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Diagnostic Efficiency of a New *Candida albicans* Rapid Test Device and the Importance of some Risk Factors of Vulvovaginal Candidiasis in Mosul City

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ABSTRACT

The objective of this study is to evaluate the importance of *Candida albicans* rapid test device and to evaluate the importance of some risk factors in the diagnosis of *Candida albicans* in vaginal discharge and to compare this test with other diagnostic methods. One hundred sixty women were investigated in this study. Results showed no statistical relationship between the age and the occupation of the patient and the incidence of fungal vaginitis. Relationship between recent use of antibiotics and infection was statistically evident. The sensitivity of the rapid test device for the diagnosis of VVC was 87.9 % versus direct microscopical examination which has 64.5%sensitivity. We conclude that the rapid test device provided a rapid and better results than conventional microscopy and culture for the diagnosis of VVC. This easy-to-perform diagnostic test will be useful to practitioners treating women with symptoms of fungal vaginitis.

Keywords : *Candida albicans*, diagnosis, vaginal discharge, Rapid test device.

الكفاءة التشخيصية للفحص السريع للمبيضات البيضاء وأهمية بعض عوامل الخطورة لداء المبيضات الفرجي المهبلي في مدينة الموصل

الملخص

الهدف من هده الدراسة هو لغرض تقييم اهمية الفحص السريع للمبيضات البيضاء و تقييم أهمية بعض عوامل الخطورة في تشخيص المبيضات البيضاء في الإفرازات المهبلية ومقارنة هذا الفحص مع الطرائـق التشخيصية الأخرى. تم فحص مئة وستين امرأة تشكو من افرازات مهبلية. وقد أظهرت النتائج عدم وجـود علاقة بين عمر المريضة او وظيفتها وحدوث الالتهاب الفطري المهبلي. في حين أظهرت الدراسـة وجـود علاقة بين الاستخدام المسبق للمضادات الحيوية والاصابة بالمرض. وقد وجد أن حـساسية هـذا الفحـص السريع لغرض تشخيص داء المبيضات الفرجي المهبلي هي 87.9 % بينما كانت حساسية الفحص المباشـر 64.5 %.

تم الاستنتاج بان هذا الفحص السريع يوفر نتيجة اسرع واحسن من الفحص المباشر التقليدي والزرع لغرض تشخيص داء المبيضات الفرجية المهبلية. وهو سهل التطبيق وسيكون مفيـد لمعـالجي النـساء المـصابات باعراض التهاب المهبل الفطري.

الكلمات الدالة: المبيضات البيضاء، تشخيص، الإفراز المهبلي، فحص السريع.

INTRODUCTION

Candida albicans is a diploid fungus and a causal agent of opportunistic oral and genital infections in humans, (Denfert and Hube, 2007). Vulvovaginal candidiasis is the second most common cause of vaginal symptoms after bacterial vaginosis (Nester *et al.*, 2007). *Candida albicans* accounts for approximately 80-90% of all vulvovaginitis cases. (Adeb *et al*., 2011). predisposing factor include, pregnancy, use of high-oestrogen oral contraceptives, Diabetes Mellitus, taking of wide spectrum antibiotics and corticosteroid, premenstrual phase of the menstrual cycle, depressed cell mediated immunity (e.g. AIDS) and obesity. (Nviriesy, 2008) Infection of the vagina or vulva may cause severe itching, burning, soreness, irritation, and a whitish or whitish-gray cottage cheese-like discharge, often with a curd-like appearance. **(**Warren, 2010).

The necessary investigations to be performed in *Candida* vulvo-vaginitis include; pH remains normal (4-4.5) (Thulkar *et al*., 2010). The vaginal discharge is subjected to both unstained and stained preparation. Unstained preparation include a saline wet mount to rule out *Trichomonas* or a KOH wet mount. Absence of pus cells or their presence in small numbers is notable in *Candida* infections. Stained preparations include Gram stain and Papanicolaou stain to rule out *Trichomonas*. Confirmation of the fungal etiological agent is achieved by culture on Sabouraud's dextrose agar. (Yehia, 2009).

