

Original Research Article

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Co-infection of *Candida albicans* and *Trichomonas vaginalis* among Pregnant Women in Tertiary Care Hospital

Surbhi Mishra and Arti Jain *

Department of Microbiology, People's College of Medical Sciences & Research Center,
Bhanpur Road, Bhopal -462037, Madhya Pradesh, India

*Corresponding author

ABSTRACT

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Vaginal infections are common among women of reproductive age. The purpose of this study was Co-infection of *Candida albicans* and *Trichomonas vaginalis* among pregnant woman in Tertiary care hospital. The prospective Analytical study was conducted in the Department of Microbiology, attached to a Tertiary Care Hospital in Central India after obtaining ethical clearance over a time period of one year. Out of these 320 samples, 200 sample were randomly selected (by Chit system) in our study. Out of 200 samples 46 (23%) samples were culture positive. VVC, Trichomoniasis and other vaginal infection can resolve within a short period of time ,if not treated timely then, it may enhances the growth of co-infection and it is a major cause of morbidity in pregnant women in developing countries like India. Present study highlights Rapid test should be mandatory routinely done for all pregnant women or in patients suffering from vaginal discharge and thus, help in early diagnosis and prompt treatment of patients.

Introduction

Vaginal infections are common infection in women. They can be caused by sexually transmitted microorganisms or by some disorder resulting in the proliferation of endogenous microbials¹. Among the more frequent microorganisms are the protozoan *Trichomonas vaginalis* (*T. vaginalis*)² and *Candida albicans* (*C. albicans*) responsible for vaginitis specially in women of childbearing age³.

These organisms may occur as single infection or co-infections. Mucosal candidiasis, especially vulvovaginal

candidiasis (VVC), is one of the most population suffers at least one episode during their lives⁴ Pregnancy, diabetes mellitus and antibiotic treatment are the most common predisposing factors^{5,6} Some studies have shown that vulvovaginitis has increased in the past three decades due to antifungal resistance in the *Candida* species and a change in women's health quality.^{7,8,9}

Candidiasis is associated with vaginal discharge and pruritis. The discharge appears to be like curded milk or white discharge, soreness, dyspareunia, irritation and itching and deep erythema of vulva and vagina is often seen. The incidence of the infection is

almost double in pregnant women particularly in the third trimester, compare to non-pregnant women.¹⁰

Vaginitis due to *Trichomonas vaginalis* clinically manifests with symptoms of vaginal itching, odor, purulent, foul-smelling and vaginal discharge, which is associated with pruritis, dysuria and dyspareunia.¹¹

In various clinical settings, culture is not available for detection of *T. vaginalis* and it requires additional resources. Wet mount is the most widely used method for the detection of *T. vaginalis* in clinically suspected cases of vulvovaginitis.¹²

Although, wet mount examination is most cost-effective diagnosis test, but it has low sensitivity resulting in under diagnosis of the diseases. Therefore, to overcome this problem, various staining techniques like Giemsa and Acridine orange can be used along with wet mount examination for diagnosis of *T. vaginalis* infection.

This study was emphasizing the importance of screening and treating infected pregnant women to prevent these unwanted infections. Therefore, this study was conducted to determine the Co-infection of *Candida albicans* and *Trichomonas vaginalis* among pregnant woman attending antenatal clinic in Tertiary care hospital.

Materials and Methods

This Prospective analytical study was carried out in the Department of Microbiology, in college associated with Tertiary care hospital, in central India over a time period of one year after obtaining Ethics Clearance

All pregnant women presenting with signs and symptoms of vaginitis reporting in OBGY were enrolled.

Collection of Samples

A total 200 vaginal samples were collected by clinicians in suspected cases of vulvovaginitis after written informed consent.

The clinicians were obtained three vaginal samples/swabs [Hi-media laboratories Pvt. Limited, Mumbai]

First swab was used for detection of motile *Trichomonas* & budding yeast cells by wet-mount microscopy & second swab was used to prepare smear for Giemsa staining, Gram staining & Acridine orange staining and third swab was used for culture onto Blood agar and Sabouraud's dextrose agar for detection the growth of candida from each patient by swabbing the vaginal vault with the help of vaginal speculum and then send to laboratory within 10 min of collection.

Procedure for sampling

Three vaginal samples were collected by clinician. The samples were transported immediately to the microbiology laboratory within 10 minutes of collection.

After the receiving of sample in the laboratory, will be processed as follows-

Macroscopic Examination-each sample was examined for color, appearance & odor.

