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Original Article

A study of clinical and sociodemographic features of trichomoniasis in symptomatic and asymptomatic female patients attending the STD clinic using wet mount and culture as diagnostic tools

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ABSTRACT

Background: Trichomoniasis is a sexually transmitted infection caused by the protozoan *Trichomonas vaginalis*, which accounts for more than half of all curable STIs (sexually transmitted infections) worldwide. **Objectives:** The study was performed to determine the disease characteristics and prevalence of trichomoniasis in asymptomatic and symptomatic female patients using wet mount and culture as the diagnostic methods. **Materials and Methods:** A cross-sectional study was conducted at the OPD of the Institute of Dermatology and Venereology at a tertiary-care center in South India for a period of nine months. 500 female patients aged > 18 years and < 55 years were enrolled in the study. 250 symptomatic and 250 asymptomatic patients were selected randomly. **Results:** A total of 8% of the women had trichomoniasis; 31 (12.4%) were in the symptomatic group and 9 (3.6%) were in the asymptomatic group. The risk factors associated with trichomoniasis in our study were women from urban areas with low socioeconomic status, who were single and separated, women with multiple sexual partners, and women whose husbands had extramarital contacts. **Conclusion:** Trichomoniasis is a marker for high-risk sexual behavior and the relatively high prevalence rate in the asymptomatic group indicates the need for routine screening of women in their reproductive age group to reduce the risks of acquiring other STIs and HIV infection.

Key words: Diamond's medium, *Trichomonas vaginalis*, wet mount, culture

INTRODUCTION

Trichomoniasis is caused by the pathogenic protozoan *Trichomonas vaginalis*, and it is one of the commonest non-viral sexually transmitted diseases (STDs) [1]. Trichomonads are flagellated eukaryotic microbes that belong to the protozoan order Trichomonadida. Typically Trichomonads are pyriform shaped, 7-32 micrometer long, 5-12 micrometer wide and roughly the size of leukocyte [2]. It causes approximately more than 180 million infections worldwide annually. Many infected individuals remain mostly asymptomatic; when symptomatic, they present with vaginal discharge, cervicitis, pelvic inflammatory disease, and infertility in women and non-gonococcal urethritis in men [3]. A higher prevalence of trichomoniasis has been seen in women who are multiparous, married at a very early age, and pregnant women [4]. Other risk factors associated are multiple sexual contacts, poor personal hygiene, and low socioeconomic status [5]. Coinfections with other sexually transmitted disease caused by pathogens such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema*

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pallidum, human papillomavirus, or herpes simplex virus types 1 and 2 [6] are common in women with trichomoniasis. Coinfection of trichomoniasis and HIV infection may facilitate viral transmission and acquisition by eliciting an inflammatory response and the recruitment of CD4 cells in the vaginal epithelium; also, there is an increased risk of HIV transmission in *Trichomonas vaginalis*-infected patients [7,8] by 2- to 3-fold, and the prevalence rate of *T. vaginalis* infection in HIV-affected patients ranges from 9% to 30% [9]. Trichomoniasis as such is highly prevalent in sexually active women, varying from 5% to 74% and 5% to 29% in men. About 10% to 50% of patients harbor trichomonads without developing any symptoms, which serve as a major reservoir of infection [10]. In this background, we planned to conduct a study to determine the sociodemographic, and clinical characteristics, and to diagnose as well as compare *Trichomonas* infection in asymptomatic and symptomatic female patients attending the STD OPD, using wet mount and culture as the diagnostic methods, which are the gold standard [6].

MATERIALS AND METHODS

A cross-sectional study was conducted at a tertiary-care hospital at the Institute of Dermatology and Venereology in South India for the period of nine months. 500 female patients aged > 18 years and < 55 years were enrolled in the study. Female patients with complaints of vaginal discharge, pruritus, dysuria, dyspareunia, and lower abdomen pain were included in the symptomatic group, and patients attending the STD OPD for routine checkups and screening were included in the asymptomatic group. Patients on any antibiotics, including metronidazole, taken two to three weeks before the study enrollment and those with severe medical comorbidities were excluded from the study. A detailed history was obtained on the following parameters: age, occupation, socioeconomic status, educational status, marital history, sexual, contraceptive, obstetric, past, personal, recent treatment history, history suggestive of systemic ailments, and symptoms related to STIs. Detailed genital examination was done using Cusco's self-retaining bivalve speculum, and the abnormalities in the vulva, vagina, and cervix were noted. The amount, odor, color, and consistency of vaginal discharge were noted. Four vaginal swabs were taken from the posterior fornix or collected directly from the discharge. One swab was used for the wet mount preparation, another was inoculated directly into the Diamonds TYIS-33 medium, and the other two swabs were used for Gram's staining and KOH preparation, respectively. Cervical smear was also taken from all patients. The institutional ethical committee clearance was taken, and written informed consent was obtained from all recruited women. All participants underwent culture for *Gonococcus*, blood VDRL, and HIV.

