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Original Article

Candida Isolation and Identification from Pregnant Women in Kirkuk City

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Abstract

Background: One of the most common fungal species that have been discovered as a component of the normal flora in different human body sections is *Candida* spp. it is the only major opportunistic fungus that exists in both commensal and pathogenic forms. **Objective:** This study was prepared to find out the prevalence of candidiasis among pregnant women in Kirkuk city in addition to diagnose and identify its important species. **Materials and Methods:** This study was carried out on 154 patients whom suspected of having candidiasis and attended at (Azadi Teaching, Kirkuk General, and Al-Nasar Maternity) hospitals from December 2021 till April 2022. Specimens were carried out by taking a vaginal swab from all pregnant women. The swabs were first examined directly by using potassium hydroxide (10%) then inoculated onto Sabouraud's dextrose agar supported with Amikacine antibiotic. The mycological examination has revealed 62.33% positive candidiasis, by direct microscopy examination through the recognition of pseudohyphae or yeasts. Hi Chrom agar medium was used to detect Candidal species identification. **Results:** Four species have been isolated each with an incidence of 67.7% for *Candida albicans*, 13.5% for *Candida glabrata*, 11.5% for *Candida tropicalis*, 7.3% for *Candida krusie*. **Conclusion:** It can be concluded that *Candida* is very common among pregnant women and being the most predominant species.

Keywords: Candidiasis, isolation, Kirkuk, pregnant women, vulvovaginitis

Introduction

Candida spp. is one of the most prevalent types of fungi which was found as a normal flora in various parts of the human body.[1,2] As a genus, *Candida* contains more than 200 species that all of them belong to the family: Saccharomycetaceae.[3] It is regarded as one of diploid eukaryotic fungi that can take a different shape varying from cocci, cylindrical, oval to elongate shape with its ability to perform changing its shape, as in other dimorphic fungi, from yeast to pseudohyphae or to true hyphae depending on the environment factors such as pH or temperature.[3]

The cell wall of *Candida* spp. mostly composed of polysaccharides structure such as mannans, glucans, and small amount of chitin.[4] It can normally live on the vaginal surface layers as one of vaginal mycobiota.[5] Growing of *Candida* spp. on vaginal surface which is usually controlled by many factors such as the activity of other microorganism, especially *Lactobacillus* spp. which is usually competing with fungi to adhere with epithelial layers and it is preventing yeast overgrowing by forming organic acids (lactic acid) that lead to lower vaginal pH.[6] The pregnant women are more risky for developing a vaginal infection that great challenges for many obstetricians today. The discharge of the vagina may be either physiological or pathological in nature.[7] The infections due to fungi belonging to the genus *Candida* are increasingly reported in recent years. *Candida* spp. is the only opportunistic fungi that exist both as a pathogen or commensal. In addition to that, it is also unique among mycotic pathogens because it causes a wide range of clinical manifestations varying from more mucocutaneous overgrowth to life threatening systemic infections.[8] Many recent studies from various parts of world have reported that there is a shift from pervasive *Candida albicans* to

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"cryptic" Non-Albicans Candida (NAC) species. The NAC spp. are closely correlated to *C. albicans* and cause the same features but different with respect to virulence factors and epidemiology, and the most necessary aspect is the pattern of susceptibility to antifungal drugs.[9] The risk of vulvovaginal candidiasis (VVC) is very high in women complaining from Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome, diabetes mellitus, oral contraceptives, people who are using broad spectrum antibiotics therapy, and in women who involved in receptive oral sex.[10]

The human vagina normally has a predominance of lactobacillus that helps to protect and prevent colonization of the vagina by other potentially harmful risky microorganisms.[11,12] During pregnancy, the estrogen and progesterone hormones alterations lead to changes in vaginal acidity resulting in overgrowth of anaerobic bacteria and other various harmful microorganisms in the vagina.[13] The virulence of a microbial species can be defined as a measure of the final outcome of the microbe–host interactions, rather than a fixed property of the microorganisms it selves. As opposed to primary pathogens which do not require an injured host to developing a disease formation, opportunistic/facultative pathogens such as *Candida* spp. can lead to disease especially in a susceptible hosts. The main strategies that are used to prevent and fight against the natural defense mechanisms of the hosts are highly affected by the environment. The virulence itself is not a constant property, as it can be elevated and increased or lost, and even restored in various conditions.[14] A similar research on vulvovaginitis was done in Baghdad, Iraq, found that out of the 250 vaginal smears collected, 141 had it (56.4%), among which 81 (57.4%) were *C. albicans* and 60 (42.6%) were non-albicans. The most common prevalent species were *C. albicans* (57.4%).[15] Another updated research was done in Kirkuk city, Iraq, to determine Candidal organisms in women's vaginal fluid by taking a total of 200 women vaginal swabs which were collected from Gynecological Clinic in Azadi Teaching Hospital and some private clinics around the hospital. It was found that 57 (28.5%) isolates were detected which comprised four different *Candidal* species, namely *C. albicans* (23%), *C. glabrata* (2%), *Candida krusei* (1.5%), and other species (0.5%), respectively.[16]