The clinical symptoms of vulvovaginal candidiasis (VVC) are nonspecific, and misdiagnosis is common, leading to a delay in the initiation of antifungal treatment. (Marot-leblond *et al*., 2009). So several rapid diagnostic tests have been developed over the past 25 years in an attempt to speed up the diagnosis of VVC. (Reed *et al*., 1992).Latex particle agglutination (LPA) was found to be more sensitive than KOH microscopy and was more specific than other diagnostic criteria **(**Evans *et al*., 1986). In the present study the Rapid test device was used for the rapid diagnosis of VVC and to evaluate this test in comparison to other test including compaired microscopy and culture. As far as we know from the literature cited ,this is the first time in Iraq that the Rapid test device is used for the diagnosis of *Candida* in vaginal discharge. The *StrongStep® Candida albicans* Rapid Test uses color immunochromatographic, capillary flow technology. The test procedure requires the solubilization of the *Candida* proteins from a vaginal swab by mixing the swab in Sample Buffer. If *Candida* is present in the sample there will be a visible test line along with the control line will indicate a positive result.

MATERIAL AND METHODS

The study was carried out during the period from January 2011, to July 2011 at Al-Batool and Al-khansaa Teaching Hospitals in Mosul City. Full informational data were recorded from 160 female attending out Patient Clinics of these hospitals complaining from

vaginal discharge. The collected data included name, age, residency, occupation and vaginal discharge including it's amount, color, consistency, odor and duration. Data was also analyzed in term of the use of antibiotics. Three vaginal swabs were taken from each patient. The first swab was placed in tube containing 2 ml of normal saline for wet mount examination, It was examined under X10 and X40 magnification of light microscope. *Candida albicans* were identified by its typical hyphe and budding spores (dimorphic fungi). The second swab were cultured on. Sabouraud's Dextrose Agar which incubated at 37^{o}C for 24-48 hours (Yehia, 2009).

In order to identify the *albicans* species; Germ tube test was done by Using a sterile loop and a small portion of a pure colony of *C. albicans* was inoculated in to sterile test tubes containing 0.5ml human sera. The resulting mixture was incubated aerobically at 37oC for 2-3 hours then, a drop of the yeast-serum mixture was placed on a clean slide and examined microscopically. The appearance of small filaments projecting from the cell surface confirmed formation of germ tubes. (Isibor *et al*., 2005)**.**

The third swab was used for subsequent evalution of *Candida albicans* rapid test kit. This is test is used for qualitative detection of *Candida albicans* within 10-20 minutes .Its an important advance in improving the diagnosis of VVC. It is produced by (Liming Bio-Products Co., Ltd.,) in China**.**

The test procedure requires the solubilization of the *Candida* proteins from a vaginal swab by mixing the swab in Sample Buffer. (Fig. 1) Then the mixed sample buffer is added to the test cassette sample well and the mixture migrates along the membrane surface. If *Candida* is present in the sample, it will form a complex with the primary anti- *Candida* antibody conjugated to colored particles. The complex will then be bound by a second anti-*Candida* antibody coated on the nitrocellulose membrane. A negative result show only the control line. (Fig. 2) While the appearance of a visible test line along with the control line will indicate a positive result. (Fig. 3). The result was statically analyzed using the following stastical methods: chi –square *(x*^2) test was used to find stastical difference between the study group using (Minitab Program). P value less than 0.05 considered to be significant. The validity indicators used for comparisons between different methods used for the examination of *C. albicans* were sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPP) as stated by Gordis (1996).

Fig. 1: Extraction tubes contain buffer solution to be mixed with the vaginal swab. (10.19 *12.7 cm)

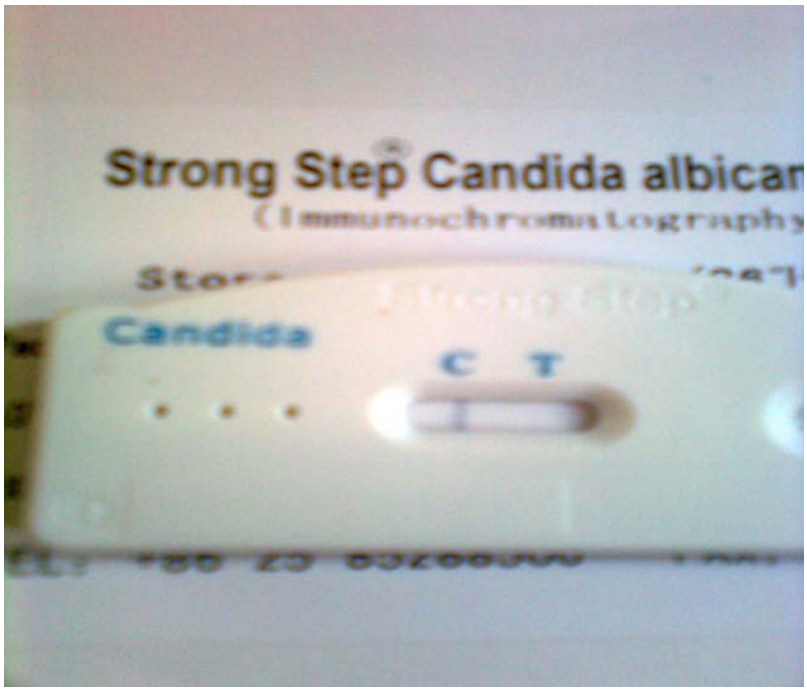

Fig. 2: A negative *Candida albicans* rapid test device (one blue line in control region). (10.19*14.72 cm)

