



DIAGNOSIS OF VULVOVAGINAL CANDIDIASIS IN PREGNANT WOMEN BY GRAM STAINING AND FUNGAL CULTURE- A COMPARATIVE STUDY

KEYWORDS

VULVOVAGINAL CANDIDIASIS, GRAM STAIN, FUNGAL CULTURE, PREGNANT WOMEN

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ABSTRACT

Vulvovaginal Candidiasis (VVC) is one of the important causes of morbidity in pregnant women. Gram staining is routinely followed in peripheral setup whereas culture is the most accurate technique for the diagnosis of VVC. This study was conducted to compare these two methods. High vaginal swabs were collected from 50 pregnant women and processed by Gram stain and culture on SDA. Gram staining was positive in 15 (30%) and culture in 18 (36%). Among 18 cases positive by culture, 12 (66.7%) were *Candida albicans* and 6 (33.3%) were Non *Candida albicans*. In peripheral healthcare setups of developing countries culture may not be always possible, Gram staining can serve as screening method for diagnosing VVC.

INTRODUCTION

Among the many causes of vaginitis, Vulvovaginal candidiasis (VVC) is the second most common after bacterial vaginosis and is diagnosed in up to 40% of women with vaginal complaints in the primary care setting. VVC is defined as signs and symptoms of inflammation in the presence of *Candida* spp. and in the absence of other infectious etiology¹. VVC is caused by *Candida albicans* (46.9–75%), or one of other *Candida* species: *Candida glabrata* (14–36.7%), *Candida parapsilosis* (10.2%), *Candida tropicalis* (2.8–7%), *Candida krusei* (1.4–3.5%), and *Candida kefyr* (1.9%)².

Pregnancy, diabetes mellitus, and antibiotic treatment are the most common predisposing factors of VVC³. Higher estrogen levels and higher glycogen content in vaginal secretions during pregnancy increase risk of candidiasis in pregnant women⁴.

VVC, a frequent companion of pregnancy greatly complicates the course of the pregnancy and jeopardizes the health of both mother and child⁵. During delivery, transmission can occur from the vagina of infected mother to the newborn, giving rise to congenital candida infection⁶.

A reliable diagnosis cannot be made from the signs and symptoms alone, and the sensitivity and specificity for self-diagnosis are 35% and 89% respectively⁷. The clinicians are required to make immediate and specific diagnosis for appropriate and timely treatment.

Gram stain smear is reliable and rapid method in order to treat the patient at her initial attendance⁸. However, vaginal culture is the most accurate method for the diagnosis of VVC. It is indicated if microscopy is negative but VVC is suspected¹.

Therefore, we conducted this study to compare Gram staining and fungal culture in the diagnosis of VVC in pregnant women to ensure timely treatment.

MATERIALS AND METHODS

Institutional Ethical Committee approval was obtained and study was conducted in the Department of Microbiology, Mandya Institute of Medical Sciences, Mandya.

Type of study: Cross-sectional study

Duration of study: 2 months

Study Population: 50 pregnant women

We included pregnant women of any age in any trimester of pregnancy and excluded non pregnant women as well as repeated sampling

METHODOLOGY

50 pregnant women who attended antenatal care on the days of study were randomly selected irrespective of age, symptoms and parity for the study. Informed written consent was obtained from them. Vulva and vagina were inspected for signs of inflammation and discharge under aseptic precautions. Sterile speculum examination was performed. Two high Vaginal Swabs were collected from each woman under aseptic precautions and was labelled appropriately. They were processed at the earliest on the same day in the Microbiology Laboratory. One swab was used for Gram staining and the other swab was used to inoculate Sabourauds dextrose agar (SDA) with 0.05mg/mL chloramphenicol. The inoculated culture tubes were incubated at 37°C for 48 hours. Colonies which were pasty, creamy and smooth were considered as yeast and were subjected to Gram stain. Gram positive budding yeast cells were considered as *Candida* species⁹.

These colonies were inoculated into 0.5 ml of human serum and incubated at 37°C for 2 hr. After incubation, a loopful of this serum was examined for germ tube. *Candida albicans* was identified by the formation of germ tubes seen as long tube like projections extending from the yeast cells. The inoculum of yeast colony was streaked on the corn meal agar plate and coverslip was placed over it such that the streak projected beyond the coverslip. The plates were incubated for 24 to 48 hrs at 25°C. Edges of the coverslip were examined for the formation of large, highly refractive and thick walled chlamydospores¹⁰.

The results were analysed using descriptive statistics and expressed as percentages.

RESULTS

The study comprised of 50 pregnant women in the age group of 18-30 years.

Culture showed 18 (36%) of the 50 examined specimens yielded *Candida* species and thus were diagnosed as having VVC. 8 (44.4%) of the 18 positive specimens were positive by Gram stain too. But, 10 (55.5%) culture positive cases were negative in Gram stain.