Aim of Study

This study was prepared to find out the prevalence of candidiasis among pregnant women and also to diagnose and identify its species in Kirkuk city.

Materials and Methods

Study setting and period

This study was done in Kirkuk city in three main hospitals (Azadi Teaching, Kirkuk General, and Al-Nasar). In order to collect the necessary information and samples, 154 patients were included in this study from December 2021 to April 2022.

Study design

It is a cross-sectional study done on (154) samples taken by vaginal swabs with an aide of speculum which was inserted into the vagina, and a sterile cotton-tipped stick was used to transport the sample of discharge to the laboratory unite. Each samples was subjected to microscopic examination before being cultured on Sabouraud dextrose agar (SDA).

Study subjects

This study was carried out on 154 cases who were suspected of having candidiasis and attended the hospitals.

The clinical presentation of candidial vaginitis was defined as having two or more of the following symptoms of vaginal etching, which is a thick odorless cottage cheese-like discharge, and soreness. The second major risk factor for developing vaginal infection is the diabetes mellitus in which the likely reasons are due to uncontrolled blood sugars and also the use of unsuitable antifungal agents. The use of antibiotics acts as a short-term risk factor for the symptomatic VVC due to the loss of lactobacilli and other normal flora in the vaginal surface. Vulvovaginitis is a general problem attacking large numbers of women around the world, and can be characterized as signs and symptoms of inflammation of the vulva and vagina with the presentences of *Candida* spp.[17]

Statistical analysis

Number and percentage were used for the calculation of the diseased and control patients.

Chi-square test was used to determine with the correlation between the studied variable and *P*-value <0.05 was regarded a significant.

Sampling procedures

Detection and isolation

Direct microscopy From January 1993 to July 1995, 646 consecutive patients presenting for routine checkup or for presumed genital infection underwent a standardized vaginal speculum examination. An unmoistened speculum was inserted before any other vaginal examination was performed. Vaginal fluid was taken from the posterior vaginal vault with a sterile cotton-tipped stick was used to transfer the sample of discharge to the laboratory and spread on to glass slides, a droplet of 0.9% NaCl in water was applied and covered with a glass coverslip for immediate microscopic was made within 5 min of collection of the specimen.[18] *Candida* infections were recognized by the presence of pseudohyphae or yeasts.

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Culture media The basic culture media, used in isolating clinical *Candida* species, was Sabouraud's Dextrose Agar (SDA). After direct microscopic examination, irrespective of demonstration of fungal elements. The specimens were inoculated onto Sabouraud's dextrose agar supported with Amikacin 1 g and incubated at 37ºC for 48 h,[19] and if growth is positive for culture additional steps were done for species identification.

Dextrose agar with amikacin antibiotic SDA was created in 1892 by Raymond Sabouraud. SDA is used to grow grow fungi (yeasts, molds) ; 65 g of Sabouraud dextrose powder was suspended in 1000 ml of distilled water, disolved the medium completely by heating to boiling, then autoclaved at 121ºC for 20 monutes, then added antibiotic Amikacine added to inhibit the bacterial overgrowth of competing microorganisms while alliwing the successful isolation of fungi and yeasts, and mixing well after cooling to 45-50ºC,the suspension awas added into sterial pitridish then refrigerator to store. On the surface of Sabouraud's agar the vaginal swab was streaked then incubated the plate in incubator at 37ºC for 24–48 h.[20]

Identification of Candida species

Hi Chrom agar media The chromogenic media are frequently used in direct and quick isolation and identification of yeasts because different *Candida* species from mixed cultures in clinical samples produce unique colors on these media. 42.72 g of powder medium was suspended in 1000 mL distilled water, boiled to dissolve the medium completely. Then it was cooled to 45–50ºC; after mixing the suspension well, it was poured into sterile petri plates. Colonies from Sabouraud's agar after incubation growth for 24 h were inoculated by streaking on Hi Chrom agar and incubation at 37ºC for 24–48 h.[21,22] The media were maintained for 96 h.