Ist swab - was placed in screw capped sterile plastic tube containing 0.5 ml of 0.9% saline and used immediately for detection of motile *Trichomonas* and budding yeast cells to rule out the *Candida* by wet-mount microscopy.

The *Trichomonads* were identified by their size (10-20 μm), round or oval shape, and characteristic twitching motility and *Candida* were identified by their size (3-5 μm), ovoid or spherical budding yeast cells by wet mount examination.

II swab was used to prepare smear for Giemsa staining, Acridine orange staining for detection of *Trichomonas vaginalis* and Gram staining detection of candida morphology (Gram positive budding yeast cells) (Procedure as per manufactures).

Giemsa Staining

The prepared smear was scanned for *Trichomonas vaginalis* at 10X magnification and then at a magnification of 40x. At least 30 fields were examined before a negative finding was recorded.

Both the internal and external structures of the organisms were clearly visualized. The former stained dark blue with a red nucleus and the latter was sharply being outlined, showing clearly the flagella and the undulating membrane.

Acridine Orange Staining

The prepared smear was fixed with methanol for 2-3 min and allow to dry it. It was then stained with Acridine Orange Staining as per as manufactures instructions. After staining, the slide was examined under a fluorescence microscope (with a 470-490 nm filter) at a magnification of 40Xf.¹³ Epithelial cells fluoresced light green with a bright green nucleus. The nuclei of leucocytes (pus cells) fluoresced bright green and bacteria stained bright red color. Trophozoites of *T. vaginalis* were seen as characteristic brick red color with a yellowish green nucleus.

Gram staining

The prepared smear was stained by Gram staining as per as manufactures instructions. After staining the slide were examined by light microscope for detection of Candida morphology (Gram positive budding yeast cells)

III swab was used for culture onto Blood agar and Seaboard's dextrose agar for detection the growth of candida. Procedure was as follows:

The colonies on BA & SDA were further be identified and species identification by CHROM agar then antifungal susceptibility was performed by standard procedure.

Results and Discussion

A total of 200 vaginal swabs were collected from patient of reproductive age group (15-45 years) after Institutional Ethics committee clearance.

A total of 3158 pregnant women were seen at the OBGY department of a Tertiary care centre, over a period of 12 months from (1st May 2017 to 31st May 2018). Out of these 320 women, (10%) presented with vaginal discharge and were sent for high vaginal swabs microscopy, culture and sensitivity. Out of these 320 samples, 200 sample were randomly selected (by Chit system) in our study. Out of 200 samples 46 (23%) samples were culture positive.

The Age wise distribution of patients having vaginal discharge among pregnant women were collected is as given in Table 1.

Frequency of Candidiasis

Out of 200 samples, 42 (21%) cases of vaginal infection were positive for candidiasis among these women.

Maximum number of candidiasis were positive in the age group of 20 to 30 years 21 (10.5%), followed by age group 30 to 40 years 17(8.5%). There were 4 (2.5%) in 40-45 years

The observation by Direct wet mount, Gram staining, Culture results of Candidiasis among pregnant women were as shown in Table 2.

Out of 42 positive candidiasis, *Candida albicans* were maximum 36 then non-albicans that was only 6.

Detection of different spp of isolated Candida by culture on CHROMagar

Out of the 42 isolated Candiada spp in pregnant women, maximum no. of candida albicans were positive (36), followed by C. dublinansis (3), C. krusei(2) and C. glabrata(1).

Percentage of Candidiasis in different trimester among pregnant women were shown in Figure 1. Anti- fungal sensitivity pattern of the predominant Candida isolated from vaginal infection among pregnant women were shown in Table 3.

Frequency of Trichomoniasis

Frequency of *Trichomoniasis* in various age

groups among pregnant women shown in Table 4.

Observation by Direct wet mount, Gram staining, Culture results of Trichomonissis among pregnant women shown in Table 5.

Percentage of *Trichomoniasis* in different trimester among pregnant women shown in Figure 2.

Frequency of co-infection

Out of 200 samples, 46 (23%) cases of vaginal infection were positive among these women. of 42 (21%) were candida infection followed by 04 (2%) trichomonas and co-infections.

Frequency of Candidiasis and Trichomoniasis in different trimester among pregnant women were shown in Table 6.

