Gram stain versus culture in the diagnosis of vulvovaginal candidiasis

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مقارنة بين ملوّن غرام والزرع في تشخيص داء المبيضات المهبلي الفرجي

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خلاصة: هدفت الدراسة لإجراء دراسة داء المبيضَّات المهبلي الفرجي أثناء الحمل وتقييم ملوّن غرام كطريقة تشخيصية سريعة. أخذت مسحات من مئة حامل وتم تلوينها بملوّن غرام وزرعها. وقد وجد لدى 39٪ منهن داء المبيضَّات المهبلي الفرجي (29٪ لديهن أعراض، و10٪ بدون أعراض) كما تم استفراد المبيضَّات البيضاء من 94.9٪ من مجمل حالات الزرع فيما لم تُستفرد أنواع المبيضَّات الأخرى إلا من 5.1٪ من الحالات. وكانت عديدات الولادات أكثر إصابة من الحوامل للمرة الأولى. أما التلوين بملوّن غرام فطريقة قيّمة في التشخيص السريع والدقيق لداء المبيضَّات المهبلي الفرجي المترافق بالأعراض، وتزيد قيمته على قيمة الزرع لأنه يظهر الأشكال المتعدِّية من هذه الخمائر. إلا أن حساسيته المنخفضة قد تحد من استخدامه في الممارسة الروتينية. إن الجمع بين التلوين بملوّن غرام والزرع هو الأسلوب المثالي لتشخيص داء المبيضَّات المهبلي الفرجي.

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ABSTRACT We evaluated Gram stain as a rapid diagnostic method for vulvovaginal candidiasis (VVC). Vaginal swabs were taken from 100 pregnant women and subjected to Gram stain and culture; 39% were diagnosed as having VVC (29% had symptomatic VVC and 10% had asymptomatic VVC). *Candida albicans* was isolated from 94.9% of all cultures while other *Candida* species were isolated from 5.1% of cases. Multigravidae were significantly more affected than primigravidae. Gram stain is a valuable method in rapid accurate diagnosis of symptomatic VVC and even superior to culture as it demonstrates the invasive forms of the yeast. However, its low sensitivity could restrict its use in routine practice. A combination of culture and Gram stain is the ideal approach for the diagnosis of VVC.

La coloration de Gram comparée à la culture dans le diagnostic de la candidose vulvo-vaginale
RESUME Nous avons évalué la coloration de Gram comme méthode de diagnostic rapide de la candidose vulvo-vaginale. Des prélèvements vaginaux effectués à l'aide d'un écouvillon sur 100 femmes enceintes ont fait l'objet d'une coloration par la méthode de Gram et ont été mis en culture. Parmi les 100 femmes, un diagnostic de candidose vulvo-vaginale a été posé pour 39 % (29 % avaient une candidose vulvo-vaginale symptomatique et 10 % une candidose vulvo-vaginale asymptomatique). *Candida albicans* a été isolé dans 94,9 % de toutes les cultures tandis que d'autres especes de *Candida* ont été isolées dans 5,1 % des cas. Les multipares étaient considérablement plus touchées que les primipares. La coloration de Gram est particulièrement utile pour le diagnostic précis et rapide de la candidose vulvo-vaginale symptomatique ; elle est supérieure à la culture car elle met en évidence les formes invasives de levure. Toutefois, sa faible sensibilité pourrait restreindre son utilisation dans la pratique courante. Une association de la culture et de la coloration de Gram constitue l'approche idéale pour le diagnostic de la candidose vulvo-vaginale.

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Introduction

Vulvovaginal candidiasis (VVC) is an opportunistic mucosal mycosis and one of the most common infections seen in general practice. Up to 75% of all women will suffer at least one episode of this condition during their lifetime, around half of them suffering a further episode [1].

The public health significance of mycoses, especially with opportunistic organisms, lies in their worldwide distribution, therapeutic resistance and tendency to recur. Moreover, the increasing number of complex medical and surgical techniques and immune suppression can predispose to systemic fungal infection, especially with *Candida albicans*, which is usually fatal [2].