-*Germ tube production test* Germ tube test is the most widely used conventional technique for the identification of *C. albicans* and *C. dubliniensis*. As it is a basic mycological workup for species identification of *Candida* isolates will start with screening its ability to produce germ tube in serum or other proteinaceous medium,[23] this test was done by placing 0.5 mL of human serum obtained from serology section of Azadi hospital in a test tube with one colony of the fungus and incubated for 3 h at 37ºC. The filamentous overgrowth (germ tubes) that arises from the blastoconidium, was identified by lacking walls without any constriction at their point of origin.[23,24] It is very essential to differentiate germ tube from pseudohyphae. In contrast to germ tube, pseudohyphae are constricted at the point of emergence from the blastoconidium.[25] Germ tube test is known as "Reynolds-Braude phenomenon." For the most important *Candida* species, they produce germ tubes.

Applied index 20 Candida (Api 20 C) AUX

Inoculation of the strip For the confirmation of *Candida* spp. Api 20 C AUX strip consists of 20 capsules containing dehydrated substrates which enable the performance of 19 assimilation tests. The capsules are inoculated with a semi solid minimal medium and yeasts will only grow if they are capable of utilizing each substrate as the sole carbon source. Reading the reaction involves comparing it to growth control, followed by identification using either the diagnostic profile index or identification software.

The cupules were filled with the suspension obtained in the ampule of Api C medium. The formation of bubbles was avoided by placing the tip of the pipette against the side of the cupule. Care must be taken not to over fill or under fill the cupules (the surface should be flat or slightly convex, but never concave). The lid was placed on the tray and incubated at 29°C ± 2°C for 48–72 h (±6°h).[26]

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients after verbal consents were taken from them. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number (42471 in December 9, 2021) to get this approval.

Ethical approval was obtained from Kirkuk Health Directorate after providing scientific seminar in the medical laboratory techniques department with complete filling information formula which were sent for interviewing and completing with scientific communities in Kirkuk Health Directorate (Appendix 1).

Results

According to our result, the Candida was prevalent among pregnant women in Kirkuk city. The overall prevalence among 154 examined samples was positive by culture with a rate of 62.3%. whereas 58 (37.7%) were negative [Table 1].

Table 2 shows that the most prevalent infection among diseased women was between the age 20–30 years (48%) compared to (60.3%) among control group with a *P*-value = 0.983. According the material history table, more disease-affected pregnant women (52.1%) than (53.4%) were married for aperiod of 1-5 years *P*-value 0.297 from the control group. (67.7%) of diseased women

Table 1: Frequency of *Candida*

No. of examined samples	No. of positive samples	No. negative samples
154	96 (62.3%)	58 (37.7%)

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Table 2: Frequency of *Candida* according to age, marital years, parity, and trimester of gestation

Parameters	Diseased case (96) No. (%)	Control (58) No. (%)	Total No. (154)	*P*-value
Age				
<20	9 (9.4%)	2 (3.4%)	11 (7.1%)	0.714
20-30	46 (48%)	35 (60.3%)	81 (52.6%)	0.983
31-40	33 (34.3%)	15 (26%)	48 (31.2%)	0.269
41-50	8 (8.3%)	6 (10.3%)	14 (9.1%)	0.670
Marital years				
<1 years	3 (3.1%)	4 (6.9%)	7 (4.5%)	0.276
1–5	50 (52.1%)	23 (39.7%)	73 (47.4%)	0.134
>5	43 (44.8%)	31 (53.4%)	74 (48.1%)	0.297
Parity				
1–3	65 (67.7%)	35 (60.3%)	100 (65%)	0.353
4–6	20 (20.8%)	12 (20.7%)	32 (20.8%)	0.983
7–9	6 (6.3%)	8 (13.8%)	14 (9%)	0.114
>9	5 (5.2%)	3 (5.2%)	8 (5.2%)	0.992
Trimester				
1st	20 (20.8%)	15 (25.9%)	35 (22.7%)	0.470
2nd	24 (25%)	14 (24.1%)	38 (24.7%)	0.904
3rd	52 (54.2%)	29 (50%)	81 (52.6%)	0.615

show a parity between 1 and 3 with a *P*-value 0.353, and (54.2%) from the 3rd trimester with a *P*-value 0.615.

