Prevalence of Candida and Trichomonas in Vaginal Discharge of Rural Females not Seeking Medical Help: Microscopy and its Role in Vaginitis

Rachna Tewari*, Motilal P. Sah and Mridu Dudeja

Department of Microbiology, HIMSR, Jamia Hamdard, New Delhi, India
*Corresponding author

A B S T R A C T

In developing countries complaint of abnormal vaginal discharge is the major problem in females of reproductive age group. However most of the rural women do not contact medical facilities in time for adequate treatment due to many social factors or lack of awareness about the disease which leads to complications, risk of increased sexual transmission and insufficient data to deal with. This study is aimed to assess the prevalence of vaginal candidiasis and trichomoniasis among rural females not attending any medical clinic, to raise awareness about its prevention and treatment to reduce its complications. A total of 200 females with complaint of leucorrhoea were selected. Speculum guided high vaginal swabs were collected and processed by KOH mount and Grams stain in field and confirmed by culture on Sabrauds Dextrose agar (SDA) in lab. Out of the 200 females examined 73 (36.5%) had genital infection. Among the positive cases Candida sp. were found in 24 (12%) cases. 

Trichomonas vaginalis were obtained in 9 (4.5%) cases in this study. Prevalence was found significantly higher in lower qualification (p< 0.01). Microscopy is simple, cheap and reliable screening method to detect the prevalence of vaginitis in rural areas. The high rate of unnoticed genital infections warrants the need of constant health care and sensitization to prevent complications and acquisition and transmission of sexually transmitted diseases. So, it is essential to detect the disease and treat the cases in time in neglected and knowledge deprived areas.

K e y w o r d s
Candida, Trichomonas, Vaginal discharge.

Introduction

Abnormal vaginal discharge with or without other symptoms, is one of the major problem in developing countries. It can be infective or non-infective.

In rural areas it has been noted that this morbidity is tolerated silently by women due to various social factors as poor awareness, gender inequalities, lack of proper education and lack of focused counselling and financial dependence. Candidiasis and Trichomoniasis are two of the most common causes of vaginal discharge\(^{(1-6)}\). Candida is a yeast like fungus, normally found as the lower genital tract flora in 20-50 % of healthy asymptomatic females \((7-8)\). Carrier rates are higher in women using broad spectrum antibiotics \(^{(9)}\), pregnant, diabetic women \((10-11)\) and women with HIV/AIDS \((8,12,13)\). \textit{Candida albicans} is the commonest cause for most cases of vulvovaginal candidiasis (VVC) than other species of Candida. It is characterized by severe itching, burning sensation, redness and curdy white discharge \((9,14)\). In unattended
pregnant females can lead to preterm labour and low birth weight. Also these females are at risk of acquiring other STDs more.

Trichomoniasis is caused by a protozoa, *Trichomonas vaginalis* which is a sexually transmitted parasite resulting in discomfort, frothy yellow greenish vaginal discharge, irritation, pain in vulva, perineum, dyspareunia, dysurea and psychosocial distress in the infected patients. It has also been reported to cause complication in pregnant women like post abortion infection, post caesarean infection, preterm birth, low birth weight infants and preterm labour (15,16).

Asymptomatic or mild cases of Candidasis and Trichomoniasis may not be taken seriously by rural patients and may lead to severe complications like infertility or complications associated with pregnancy and make them vulnerable to other type of sexually transmitted diseases (STDs).

Keeping the above fact in view this study was carried out to see the hidden infection by organizing camps and door to door service to find out affected women who need treatment and counseling for these infections at right time so that spread and complications can be prevented.

**Materials and Methods**

A cross sectional study was conducted by the Department of Microbiology, HIMS, New Delhi, in March 2017. Camps were organized in slums and rural areas. A total of 200 women with complaints of vaginal discharge were recruited. After taking consent complete history and general and gynaecological examination was carried out. Three high vaginal sterile swabs from lateral wall and posterior fornix were collected using a speculum during comprehensive pelvic examination. The nature of discharge (amount, colour, character, and smell) was evaluated. First swab is used to prepare Wet mount to see motility of *Trichomonas vaginalis*, fungal buds, bacteria and pus or other type of cells and to make KOH preparation. Second swab of each patient was used to make smears for Grams and Giemsa stain. Third swab was inoculated in normal saline and carried to microbiology lab at sterile conditions for culture. Blood Agar (BA) and Sabrauds Dextrose agar (SDA), Hi Media, Mumbai was inoculated for swabs showing budding yeast in Grams stain and KOH mount (17-18).