Statistical analysis: The data was collected and tabulated in a Microsoft Excel worksheet. Computer-based analysis was performed using SPSS 13.0 software (SPSS, Chicago, IL, USA). The categorical variables were summarized as proportions and percentages. The continuous variables were summarized as means and standard deviations. For comparison of means, the unpaired *t*-test and one-way ANOVA were used for two and more than two groups, respectively. For the comparison of proportions, the chi-squared test was employed. If the cell values were less than five, Fisher's exact test was used. For comparison within the group, the chi-squared goodness-of-fit test was used.

The diagnosis of trichomoniasis was established on the basis of the following tests.

Wet mount: A drop of normal saline was put over a clean, grease-free microscopic slide. To this, a drop of vaginal fluid was added and mixed well. A coverslip was put over the mixture to allow a uniform spread without air bubbles. The slide was observed under 40x magnification.

Reading: Pear-shaped flagellated organisms approximately the size of a lymphocyte (10–20 μm) or that of a small neutrophil with the characteristic jerky motility were noted.

Culture: The wet mounts were prepared from the drop of Diamond's culture media and examined for the presence of motile trichomonads after 48 hours of incubation and on the 3rd, 5th, and 7th day. The Diamond's culture media tubes with 5 mL of the broth were incubated in an anaerobic atmosphere at 35°C.

Mucopurulent cervicitis: Diagnosed in those patients with Gram stain of cervical smear, showing polymorphonuclear leukocytes more than 30 per oil immersion field in the cervical mucus.

Pelvic inflammatory disease (PID): The diagnosis of PID was made if, in addition to the presenting symptoms of abnormal vaginal discharge, lower

abdominal pain, adnexal structures involvement elicited on clinical examination.

Ethics Statement

Ethical clearance was obtained from the ethical committee of the institution prior to the study.

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RESULTS

The mean age of the patients in the symptomatic group was 33.85 ± 9.65 years, and in the asymptomatic group, it was 34.25 ± 9.36, (Fig. 1). In our study, 8% of women had trichomoniasis out of the 500 women examined. 31 out of the 250 women (12.4%) in the symptomatic group had an infection with *T. vaginalis* by culture and/or wet mount, and 9 out of 250 (3.6%) asymptomatic women had trichomoniasis by culture and/or wet mount. The wet mount test was positive in 67.74% of the symptomatic participants and 55.5% of the asymptomatic (Figs. 2a and 2b). The wet mount preparation using normal saline had low sensitivity and high specificity. Culture done using Diamond's liquid media had sensitivity and specificity of 100%, PPV 100%, and NPV 100% when observed within three days of inoculation (Fig. 3). The culture examined on the 7th day of inoculation had a sensitivity of 77.50%, a specificity of 100%, a PPV of 100%, and an NPV of 98.5%. The comparison between wet mount and culture is shown in Table 1. The mean age group of *Trichomonas*-positive patients in the symptomatic group was 32 ± 7 years, whereas in the asymptomatic group, the mean age was 34 ± 8 years. 74.2% and 62.50% in the symptomatic and asymptomatic groups were married, and 25% were single among both the symptomatic and asymptomatic patients. 21 patients (67.7%) in the symptomatic group and 6 patients (67%) in the asymptomatic group lived in urban areas. 54.80% and 75% of the patients in the symptomatic and asymptomatic groups, respectively, belonged to the lower class of socioeconomic status, followed by 32.3% of the symptomatic patients and 25% of the asymptomatic patients belonging to the upper lower class. Most of the patients in both groups (45.20%) had an education level till high school, and 12% in the symptomatic group were illiterate. 14 (45.2%) patients in the symptomatic group and 4 (40%) patients in the asymptomatic group had a history of pre/extramarital contact. 22.6% of the partners of the symptomatic group

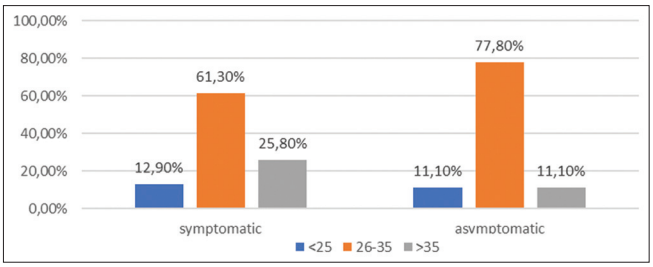

Figure 1: Age distribution of *Trichomonas*-positive cases.