Fig. 3: A positive *Candida albicans* rapid test device (one blue line in control region and a red line in the test region). (10.19 *12.77 cm).

RESULTS AND DISCUSSION

The frequency of *Candida albicans* infection according to the age

The highest frequency of *Candida albicans* infection was most commonly seen among the 21-30 years age group (38.7%) and followed by 31-40 years age group (32.3%) while the lowest incidence of infection was found among ≤ 20 years age group (6.5%). There was no statistical difference between the positive and negative cases at different age groups as shown in (Table 1). This is in agreement with the results obtained by Begum (2004) who observed peak age of having *C. albicans* among the 25-29 years age group comprising (37.8%) of cases. Other investigators reported similar results, Al-Quaiz (2000) conducted a study with 2719 females attending university Primary Care Clinic in Riyadh, who underwent screening for vaginal discharge; and reported that the peak age group for vaginal infection was 25-30 years. Another study in Syria showed that vaginal candidiasis was more common among women aged between 30-45. (Al-Karim *et al*., 2007). It seems that during this reproductive period with highest sexual activity the chance of having *C. albicans* infection is increased.

Table 1: The frequency of *Candida albicans* infection according to the age (n=160).

Age (years)	*Candida albicans* Positive cases		*Candida albicans* Negative cases		Total	
	No.	%	No.	%	No.	%
≤ 20	2	6.5	16	12.4	18	11.3
21-30	12	38.7	46	35.7	58	36.3
31-40	10	32.3	27	20.9	37	23.1
≥ 41	7	22.5	40	31	47	29.4
Total	31		129		160	

$\chi^2 = 2.842$; DF = 3; P-Value = 0.417(not significant)

The relation between *Candida albicans* and the occupation:

It was found that from 31 positive cases of *Candida albicans*, 21(67.7%) were housewives and 10(32.3%) were employees. There was no statistical difference between the positive and negative cases among housewives and employees as shown in (Table 2). This is in accordance with the results obtained by Al-Quaiz (2000) who found that 19 (11%) of these patients were employed and 156(89%) were unemployed (housewives). This result may be due to the better hygienic habits of the employees and since they are prone to ask for medical advice than those housewives who may deal with vaginal discharge and minor irritation as being trivial or normal.

Table 2: The relation between *Candida albican*s and the occupation (n=160).

Occupation	*Candida albicans* Positive cases		*Candida albicans* Negative cases		Total	
	No .	%	No.	%	No.	%
House wife	21	67.7	104	80.6	125	(78.1)
Employee	10	32.3	25	19.4	35	(21.9)
Total	31		129		160	

$\chi^2 = 2.426$ DF = 1; P-Value = 0.119(not significant)

Frequency of *Candida albicans* according to the antibiotic used:

Among the cases who have a history of recent use of antibiotics 20 (64.5%) cases were found to have *C. albicans* infection. There was a statistical difference between positive and negative cases according to the antibiotic use as shown in (Table 3). This is more than what have been reported by Begum (2004) who found that among the cases who had a history of recent use of antibiotics, 5(21.7%) cases only were found to have *C. albicans* infection. Among the recent antibiotics non-users, *C. albicans* was present in 11 (35.49%) which is more than that reported by Begum (2004) who stated that among this group *C. albicans* was found in 40(14.4%). The present findings is in general agreement with the studies done by Al –Quaiz (2000) who found that the vaginal infection rate among those who used antibiotic were 71.4%. This is because antibiotics affect the balance of bacteria in the vagina allowing organisms as *C. albicans* to multiply more rapidly. This study is also in accordance with the result obtained by Al- Karim (2007) who found that from 66 cases of positive yeast culture only 21(31.82%) had a history of antibiotic uses.

Table 3: Frequency of *Candida albicans* according to the recent use of antibiotic.