**Inclusion criteria**

Females of age 18-48 years with complaint of excessive vaginal discharge.

Those who were willing for speculum examination

**Exclusion criteria**

Not giving consent for speculum examination.

Women who were menstruating or have attained menopause.

Bacterial and non-infective cases were excluded.

**Identification of organisms**

For Candida

KOH preparation – Little vaginal secretion was taken on clean slide and a drop 10% KOH was mixed and covered with cover slip. Microscopy under 10x and 40x was performed. Round and oval shaped budding yeast cell were identified as Candida.

Grams stain- Gram positive budding yeast cell were identified as Candida in Gram stained vaginal smear.
Culture – It was done on SDA (Sabrauds Dextrose Agar) in lab. Cream coloured, smooth, moist colonies were confirmed as Candida by making their Grams Stain.

GTT – Isolated candida colonies were transferred to human sera and seen for tube like yeast cell and pseudohyphae were seen under microscope (18).

For Trichomonas vaginalis

Wet mount: motile trophozoites were identified.

Giemsa Stain: oval trophozoites were seen (18).

Ethical clearance was obtained from the department. Eligible females were informed and counselled about the benefits and risk of studies. The consent of all patients were verbally taken before collecting sample and data. Data were analysed using MS excel and appropriate statistical methods.

Results and Discussion

In the current study of two hundred females, with complaint of vaginal discharge following observations were noted. Out of these 200 women 73 (36.5%) were diagnosed to be having infective vaginitis. The prevalence of candidiasis was found in 24 (12%) females out of which majority i.e. 22 (90%) were confirmed as Candida albicans and 10% as other Candida species. Trichomonas vaginalis was identified in 9 (4.5%) females. No case of mixed infection was found (Table 1).

Candida was found in high frequency (52.7%) in age group 39-48 years of females. Prevalence of vaginal candidiasis and Trichomoniasis was found higher in unemployed 25 (20.4%) than employed women 8 (10.1%). No significant correlation was found with educational status in these areas. Lower incidence 14.8% of infection was found in not educated females where as 18% in higher qualified and 21.6% in mediocre (Table 3).

24 (12%) patients were identified as having infection with Candida species. By performing microscopic examination in field 75% strains can be isolated by KOH mount and 83.3% by Grams staining when we compared it with culture all (100%) (Table 3). Germ tube test was found positive for 91.6% strains. Trichomonas vaginalis was identified in 9(4.5%) patients by microscopic examination by wet mount and Giemsa Stain (Table 4).

Infection in the genital tract produce serious complications and can be transmitted to others (19-20). The doctors or health workers commonly encounter the females associated with vaginal discharge with or without itching or foul smell. Infectious vaginitis primarily caused by bacteria, yeast and protozoa account for 90% of the disease. Vulvovaginal candidiasis and trichomoniasis are the leading cause of vaginitis (21-22).

Mostly these studies have been conducted in hospitals in urban area. In our study we are reporting about the females who themselves have not contacted doctors but have been contacted by the health care workers to find out the hidden infection in the community which should also be prevented and treated in time to control the transmission. So this prevalence should be added to the prevalence being reported till now.

In our study we have reported 12 % females having candidiasis which is higher than reported by other studies(23-24) which may be due to their large sample size. However it was slightly less as compared to 22% reported by Venugopal et al., (25).
Table 1. Etiology of vaginal discharge and its prevalence

| Aetiology                | Number (Total -200) | Percentage |
|--------------------------|---------------------|------------|
| *Candida albicans*       | 17                  | 8.5%       |
| *Candida species*        | 07                  | 3.5%       |
| *Trichomonas vaginalis*  | 9                   | 4.5%       |
| Other infection          | 40                  | 20%        |
| No organism found        | 127                 | 63.5%      |