Table.1 Age wise distribution of patients having vaginal discharge among pregnant women

Age Group	No. Of patients	Percentage
20 – 30	115	57.5
30 – 40	75	37.5
40 – 45	10	5
Total	200	100

Table.2 Observation by Direct wet mount, Gram staining, Culture results of Candidiasis among pregnant women

Organism	Samples	Wet mount showing budding yeast cells	Gram stain showing Gram positive budding yeast cells (5)	Culture	Germ tube test (GTT) positive
Candidiasis (N=42)	Positive	38(90)	42(100)	42(100)	36 (85)
	Negative	4	-	-	6

Table.3 Anti- fungal sensitivity pattern of the predominant *Candida* isolated from vaginal infection among pregnant women

Organisms	No.	NS (%)	FLC (%)	AP (%)	CC (%)	IT (%)
<i>Candida albicans</i>	36	26 (72)	31(86)	32(88)	33(91)	28(77)
<i>Candida dublinans</i>	3	02(66)	03(100)	02(66)	03(100)	03(100)
<i>Candida krusei</i>	2	02(100)	01(50)	02(100)	02(100)	02(100)
<i>Candida glabrata</i>	1	01(100)	00(00)	01(100)	01(100)	00(00)

NS=Nystatin, FLC= Fluconazole, AP=Amphotericin-B ,CC=Clotrimazole, IT=Itraconazole

Table.4 Frequency of *Trichomoniasis* in various age groups among pregnant women

Age (yrs)	Total No of sample	Total no. of positive samples (%)	Positive no. of <i>Trichomoniasis</i> (%)
20-30	115	23(11.5)	2(1)
30-40	75	18(9)	1(0.5)
40-45	10	05(2.5)	1(0.5)
Total	200	46(23)	04(2)

Table.5 Observation by Direct wet mount detection for motility, Giemsa, Acridine orange staining of *Trichomoniasis* among pregnant women

Organism	Samples	Motility	Giemsa stain	Acridine orange staining
<i>Trichomoniasis</i> (N=04)	Positive	4	2	3
	Negative	42	2	1

Table.6 Frequency of *Candidiasis* and *Trichomoniasis* in different trimester among pregnant women

Trimester	No. of sample	Positive	<i>Candidiasis</i>	<i>Trichomoniasis</i>	Co-infection (<i>Candida</i> + <i>Trichomonas</i>)
1 st	20	2 (1%)	2(1%)	0 (0%)	0 (0%)
2 nd	35	6 (3%)	5(2.5%)	1(0.5%)	1(0.5%)
3 rd	145	38(19%)	35(17.5%)	3(1.5%)	3(1.5%)
Total	200	46 (23%)	42(21%)	4(2%)	4(2%)

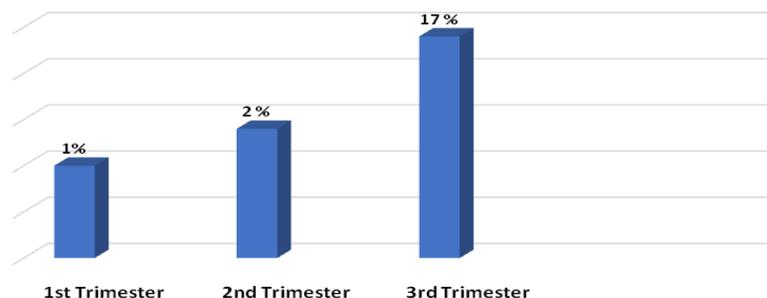
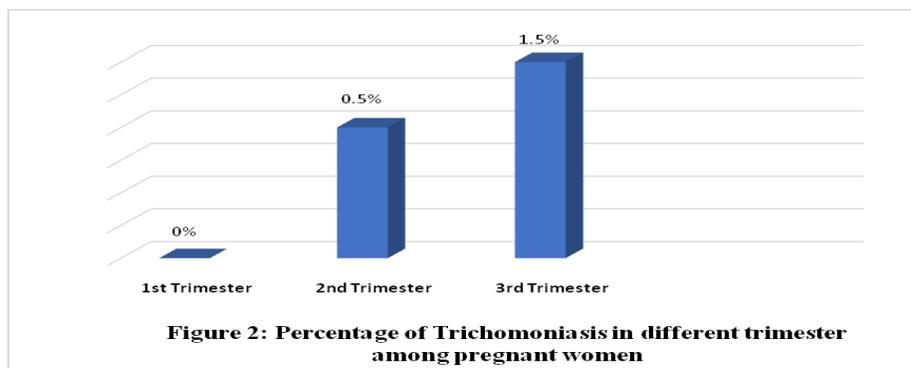


Figure 1: Percentage of *Candidiasis* in different trimester among pregnant women



This study investigated, Co-infection of *Candida albicans* and *Trichomonas vaginalis* among pregnant woman attending the Tertiary Care Hospital. The present study observed and found prevalence of candidiasis was 21 %, Trichomoniasis and co-infection (T+C) was 2%, 2% respectively.