A chi-squared test is a statical hypothesis test used in the analysis of contingency tables when the sample sizes are large.

The highest 65 (67.7%) percentage was for *C. albicans*, followed by 13 (13.5%) *C. glabrata*, 11 (11.5%) *C. tropicalis,* and 7 (7.3%) *C. krusei* [Table 3].

The microscopic examination revealed pseudohyphae or yeast like formed by Candida [Figure 1A]. After culture growth the colonies appeared as circle creamy white on sabouraude agar [Figure 1B]. For *C. albicans* germ tube was formed [Figure 1G]. About what concerning *Candida* species. On Hi Chrom agar: *C. albicans* appeared as light green colored smooth colonies [Figure 1C]. *C. galabrata* as creamy white colored [Figure 1D] *C. tropicalis* as blue colored [Figure 1E]. Candida krusie was show as a pink colored like in the [Figure 1E] Candida tropicalis as blue colored like in the [Figure 1F]. The identification was confirmed using Api 20 C AUX, and the turbidity of the suspension was depended for species diagnosis [Figure 1H].

Table 3: Frequency of isolated *Candida* species

Candida species	No. %
Candida albicans	65 (67.7%)
Candida glabrata	13 (13.5%)
Candida tropicalis	11 (11.5%)
Candida krusei	7 (7.3%)
Total	96 (100%)

Discussion

According the present study, 62.3% of the 154 pregnant women who were diagnosed had *Candida* spp. as their most common from of bacteria. A similar study was done from Sahar Mohammed Zaki Abdullah to detect the correlation between of vulvovagina infection with Candidiasis and its relation with some risk factors and the incidence of various species of *Candida* among patients with vulvovaginitis from women who were attending maternity teaching hospital in Erbil city from the period of September 2019 to February 2020. They found that the prevalence of VVC was 57%, and the highest one of positive cases were in the age range (30–34) years and the positive culture among pregnant women was 73 (63.4%) and in addition to that VVC incidence was higher among diabetic women 49 (80.3%).[16]

Another reported study mentioned that there was a strong correlation of positive cultures with pregnancy which may be because of increasing the level of female hormones which result in elevating the glycogen content in vaginal epithelial cells that are stimulating the growth of *Candida*, and various other studies showed that estrogens have a direct effect on the Candidial growth and its adherence to the vaginal epithelium.[18] Vaginal candidiasis in pregnant women is a very prevalent and usually affecting infection in the women of childbearing age; approximately (75%) of all women experience at least one episode of *Candida* infections during their lifetime.[19] At the time of gestation, which is regarded as a risk factor, the vagina is more sensitive, and the infections happen more commonly. The high incidence of vaginitis in pregnant women is directed related to estrogen level, which is mostly regarded as the main the primary risk factor for the diagnosed VVC in women.[20]

The current study shows the highest percentage of infection among the age between 20 and 30 years old group. This result was in disagreement with another study which was