Table 2. Demographic characteristics associated with infection

| Level of Education        | Number (N) | Organism          | number (n) | (Percentage) n/Nx100 |
|---------------------------|------------|-------------------|------------|----------------------|
| Less than high school     | 108        | Candida           | 12         | 11.1                 |
|                           |            | Trichomonas       | 4          | 3.7                  |
| More than high school     | 72         | Candida           | 9          | 12.5                 |
|                           |            | Trichomonas       | 4          | 5.5                  |
| Not educated              | 20         | Candida           | 3          | 15                   |
|                           |            | Trichomonas       | 1          | 6.6                  |
| Employed                  | 78         | Candida           | 6          | 7.6                  |
|                           |            | Trichomonas       | 2          | 2.5                  |
| Unemployed                | 122        | Candida           | 18         | 14.7                 |
|                           |            | Trichomonas       | 7          | 5.7                  |
| AGE in years              |            |                   |            |                      |
| 18-28                     | 121        | Candida           | 7          | 5.7                  |
|                           |            | Trichomonas       | 3          | 2.4                  |
| 29-38                     | 60         | Candida           | 7          | 11.6                 |
|                           |            | Trichomonas       | 4          | 6.6                  |
| 39-48                     | 19         | Candida           | 10         | 52.6                 |
|                           |            | Trichomonas       | 2          | 10.5                 |
| Pregnant                  | 28         | Candida           | 9          | 32.1                 |
|                           |            | Trichomonas       | 1          | 3.5                  |
| Not pregnant              | 172        | Candida           | 15         | 8.7                  |
|                           |            | Trichomonas       | 8          | 4.6                  |
Table.3 Lab result of *Candida* species

| Positive specimen | KOH mount18/24(75) | Grams stain 20/24 (83.3) | Culture 24/24 (100) | Germ tube 22/24 |
|-------------------|-------------------|-------------------------|-------------------|-----------------|
| 12                | +                 | +                       | +                 | 11              |
| 8                 | -                 | +                       | +                 | 8               |
| 4                 | +                 | -                       | +                 | 3               |

Table.4 Lab result of *Trichomonas vaginalis*

| Positive specimen | Wet mount7/9 | Giemsa staining 7/9 |
|-------------------|--------------|---------------------|
| 5                 | +            | +                   |
| 2                 | -            | +                   |
| 2                 | +            | -                   |

Table.5 Prevalence of candidiasis and *Trichomonas vaginalis* reported earlier and in present study

| Author                  | Year | % age       | Place of study     | Journal                                             |
|-------------------------|------|-------------|--------------------|-----------------------------------------------------|
| Swetha Venugopal, Kannan Gopalan | 2017 | Candida 22% Trichomonas 25% | Salem. Tamil Nadu | Indian Journal of Sexually Transmitted Diseases and AIDS Volume 38 |
| B. Anuradha*, M.C.K. Joanna and M. Praveena | 2015 | Trichomonas 22.22% | Khammam, India | IJCMAS |
| R Bansal, N Jindal, B Chand | 2013 | Trichomonas 6.1% | Punjab India | Ijmm |
| Alli et al.,           | 2011 | Candida 22% | Nigeria            | JMBR |
| Madhivanan et al.,     | 2008 | Candida 30.8% Trichomonas 8.2% | Mysore India | IJMM |

Trichomoniasis is a common STD found in humans. We have reported prevalence of 4.5% which is equivalent to reported by other studies. It has been found that prevalence varies from place to place and with social condition from study to study and reported to be low as 2-8%. Whereas high 22-45%.

This may be due to the high risk nature of group (25-27). A very high prevalence of TV 47% was reported (28). Studies have indicated it as reemerging pathogen and found to correlate with age.

Despite the various techniques available and found to be little more sensitive for diagnosing candida species and Trichomoniasis wet mount with clinical features still comes out as reliable screening test (Tables 4 and 5) in rural areas. Although many studies (29) have found more positivity by culture (13.9%) then by wet mount (11.
4%). Microscopy is reliable, inexpensive method, which can be done in field with no requirement of specialist.

Microscopy in the field, substantiated with sign and symptoms is easy, inexpensive and reliable methods to detect the infection in rural females not attending clinics for treatment. It is highly recommended that STDs should be prevented, diagnosed and treated early to avoid complications and reduce transmission. This study was done to create awareness in rural and slum area about health care facilities and self-concern in females for their health. Camps and door to door surveys should be performed to approach these types of unaware females.

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