For many years, clinicians categorized patients with vaginal candida into two groups; those who were asymptomatic carriers of candida (colonization) and those had symptomatic disease defined as candida vaginitis. More recently, the concept of vulvovaginal candidiasis has begun to replace these distinct categories. This term was introduced to emphasize the often dominant vulvar component of symptomatic infection as well as the importance of asymptomatic frank infection [2].

The condition is common in pregnancy. Moreover, a significant proportion of women with chronic or recurrent candidosis first present with this infection while pregnant [1] During pregnancy, levels of progesterone and estrogen are elevated [3]. Progesterone has suppr ssive effects on the anti-candida activity of neutrophils [4]. Estrogen has been found to reduce tne ability of vaginal epithelial cells to inhibit the growth of *C. albicans* resulting in vulnerability of pregnant women to vaginal candidiasis [5].

Occurrence of the disease during pregnancy has its consequences on the outcome. Subpartal transmission of *C. albicans* from the vagina of the mother to the neonate is often a problem; 70%–85% of the affected mothers subpartally contaminate their infants with the yeast. Infants, especially premature neonates, are severely endangered by generalized fungal infection because of their immature immune system [6]. Candida chorioamnionitis with its subsequent abortion or delivery of an infant with congenital candida infection is believed to result from candida vaginitis [7]. In addition, VVC has been reported as a causal factor of nipple candidiasis in breastfeeding mothers [8]. Hence, several investigators have recommended prepartal treatment of vaginal candidosis [6–8].

Some studies have confirmed the transmission of candida from the female with VVC to her sex partner. The male partner with genital candidiasis usually suffers from balanitis or balanoposthitis [1,2].

Due to its pathogenicity, *C. albicans* is the most frequent agent encountered in cases of VVC [2,9]. Reports of non *C. albicans* species (*C. glabrata, C. parapsilosis and C. tropicalis*) are increasing, especially in recurrent disease [6,10,11].

In Egypt, some community-based studies of the prevalence of reproductive tract infections (RTIs) among married women of childbearing age have been conducted. They documented the importance of *Candida* species as one of the major etiologies of RTIs [12,13].

Unfortunately, none of the clinical signs and symptoms of VVC either individually or collectively is pathognomonic [14]. A myriad of infectious and non-infectious factors may cause identical signs and symptoms; a reliable diagnosis therefore

cannot be made on the basis of clinical evidence without the corroborative evidence of laboratory tests [2,14]. Although culture is the most sensitive method for the diagnosis of VVC, clinicians usually recommend immediate diagnosis based on Gram-stain smear in order to treat the patients at their initial attendance [2,15]. Hence, the present investigation aimed to screen both symptomatic and asymptomatic VVC among pregnant women in Alexandria and evaluate Gram stain as a rapid diagnostic tools for symptomatic VVC.

Methods

The study was carried out on 100 pregnant women attending private medical centres for antenatal care in Alexandria, Egypt. A questionnaire including personal, general, obstetric, sexual and contraceptive history was completed by each woman. Symptoms of vaginitis (soreness, pruritis, discharge and dyspareunia) were recorded. The vulva and vagina were inspected for the presence of oedema, erythema and the nature of vaginal discharge if present.

During speculum examination, vaginal specimens were collected using sterile cotton-tipped swabs. Swabs were examined microscopically by Gram stain and also cultured on Sabouraud dextrose agar (SDA) with 0.05 mg/mL chloramphenicol and incubated at 37 °C for 48–72 hours then examined for growth of yeasts [2,10,15]. Colonies which were pasty, creamy and smooth were considered as yeasts and then further identified by germ tube test and chlamydospore production on corn meal agar [16,17]. Isolates which formed germ tubes after 3 hours incubation in serum at 37 °C and produced chlamydospores on corn meal agar after 18–24 hours incubation at room temperature were

considered as *C. albicans* [16,17]. Isolates that gave negative results were considered as non *C. albicans* species and were further identified by auxanogram (API 20C Aux, API system, Montalieu-Vercieu, France) [10,11]. The Gram-stained smears were examined for the presence of budding yeast cells, pseudohyphae and or hyphae, which indicate tissue invasion [1,2,9]. Depending on the presence or absence of symptoms combined with the results of culture, the women were diagnosed as having symptomatic or asymptomatic VVC or as being negative cases.