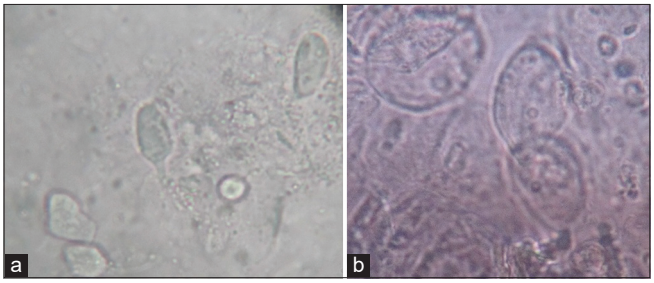

Figure 2: (a and b) Image showing *Trichomonas vaginalis*: Wet mount in low and high power.

Table 1: TV-positive cases among both the symptomatic and asymptomatic patients

Wet-Mount	Culture in Symptomatic Group		Culture in Asymptomatic Group	
	Positive	Negative	Positive	Negative
Wet-mount positive	21	0	5	0
Wet-mount negative	10	219	4	241
Total	31	219	9	241

were promiscuous. 7.7% of the partners of positive cases had a history of urethritis, dysuria, and pruritus. The disease characteristics of *Trichomonas*-positive cases in our study are as follows. The mean age of symptom onset in patients with trichomoniasis was 30 years of age, and the mean duration of vaginal discharge in patients with trichomoniasis was 4.8 months; 77.4% of women had vaginal discharge for less than a year, and 22.6% had discharge for more than a year. Associated vulval itching was found in 29% and lower abdominal pain in 9.7%. About 90.3% of women in the symptomatic group had moderate to profuse vaginal discharge, and 64.5% had foul-smelling discharge (Fig. 4) (Table 2). Out of nine positive cases in the asymptomatic group, one patient had moderate vaginal discharge on examination. 12.9% of the patients had a history of abortion when compared with negative cases, which was statistically significant. 65.5% of women had increased symptoms during menstruation. Only 12.9% of the patients had erythema on the vaginal wall. None of the patients in our study showed a strawberry cervix (colposcopy not done). A genital ulcer was noted in only

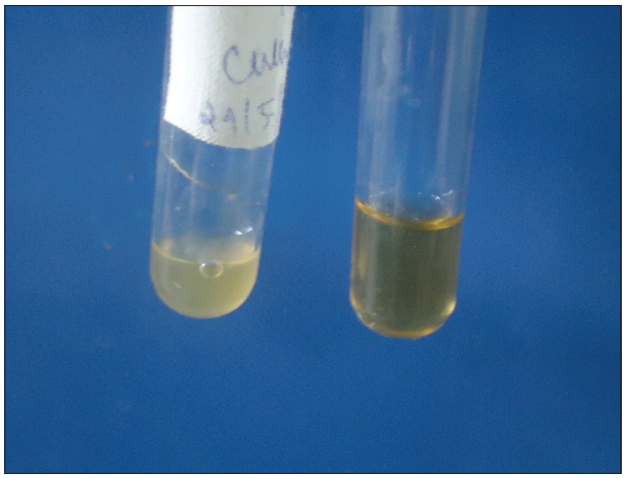

Figure 3: Image indicating the growth of Trichomonas vaginalis in modified Diamond's medium as a turbid media.

Figure 4: Image of a patient with trichomoniasis: profuse, frothy vaginal discharge.

one patient. Bacterial vaginosis was the commonest coinfection noted followed by mucopurulent cervicitis and candidiasis. Three patients in symptomatic and six patients in the asymptomatic group were positive for HIV. Table 3 lists the various diagnoses of the patients enrolled in our study. All trichomoniasis patients were treated with tablet metronidazole 400 mg twice daily for seven days, and the symptomatic partners were treated with tablet secnidazole 2 gm stat dose.

