women, and 56 high vaginal swabs were (35%) positive culture revealed *candida spp.* and higher than reported by [10] which was (30.6%).

This study found the highest incidence of VVC were in (30-34) years age range (21.6%) followed by (25-29) years with (18.7%) while the lowest incidence was in the age group (15-19) (2.3%) and this indicates that this disease is a common problem of active reproductive life and is similar to aresearch done by (Ugwa*etal*) [14] and (Yadav and Prakash) [16]. [14] reported the highest prevalence in 26-35 years age group (53%) while [16], found that the highest incidence of VVC were in age range (21-25) years then age range (40.44%) followed by (26-30) years with (32.58%).

The age range (25-35) years contains females who are young and are sexually active have low vaginal defense mechanisms towards 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, on the other hand, the aging decreases the action of estrogen hormone in women, which could lead to decrease infection rates as females advance in age, and most women over 40 years are less or not sexually active [18].

Vulvovaginal candidiasis 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 not usual in postmenopausal women unless they are consuming estrogen therapy, and it is also not common in prepubertal young ladies [11].

The incidence of *candida spp*among pregnant and non -pregnant was 73(61.3%), 98(54.1%) respectively.

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

In a study demonstrated a strong relation of positive cultures with pregnancy which could be due to increasing the level of female hormones which leads to increasing the glycogen content in vaginal epithelial cells stimulating the growth of *Candida,* and some studies observed that estrogens have a direct impact 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].

In the time of gestation, which is consider as a risk factor, the vagina is more sensitive, and the infections happen more frequently. The high incidence of vaginitis in pregnant women is correlated to levels of estrogens, which is, mostly regarded the primary factor for the detected Vulvovaginal candidiasis was more frequent in pregnant women [24].

In our study revealed that from 61 patients with diabetes, the incidence of *candida spp* was 49(80.3%), and this is higher than a study done by [11] and [25] which was (56.5%) and (53.06%) respectively.

It has been observed that elevated glucose levels in genital tissues boost yeast adhesion and growth and that vaginal epithelial cells attach to *Candida spp*. cells with a high tendency in diabetic females in comparison to non-diabetic ones [26].

In this current study showed that among 94 antibiotic users, the incidence of positive culture was 61(64.9%)

It is assumed that most often the growth-promoting impact of antibiotics is achieved by mean of destruction of the normal flora houses in the vagina and The elevated the incidence of clinical candidiasis after the treatment by broad-spectrum antibiotics has been remarked upon by numerous researchers, it has been recommended that antibiotics maydiminish phagocytosis, or directly enhance the growth of the yeast [27].

Some researchers have shown that VVC commonly after using of antibiotic, antibiotics decreasing the protective normal bacterial flora in the vagina, i.e. *lactobacillus*, in this way permitting excess of yeast It's been accounted for that (28-33%) of ladies on antibiotic therapy develop symptomatic genital candidiasis, a recent report among non-pregnant females using antibiotics for non-gynecological conditions and seen that short courses of oral antibiotics appear to elevate the prevalence of symptomatic VVC [28].

The current study showed that from a total of 66 women of oral contraceptive users, *Candida* spp was detected in 40(60.6%) cases.

A study done by (Mishra*etal*) [29] revealed that among the ladies utilizing contraceptives, the highest incidence of (69.4%) was in oral pill users coinciding with our result and [30] reported a highest rate of vaginal candidiasis was in oral contraceptives users than non-contraceptives users, this could be due to the presence of estrogen and progesterone hormones in the contraceptives that elevate glycogen in the vagina, thus display it to the activities of lactobacilli. The lactobacilli are generally assumed to play a role in the conversion of glycogen to lactic acid, in turn decreasing the pH of the vagina [31].

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

In our study showed that from the total of 300 women, 45 Intra uterine contraceptive device (IUCD) users, 31(68.9%) of the users shows positive *Candidaspp* and this result is similar to (Lubis*etal*) [33] which found that out of the 36 specimens taken from the vulvovaginal candidiasis patients utilizing the intrauterine contraceptive devices, 20 specimens were found to be yeast positive (56%).

The utilizing of the Intra uterine contraceptive device is a highly successful, cost-efficient technique of preventing pregnancy, and it is one of the most common methods of contraception in the world now a day [34].

(Chassot*et* al.) who done in vitro tests have seen that yeast cells can attaches strongly to the parts of the IUD and form biofilms and the observations in this research recommended that the formation of biofilm on the patients' IUDs act as a reservoir of yeasts and leads to recurrent infection by *Candida albicans* [35].

A study by (Lubis*et* al.,) recorded that 24 specimens show positive yeast results out of 30 specimens taken from IUD utilizer (80%), and this is higher than the result of our study [33].

Our result was concurrent to a study by (Anindita) [36] where found that contraceptive device using is a dominant risk factor for vulvovaginal candidiasis, and *Candida spp* may develop on vagina under unsanitary and damp conditions.

In 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 66(55%) which was less than the non- user which was 105(58.3%).

Simultaneously, an investigation indicated that recurrence of cleaning the vulva applied no impact on the appearance of VVC, while cleaning the vulva before or after intercourse was a favorable factor for preventing against VVC [37].

Some studies demonstrated that particular attention should be paid to vaginal cleanser, women carrying out vaginal cleanser were more likely to develop vaginal candidiasis than no-users, and that vaginal cleanser increased the case rate by 1to 4 because the females lacked professional knowledge about cleanser and used the wrong methods for using it [24].

In the current study, the identification of *Candida* spp done by the Culture method and confirmed by Vitek 2 compact.

There were different *candida* species detected in this study, with highest isolated was *C. albicans* (86.0%), and *non-albicans Candida* (NAC)(14%) of cases.

The prevalence of non-albicans species in this study was as follows *Candida glabrata* which was 12(9.4%), *Candida parapsilosis* 4(3.5%) and the lowest percentage belonged to *Candida kruis* 2(1.2%).

The study observed results similar to two studies done by (Krishnasamy*etal*) and (salvi*etal*) by which they found that the highest parentage 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 etal.) found that the most obvious non albicans species was *C. glabrata* and the other species *C. parapsilosis, C. krusei* have been detected less frequently in patients with vulvovaginitis which is almost identical to this study [(38)].

C. 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 redness, while very common, but not sufficient to diagnose vulvovaginal candidiasis in the absence of laboratory confirmation. The authors found a positive correlation between having clinically diagnosed and laboratory diagnosed vulvovaginal candidiasis. The positive predictive value was high (64.7%). In a study done by [(39)] reported the highest incidence of VVC was detected in those of the participants who had symptoms of vaginal candidiasis and a similar study was done by (Kalia *et* al) reported (82%) of candida positive females were symptomatic, and the others (18%) were asymptomatic [(13)].

Conclusions:

The higher incidence *Candida* species were in the age young group and there is a relationship 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 and *Candida albicans* is the most prevalent species among other candida species and the culture and Vitek-2 is reliable method to isolate and diagnosis the species of *Candida.*

Recommendations:

Avoidance of antibiotics using with-out physician prescription and prefer to be taken according to the result of culture and sensitivity and each patient have signs and symptoms of VVC should be screened for the causative agent of it and we need other serological and molecular investigations like PCR (Polymerase Chain Reaction) in order to know more about the pathogenesis of *Candida* species

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