Analysis of variation (ANOVA), Pearson chi-squared and Monte Carlo tests were used to analyse the data. A P-value < 0.05 was considered significant. The validity of Gram stain in the diagnosis of symptomatic VVC was interpreted in terms of sensitivity, specificity, positive and negative predictive values, and efficiency [18].

Results

We enrolled 100 pregnant women in the present study; their ages ranged from 18 to 35 years with a mean age of 24.53 ± 4.54 years. The majority of the women (65%) was newly married primigravidae without a previous history of contraceptive use. The rest (35%) were multigravidae with a history of use of contraceptive means preceding the present pregnancy (20% were users of intrauterine devices (IUDs) , 10% used contraceptive pills while 5% used other methods). All the women were in the third trimester near full term (females with early pregnancy refused vaginal examination because of the strong belief of induction of abortion if vaginal examination is done in early pregnancy). Of the 100 women, 29 (29%) had the characteristic symptoms of candida vaginitis. The main complaints

were itching and discharge [24 (82.8% of the total complaints)] while the remaining 71 (71%) were asymptomatic.

Culture revealed that 39 (39%) of the examined vaginal specimens contained *Candida* species and hence the corresponding women were diagnosed as having VVC, 29 (74.4%) of whom were symptomatic (had symptomatic VVC) while 10 (25.6%) were asymptomatic (had asymptomatic VVC) (Table 1).

Gram-stained smears gave lower results. Only 14 (14%) of the women were found positive (12 were symptomatic and 2 were asymptomatic) (Table 1). The Gram stain of the symptomatic cases showed evidence of tissue invasion in the form of hyphae and pseudohyphae (Figure 1).

C. albicans was the most frequently encountered yeast in symptomatic VVC cases and the only isolate in asymptomatic VVC cases (represented 94.9% of all isolates). Non *C. albicans* species, namely *C. glabrata* and *C. parapsilosis*, represented only 5.1% of all isolates. They were isolated from two symptomatic VVC cases (Table 2).

There was no significant age difference between the women with symptomatic

Figure 1 Gram-stained smear of vaginal discharge of a symptomatic case of vulvovaginal candidiasis case showing germinated yeasts and pseudohyphae which denotes tissue invasion

VVC, asymptomatic VVC and negative cases as the mean ages were 24.58 ± 4.84 years, 25.20 ± 5.59 years and 24.39 ± 4.27 years respectively. Multigravidae were significantly more vulnerable to both symptomatic and asymptomatic VVC than primigravidae. Although there was an association between the use of contraceptives and the occurrence of VVC, it was not significant (Table 3).

Table 4 illustrates the sensitivity, specificity, predictive values and efficiency of Gram stain in the diagnosis of symptomatic VVC.

Discussion

VVC is a common and frequently distressing infection for many women of childbearing age. Acute episodes of VVC often occur during pregnancy and during the luteal phase of the menstrual cycle as levels of progesterone and estrogen are elevated [5].

Table 1 **Distribution of the 100 pregnant women according to their diagnosis and the results of Gram stain and culture**

Diagnosis	No.	Gram stain +ve	Gram stain −ve	Culture +ve	Culture −ve
Symptomatic vulvovaginal candidiasis	29	12	17	29	0
Asymptomatic vulvovaginal candidiasis	10	2	8	10	0
Negative	61	0	61	0	61

Table 2 **Distribution of vulvovaginal candidiasis cases in relation to the isolated *Candida* species**

Vulvovaginal candidiasis	C. albicans No.	C. albicans %	Non C. albicans No.	Non C. albicans %	Total No.	Total %
Symptomatic	27	93.1	2	6.9	29	74.4
Asymptomatic	10	100	0	0	10	25.6
Total	37	94.9	2	5.1	39	100

In our study, the overall prevalence of VVC among the women was 39% (29% had symptomatic VVC while 10% had asymptomatic VVC). The main symptoms were itching and discharge. Very similar to our results, Hardy et al. reported *Candida* species isolation from 38% of pregnant adolescents aged 13 17 years during the third trimester [19]. Higher results have been recorded by other investigators. In Jordan yeasts have been isolated from 68.2% of pregnant women [20]. In Venezuela, Marcano and Feo reported the isolation of *C. albicans* from 60% of pregnant women complaining of vaginal discharge, 92% of whom were complaining of pruritus [21]. Donders et al. isolated *Candida* species from 43% of black pregnant women from