Vaginal discharge (VC)

Vaginal discharge is a major problem in India among pregnant women. It occurs in 1-4% of all women with reproductive age group throughout the world and its prevalence in India was estimated approximate 30% by Thakur *et al.*, 2010.¹⁴ The similar finding observed in our study, the prevalence of vaginal discharge was 23%.

Vulvovaginal candidiasis (VVC)

Vulvovaginal candidiasis (VVC) is a common cause of vaginal discharge among pregnant women worldwide.¹⁵

The present study shows, high incidence (21%) of vulvovaginal candidiasis among pregnant women. In similar study done by Parveen in 2008,¹⁶ shows, incidence was 38% and Rekha and Jayoti, 2010¹⁷, reported 30% , Sansui Mohhamed Ibrahim *et al.*,¹⁸ also observed - 40%, and Sobel *et al.*,¹⁹ estimated that approximately 75% of women will experience an episode of VVC.

Age wise distribution

Vulvovaginitis is a common problem globally and affects woman of all age groups.

Present study observed the most common infective age group of VVC and *Trichomoniasis* was 20-30 years than 30-40 years (Table 1).

Similar study done by Kemenbradikumo pondei *et al.*,²⁰ observed the most of VVC and *T. vaginalis* infection were in the age group of 21-30 years. In contrast study done by Alo M.N *et al.*,²¹ shows; women within the age group of 31-35 years was high infection.

Trimester wise distribution

In the present study we observed, the infection of VVC was highest at third trimester 35(24%) followed by second trimester 5(14%) then least was in the first trimester 2 (10%) (Table 6). Similar finding reported by Alo *et al.*, and Glatthaar *et al.*,^{21,22} women within their 3ed trimester had the highest VVC infection (32%)while those their 1st trimester had least infection.

It has been observed that there is high concentration of oestrogen hormones during pregnancy and this provides favourable environment for the growth of candida, hence the highest prevalence in the third trimester.

Vulvovaginal trichomoniasis (VVT)

In the present study, a low prevalence (2%) was observed for trichomoniasis which is lower than the 10.6% reported by Mbu *et al.*,²³

Age & trimester wise distribution: Co-infection with *Candida* and *Trichomonas vaginalis*

The present study, out of 200 samples, 46 (23%) cases of vaginal infection were positive among these women. of 4 (2%) were co-infection infection.

Present study observed the most common infective age group of co-infection of Trichomoniasis & Candidiasis was 20-30 years than 30-40 years (Table 6). In other studies done by Donbraye *et al.*, 2010, Dickson Shey Nsagha *et al.*,²⁴ reported the highest co-infection reported within the age group of 26-30 years and 26-40 years respectively. This could be as a result of the fact that this age group is still sexually active.

The women within their 3rd trimester had the highest co-infection 3(2%) while those their 2nd trimester had least infection-1(2%). similar finding observed by Alo M.N (2012) *et al.*,^{21,22}. In contrast study done by Baekare *et al.*, 2002²³, reported the co-infection in the 2nd trimester also corroborates.

Many studies shows, 3rd trimester are more vulnerable for vaginal infection then other two trimester, there may be repeated vaginal examination as well as pelvic examination in preparation for delivery.

In our study, most common vulvovaginal infection (VVI) is caused by vaginal Candidiasis (21%) then trichomoniasis (2%) among pregnant women. Similar Study done by Dr. Bhatia Ibrahim reported, vaginal

Candidiasis largely contributed to pathological vaginal discharge 37.1% while trichomoniasis contributed the least (2.2%)²⁶

In conclusion the pregnant women are more vulnerable to vaginal infection irrespective of their age, trimester and education level. Because of avoidance of contributory factors such as; douching, wearing tight underwear, general personal hygiene.

VVC, Trichomoniasis and other vaginal infection can resolve within a short period of time, if not treated timely then, it may enhances the growth of co-infection and it is a major cause of morbidity in pregnant women in developing countries like India. Hence there is need to create awareness education through campaign, symposium, seminar, conference and media on the detrimental effect of this dangerous co-infection like Candidiasis & trichomoniasis among pregnant women. Rapid test should be mandatory routinely done for all pregnant women or in patients suffering from vaginal discharge and thus, help in early diagnosis and prompt treatment of patients.

This will ultimately prove beneficial to patients in reducing morbidity and associated adverse health outcomes in the form of pelvic inflammatory diseases and increased risk of acquiring other sexually transmitted diseases including human immunodeficiency virus infection.

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