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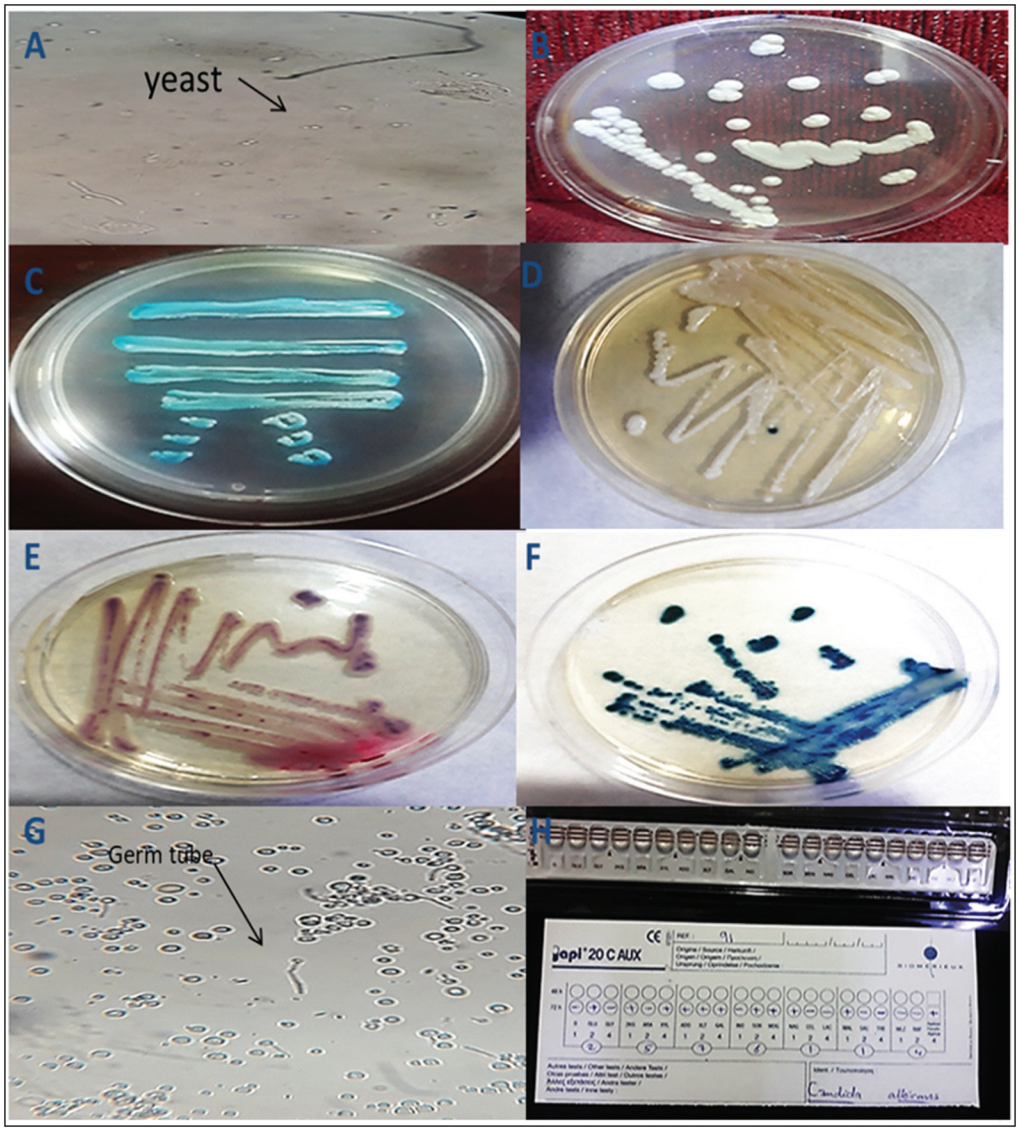

Figure 1: Candida identification. (A) Microscopic field showing yeast. (B) Growth of the fungi on Sabouraud Dextrose agar. (C) Light green colonies of *Candida albicans* on Hi Chrom agar. (D) Creamy white colored colonies of *Candida galabrata* on Hi Chrom agar. (E) Pink colored colonies of *Candida krusie* on Hi Chrom agar, dark blue colored colonies of *Candida tropicalis* on Hi Chrom agar. (G) Germ tube of *Candida albicans*, and (H) *Candida albicans* identified by Api C 20 AUX

done by Daniel *et al.*,[21] to detect the prevalence of VVC among pregnant women who attended the antenatal clinic of a tertiary health institution in North-West Nigeria to assess the accurate need for adequate treatment and diagnosis which was performed at the Department of Obstetrics and Gynecology in association with the Department of Medical Microbiology of the Usmanu Danfodiyo University Teaching Hospital, Sokoto in North-Western Nigeria at 2022. They found that the out of 288 pregnant women sampled, 175 showed a positive result for candidiasis giving a prevalence rate of 60.8%. The age of the women ranged from 16 to 45 years with a mean of 26.8 (standard deviation ± 4.93). The pregnant women who are aged between 26 and 30 years reported the highest prevalence of 37.1% (65/175). The women with multigravidae were more affected 41.7% (120/288) than the primigravidae one 19.1% (55/288). The VVC level elevated with increasing the pregnancy trimester. 73.7% of the women in the research had *C. albicans*, the most common species was isolated.[21] This could be because of higher levels of placental corticosteroids, estrogens, and progesterone, so in advanced pregnancy which decrease the vaginal defense mechanisms and enhance the growth of yeast cells. There is a slight decrease in cell-mediated immune response in pregnancy and therefore, pregnant women are more liable protozoal, fungal, and viral infections. Similar findings have been reported from Nwosu in South-East Nigeria and in Brazil.[23]