Table 3 **Distribution of the 100 pregnant women according to their diagnosis, age, gravidity and use of contraceptives**

Variable	Symptomatic VVC (n = 29) No.	Symptomatic VVC (n = 29) %	Asymptomatic VVC (n = 10) No.	Asymptomatic VVC (n = 10) %	Negative (n = 61) No.	Negative (n = 61) %	Significance tests
Gravidity							$\chi^2 = 6.04^*$
Primigravida	16	24.6	4	6.2	45	69.2	$P = 0.0487$
Multigravida	13	37.1	6	17.1	16	45.7	
Contraceptives							Monte Carlo = 6.888
None	16	24.6	4	6.2	45	69.2	$P = 0.327$
Intrauterine devices	7	35.0	4	20.0	9	45.0	
Pill	4	40.0	1	10.0	5	50.0	
Other	2	40.0	1	20.0	2	40.0	

The mean age ± standard deviation for those with symptomatic VVC was 24.58 ± 4.84 years, for those with asymptomatic VVC was 25.20± 5.59 years and for those who were negative was 24.39 ± 4.27 years (ANOVA = 0.136, P = 0.873).
Significant at P < 0.05.
VVC = vulvovaginal candidiasis.

Table 4 **Sensitivity, specificity, predictive values and efficiency of the Gram stain in the diagnosis of symptomatic vulvovaginal candidiasis**

Evaluation	%
Sensitivity	63.2
Specificity	97.2
Predictive value positive	85.7
Predictive value negative	80.2
Efficiency	81.0

endocervical culture [22]. These higher results may be attributed to the selection of pregnant women complaining of vaginal discharge in most of these studies, while our patients were investigated during their routine antenatal care. However, lower results have been reported by others. In Malaysian women, *C. albicans* was isolated from 27% of pregnant women with vaginitis and 14% of pregnant women without overt vaginitis [23]. Kukner et al. in Turkey detected vaginal thrush in 25% of pregnant women [24]. Meis et al. in the United States reported that the rate of VVC at 28 weeks of gestation was 19.5% [25]. In Spain, the prevalence of VVC during pregnancy was only 18% [26]. Some of these lower findings were reported from the industrialized world where RTIs are less common than in developing countries. Women in developing countries, including Egypt, are less likely to seek early health care for their conditions. The low level of education and health information leads to misconception about illnesses [27].

In Egypt, a study was carried out at El Shatby Maternity University hospital in Alexandria. It included two groups of pregnant women. One group was complaining of vaginal discharge and the other was as-

ymptomatic. The overall prevalence of VVC in the two groups was 32%. Among the symptomatic cases, *C. albicans* was isolated from 40% while among the asymptomatic group, *C. albicans* was isolated from only 20% of the women [28].

Two large community-based studies have been conducted in different regions in Egypt to study the prevalence and the different etiologies of RTIs among women of reproductive age [12,13], one of which was carried out in rural Giza and included 509 women. Vaginitis was found in 44% of the women and *C. albicans* was isolated from 11% [12]. The other study was conducted in three governorates of Upper Egypt. It included 1344 women from both urban and rural areas. The overall prevalence of RTIs was 52.8%. The most prevalent organism isolated was *C. albicans*, isolated from 28% of the women [13].

The prevalence of VVC among the women in our study and in the other studies conducted in different areas in Egypt (both the community-based and hospital-based studies) [12,13,28] reflects the significance of the disease among Egyptian women. The disease burden could be attributed to a lack of early diagnosis and management of the disease. Reasons other than women's delay in seeking medical care include lack of time, poor health awareness, stigma attached to RTIs and a culture of silent endurance [29].

C. albicans was the dominant isolated species in our study. The same finding has been documented by other investigators [18,30–33]. For *Candida* species to colonize the vaginal mucosa, they must adhere first to the vaginal epithelial cells; *C. albicans* adheres in significantly higher numbers than do other *Candida* species. This could explain the relative infrequency of the latter in VVC. Non *C. albicans* species were isolated from two of our symptomat-

ic VVC cases (Table 2). These species are important because of their tendency to cause recurrent vaginitis and their resistance to the conventional antifungal treatments [1,10,11].