32 (64%) specimens out of 50 were negative for *Candida* by culture. 25 (78.1%) were negative by both Gram stain and culture. 7 (21.9%) were negative only by culture, but positive in Gram stain.

Gram stain was positive in 15 (30%) women. 8 (53.3%) of the 15 positive specimens were positive by culture too. 7 (46.6%) Gram stain positive cases were negative in culture.

35 (70%) specimens were negative for *Candida* by gram stain. 25 (71.4%) of these were negative by both Gram stain and culture. 10 (28.6%) were negative only by Gram stain. (Table 1)

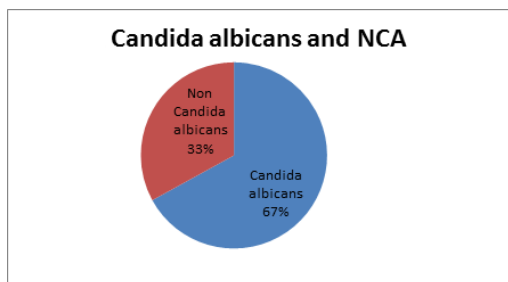
Table 1: Comparison of gram stain & culture for Candida

Gram Stain	Culture		Total
	+	-	
+	8	7	15
-	10	25	35
Total	18	32	50

Sensitivity of Gram staining= $8/18=44.4\%$ Specificity of Gram staining= $25/32=78.1\%$ Accuracy= $33/50=66.7\%$

Predictive value of positive test= $8/15=53.3\%$ Predictive value of negative test= $25/35=71.4\%$

Among 18 cases positive by culture, 12(66.7%) were *Candida albicans* and 6(33.3%) were Non *Candida albicans*



DISCUSSION

Candidiasis is an important yeast infection that is especially common in pregnancy with the attendant risk of the pregnant woman harbouring *Candida* spp. and infecting their babies during the perinatal period¹¹. VVC can cause abortion, candida chorioamnionitis and subsequent preterm delivery⁶.

Our study found that VVC was positive in 36%(18) by culture. Olowe et al¹¹ reported a prevalence rate of 36%, Guzel AB et al¹² 37.4% ,Parveen N et al¹³ 38% and Omar AA⁸ found 39% had VVC. All these studies are comparable to our study. However, Kanagalet al⁶ obtained a higher prevalence rate of 42.37% in their study.

Among 50 pregnant women, 18(36%) were positive by culture and 15(30%) were positive by Gram staining. We found that out of 18 culture positive cases, 8(44.4%) were positive by Gram staining & 10(55.6%) were negative by Gram staining.

Culture is the gold standard in the diagnosis of VVC. The difference in the sensitivity of direct microscopy could be because of the difference in yeast concentration in vaginal secretions. Direct microscopy is reliable only if the infection is heavy¹⁴. M Aslamet al¹⁵ reported that microscopy by Gram stain / KOH mount revealed positivity in 19(38%) as compared to 24(48%) women positive for VVC by culture. Omar AA⁸ found that 39 cases(39%) were positive by culture on SDA and 14(14%) were positive by Gram staining.

Out of the 18 cases positive by culture, 12(66.7%) were *Candida albicans* & 6(33.3%) were Non *Candida albicans*. Doddaiiah Vijaya et al¹⁶ obtained similar results in their study which showed presence of 66% of *Candida albicans* species and 34% of Non *Candida albicans* (NCA). Results of a study by Oviasogie.F.Eet al¹⁷ revealed *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. stellatoidea*, *C. parapsilosis* with 61.5%, 17.9%, 7.7%, 5.1%, 2.6% frequency of isolation respectively.

Candida albicans produces protease, phosphatase and carbohydrates which enhances its attachment to vaginal epithelium.¹⁷ This explains the increase occurrence of infections by *Candida albicans*. Current trend shows an increase in the prevalence of *NCA* vaginitis. The nonalbicans are appearing more frequently because of the widespread abuse of over the counter antifungals, use of single dose oral and topical azole regimens, and long term maintenance

regimens of oral azoles¹⁴

CONCLUSION

Gram staining is the rapid and reliable technique in the diagnosis of VVC, though culture is the most accurate method. In our study, among 18 culture positive specimens, 8(44.4%) were positive by Gram staining. Thus the best approach towards the diagnosis of VVC is combining the two to provide immediate and appropriate treatment in routine practice. However, in peripheral healthcare setups of developing countries where facility for culture may not be always available, Gram staining can serve as screening method for diagnosing VVC at initial attendance of pregnant women.

LIMITATIONS OF THE STUDY

Follow up could not be done in our study.

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CONFLICT OF INTEREST

None to declare

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