The present study obtained 50 (52.1%) of studied women who married with marital years ranged from 5 years. This

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result was similar to a study done by Morris *et al.*[24] to verify if the risk factors for bacterial vaginosis (BV) are permanent with it being a sexually transmitted infection through the comparison between the features of BV patients to those of women with sexually and non-sexually transmitted infections.[24]

According to the current study women with aparity of betweenone and three were highly prevalent which is in agreement with Sule Odu et al.'s related research. At the antenatal clinic and labor ward of Olabisis Onabanjo University Teching Hospital Sagamu, Ogun State, Nigeria, 2020, 408 pregnant women were collected to determine the prevallence of vaginal Candida infection, the main type of it that was isolated from the genital tract of pregnant women during early labor and the antenatal period, and the related fetal impact. The samples were analyzed to isolate *Candida* species. The prevalence of *Candida* infection was significantly higher in early labor (46%) than other period of the antenatal counseling (38%) (P = 0.02), and 165 study women (40.4%) were nulliparous, whereas 199 (48.8%) had parity of 1–2.[25] In this study, women who had positive *Candida* cultures had a significantly lower mean birth weight and gestational age at delivery when compared with *Candida* negative women. The regression analysis revealed there was an increase in likelihood of low birth weight babies among women with vaginal *Candida* infection. In spite of that result there was a higher odd ratio of preterm delivery among women with vaginal *Candida* infection in labor, the trend was not statistically significant, whereas other researchers have reported and mentioned that *Candida* colonization was not correlated with preterm delivery.[26]

In the existing study, there was a significant difference between *Candida* species which were detected and the more prevalent type was *C. albicans*. All *Candida* species found significantly more among women who are using contraceptive, and constitute the more type. These results are supported by other studies.[27] The use of oral contraceptives, the luteal phase of the menstrual cycle,hormonal changes during pregnancy, and the fact that it was the one species of Candida that was most prevalent in VVC are just a few of the various leading to reasons for the condition.

Khattab[28] reported a similar study to diagnose the main species of vaginal candidiasis and the *C. albicans* genotypes isolated from vaginal samples among pregnant women in the Kurdistan region, Iraq, at the Maternity hospital in both Sulaymaniyah and Erbil cities from March 2016 to December 2018. He found that among 340 tested women, 114 (33.53%) showed a positive result of vaginal candidiasis and only five species of *Candida* were identified; they are *C. albicans* which constitute (56.14%), *C. glabrata*, *Candida kefyr*, *Candida tropicalis*, and *Candida krusei* and has been found that *Candida albicans* was significantly higher than other types of non-albicans species with a ($P < 0.01$).

This variation of VVC rate in pregnant women may be caused by increasing the risk factors during pregnancy such as increased vaginal glycogen production mechanism, immunologic alterations, and increased estrogen levels.

Another study was done by Krishnasamy *et al.*[29] to determine the prevalence of VVC among the patients attending the tertiary care hospital, Chennai form January, 2017 to December 2017. The study included 160 women aging between 15 and 65 years with suggestive signs and symptoms of vulvovaginitis by taking a higher vaginal swabs and examined through direct microscopy, cultured by SDA and Hi Chrome *Candida* differential agar. *Candida* species were determined by standard microbiological methods and the results were confirmed by an automated VITEK2 compact. *Candida* species were detected from 56 patients which included *C. albicans* (25), *C. tropicalis* (20), *C. glabrata* (6), *Candida parapsilosis* (4), and *C. krusei* (1).

There was a statistically significant relation of positive cultures with pregnancy which could be due to high levels of reproductive hormones which inducing higher glycogen content in vaginal epithelial cells enhancing the growth of *Candida*. Some studies said that estrogens have a direct effect on the growth of *Candida* and its attachment to the vaginal epithelium which explains the elevated incidence in women using who are using oral contraceptive pills.[30,31]

The use of antibiotics acts as a short-term risk factor for the symptomatic VVC because of the loss of lactobacilli and other normal flora present in the vaginal surface.

Conclusion

- Candidiasis is a common fungal disease in pregnant women in Kirkuk city.
- *Candida albicans* is the most common species of fungi causing vulvovagins in in pregnant women.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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