In our study, multigravidae suffered significantly more from VVC than did the primigravidae (Table 3). This finding could be explained on the basis that multigravidae have a longer sexual history than the newly-married primigravidae and so they are more prone to VVC than the latter. A self-reported history of physician diagnosis indicated a marked increase in the frequency of VVC at the time most women begin regular sexual activity [2].

Another important risk factor with the multigravidae is the use of contraceptives in the period preceding the current pregnancy. In our investigation, although there was an association between the use of contraceptives and the occurrence of both types of VVC in the multigravidae, it was statistically insignificant. Barbone et al. reported that the use of IUDs increases the risk of VVC [34]. The rarity of *Candida* species isolation in premenarchal women and low prevalence of candida vaginitis after the menopause emphasize the hormonal dependence of the infection [35]. Many studies have found a significantly higher risk of VVC in women who use contraceptive pills. This risk of infection may be greater with the use of high estrogen-containing first-generation oral contraceptives than those with low estrogen content [31,36–38].

Although Hurley [39] reported that *C. albicans* was never a commensal in the vagina and always a pathogen, other investigators have not corroborated this view and have demonstrated that many women carry *C albicans* in the vagina without symptoms [1,2,32]. However, the extent to which *C. albicans* can be regarded as normal inhabit-

ant of the genital tract is unclear. On the one hand, Carroll et al. found that clinical signs of infection could be detected in all pregnant women from whom the fungus was isolated [40]. On the other, some reports describe *C. albicans* in vaginal swabs taken from women without any vaginal signs or symptoms [2,23]. In our study, 10% of the women had asymptomatic VVC. During vaginal examination, 6 of them had local signs of vaginitis while 4 had no signs. Glover and Larsen in their longitudinal and observational study throughout the course of pregnancy reported that asymptomatically colonized women were at a threefold greater risk of developing symptoms than were uncolonized women [42]. Hence asymptomatic VVC should be considered during pregnancy as the risk of the development of symptomatic disease is substantial.

There are many problems in the diagnosis and treatment of VVC. The condition is routinely diagnosed without the benefit of microscopy or culture and in many cases thus diagnosed, the women may be uninfected or have another condition.

The most reasonable approach to the diagnosis of VVC is to consider both symptoms and signs with the result of culture of vaginal specimens or of microscopic examination of vaginal smears or both [1,2,42,43]. Many investigators have declared that patients with symptomatic VVC can be diagnosed on the basis of simple rapid microscopic examination of vaginal secretions, and it is extremely valuable when demonstrating germinated yeast and pseudohyphae. It is widely believed that the presence of pseudohyphae indicates tissue invasion. Hence, if the Gram stain is positive, it allows the physician to start treatment without delay. Moreover, investigators consider positive microscopic examination is more diagnostic of vaginitis

than culture as the latter cannot differentiate between colonization and infection [1,2,44].

However, culture is not only important in establishing the correct diagnosis but also in obtaining additional information about speciation. Reports of non *C. albicans* species causing recurrent VVC and being resistant to conventional regimens of therapy are increasing [1,2,10,11]. These non *C. albicans* species can only identified by culture. Unfortunately, reports about Gram stain sensitivity indicate low values ranging from 30% to 50% [2,44,45]. In our study, the sensitivity of the Gram stain in the diagnosis of symptomatic VVC was higher than in previous reports (Table 4).

In most patients with asymptomatic VVC, the results of microscopic examination of vaginal secretions tend to be negative and infection is usually identified on the basis of positive culture [2]. This microscopic profile reflects the low number of the organisms in an asymptomatic host. On that basis, we can explain the negative results of the Gram stain in most of our asymptomatic VVC cases (Table 1).

We conclude that VVC is a disease of considerable importance during pregnancy as 39% of the pregnant women investigated suffered from it. Culture is a sensitive and reliable method for diagnosis of VVC as it is positive in both symptomatic and asymptomatic cases. It can identify the species responsible for infection, and this identification has several diagnostic and therapeutic implications. Although Gram stain is a valuable method in rapid specific diagnosis of symptomatic VVC and even superior to culture, its low sensitivity could restrict its use in routine practice. Hence the combined use of microscopic examination and culture for accurate diagnosis of VVC is recommended.

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