Recent Use of antibiotics	*Candida albicans* Positive cases		*Candida albicans* Negative cases		Total	
	No	%	No.	(%)	No.	(%)
Yes	20	64.5	28	21.7	48	(30)
No	11	35.5	101	78.3	112	(70)
Total	31		129		160	

$\chi^2 = 21.813$ DF = 1; P-Value = 0.000(Highly significant)

A comparison between different diagnostic methods for the detection of *Candida albicans*:

The results showed that *C. albicans* rapid test kit for the detection of *C. albicans* antigen showed an infection rate of 29 from 31(93.5) cases and culture showed infection rate of 31 cases (100%) while direct microscopical examination had 22(70.9%), as presented in (Table 4**).**

Table 4(a): A comparison between different diagnostic methods for the detection of *Candida albicans*:

Test	*Candida albicans* Positive cases**		*Candida albicans* Negative cases		Total
	No .	(%)	No .	(%)	
Direct microscopical examination	22	70.9	138	86.3	160
Culture*	31	100	129	80.6	160
C. albicans rapid test kit	29	93.5	131	81.9	160

* The growth of *Candida albicans* species was confirmed by (Germ tube test) Non *albican*s species were neglected.

** From total positive cases (i.e. 31)

Table 4 (b): Comparison between Sensitivity and Specificity of wet mount, and the rapid kit for diagnosis of *C. albicans* (the culture is taken as a gold standard).

Test	Sensitivity %	Specificity%	NPV %	PPV %
Wet mount	64.5	98.4	92.02	90.9
The rapid test	87.9	98.4	96.9	93.1

Wet mount

Visualization of pseudohyphae and budding yeast cells are typical of many *Candida* species. Wet mount for detection of *C. albicans* in this study can detect 70.9% of the positive cases. This is in accordance with the study done by Al- Karim *et al*., (2005) in Syria, who found that direct microscopical examination can diagnose 66.67 % of the cases of VVC and the study from India in which 77% of the cases diagnosed by this methods, however, a study done by Goswami *et al*., (2000) reported even more positively by this test (94.8%). This is depend on the experience of the examiner.

Culture

Vaginal culture for *C. albicans* is useful if a wet mount is negative for hyphae but the patient has symptoms and discharge or other signs suggestive of vulvovaginal candidiasis on examination (Linda and Eckert, 2006). The disadvantage of cultures are that they require at least 2 days to complete and get the diagnosis which delay initiation of treatment. (Sobel, 1998 ; Ozturk *et al*., 2006)

In this study by using the culture method, the positive cases of *C. albicans* was increased from 70.9 % by wet mount to 100% by culture which is in accordance with other reports such as that done by Al-Karim *et al*., (2005) in which (21 cases from 21 swab) 100% of the VVC cases can be diagnosed by culture in comparison to (14 cases from 21 swab) 66.67% by wet mount. Sabouraud dextrose agar, remain the gold standard for diagnosis of fungal infection (Guzel *et al*., 2011).

***Candida albicans* Rapid Test Device**

In our study the rapid test was more sensitive than wet mount (87.9 % versus 64.5 % respectively) and had the specificity of (98.4 % versus 96.9 % for culture). The negative predictive value was 96.9 % and the positive predictive value of 93.1%. This is in accordance with the study done by Marot-Leblond *et al*., (2009) in France who studied the efficient diagnosis of Vulvovaginal Candidiasis by using a New Rapid Immunochromatography Test (ICT). They found that the test had a significantly higher sensitivity (96.6 %) than microscopic examination (61.6%) and a higher specificity (98.6%) than fungal culture, they found that the sensitivities of microscopic examination, culture, and ICT for the diagnosis of VVC were 61%, 100% and 96.6%, respectively, while the specificities of the three methods were 100%, 82%, and 98.6%, respectively and found that ICT had a negative predictive value of 98.6%, a positive predictive value of 96.6%. They found also that there is no cross-reactivity with human molecules or other microorganisms. But the limitation of the *StrongStep® Candida albicans* Rapid Test is that this test does not differentiate between viable and non-viable organisms and between individuals that are carriers and individuals that have an acute infection. A negative result may be obtained if the specimen collection is inadequate or if antigen concentration is below the sensitivity of the test. So the culture remain the gold standard but it needs 2-3 days to complete and get the diagnosis which delay the intiatation of the treatment.

CONCLUSION

The *StrongStep Candida albicans* Rapid Test is a point-of-care test for qualitative detection of *Candida albicans* in vaginal swabs within 10-20 minutes. It is an important advance in improving the diagnosis of women with VVC, in addition this test is easy to perform, and the results are simple to interpret. The test is convenient for use by physicians during patient consultations**.**

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