DISCUSSION

Trichomoniasis is a common non-viral sexually transmitted infection. The prevalence rate of *Trichomonas vaginalis* infection largely varies among the female population all over the world, depending on the group of population studied and the method of investigation employed in the diagnosis, ranging from 5% to 74%. The highest rate of prevalence was reported

Table 2: Vaginal discharge characteristics of the TV-positive cases

Vaginal Discharge		Patients			
		Symptomatic Group		Asymptomatic Group	
		Trichomoniasis		Trichomoniasis	
		Positive		Positive	
		N	%	N	%
Discharge amount	Scanty	3	9.7	8	88.8
	Moderate	15	48.4	1	11.1
	Profuse	13	41.9	0	0
Discharge odor	Odorless	9	29.0	6	66.7
	Foul-smelling	20	64.5	2	22.2
	Fishy	2	6.5	1	11.1
Discharge consistency	Curdy	0	0.0	0	0.0
	Flocculent	1	3.2	1	11.1
	Thick, homogeneous	1	3.2	0	0.0
	Frothy	18	58.1	2	22.2
	Watery	5	16.1	2	22.2
	Mucoid	6	19.4	4	44.4
Discharge color	White	18	58.1	6	66.7
	Greyish	1	3.2	0	0.0
	Purulent	12	38.7	0.0	0.0
	Serosanguinous/ white	0	0.0	3	33.3
	Total	31	100.0	9	100.0

Table 3: Diagnosis of the study groups

Disease	Symptomatic Group	Asymptomatic Group
1. Trichomonas vaginalis	31 (12.4%)	9 (3.6%)
2. Bacterial vaginosis	54 (21.6%)	27 (10.8%)
3. Candidiasis	32 (12.8%)	29 (11.6%)
4. Mucopurulent cervicitis	40 (16%)	26 (10.4%)
5. Physiological/non venereal	69 (27.6%)	145 (58%)
6. Herpes simplex	6 (2.4%)	0
7. HIV	3 (1.2%)	6 (2.4%)
8. Wart	2 (0.8%)	0
9. Genital scabies	1 (0.4%)	0
10. Cervical cancer	5 (2%)	0
11. Cervical growth	1 (0.4%)	0
12. Others	6 (2.4%)	8 (3.2%)

among STI clinic attending patients and among high-risk populations [10,11]. In our study, a total of 8% (40 out of 500) of women had trichomoniasis, 31 (12.4%) women in the symptomatic group and 9 (3.6%) women in the asymptomatic group diagnosed by culture. The prevalence of trichomoniasis observed in our study was comparable to the study done by Mahmoud et al., which was a hospital-based study done in Egypt with a sample size of 450, among which 290 were symptomatic and 160 were asymptomatic. The prevalence of trichomoniasis in their study was 7.7%, among which 30 (10.34%) were symptomatic cases and 3.1% were asymptomatic [12]. In a study by Cevahir et al, which included 310 symptomatic patients, the prevalence rate of trichomoniasis was

found to be 12.9%. This is comparable to our study (12.4%) in the symptomatic group [13]. Another study was done by Chakaraborthy et al. [14] on the symptomatic and asymptomatic patients from rural and urban areas (102 cases) in Surat, India, showing a total prevalence of 34.4%, which was significantly higher when compared to our study (8%). The prevalence rate of *Trichomonas vaginalis* infection increases with age, unlike in *Chlamydia* and gonorrhea [10]. The increased prevalence of *Trichomonas* infection in older women suggests a longer duration of the infection and its predominantly asymptomatic nature [15], which was evident in our study, as the mean age at presentation for women with *T. vaginalis* infection was 32 ± 7 years in the symptomatic and 34 ± 9 years in the asymptomatic group. This was comparable with a population-based study from Vanuatu, Australia, by Fotinatos et al. [16], and a study done by Haythan et al., who reported a mean age of 36.6 years. However, a study by Leon et al. [17] reported a lower mean age at presentation, i.e., 20 to 25 years. This probably reflects the early onset of sexual activity in the high-risk population. About 74.2% of women in the symptomatic group and 62.5% of women in the asymptomatic group were married, and 25% of women were single among both the symptomatic and asymptomatic patients. The risk of infection is more likely in women living single (16.1%) as compared to *Trichomonas*-negative single women (5.5%). Klinger et al. [18] observed that the risk of trichomoniasis was significantly increased among women who were separated and was comparable to our study. Although trichomoniasis was observed in the majority of married women, the prevalence in separated women was found to be higher in their study. About 54.8% of women with trichomoniasis in our study belonged to lower (class V) socioeconomic status when compared to the upper lower (class IV) in *Trichomonas*-negative patients. The majority of women with trichomoniasis in our study had low education status till primary standard. About 48.7% of women studied till 5th standard, 35.9% were educated till 6–12th standard, whereas most of the *Trichomonas*-negative cases had (52.2%) education till 6–12th standard. 12% were illiterate in the symptomatic group. However, in a study conducted by Haythm et al., illiterates formed the majority (61.8%) [19]. Most of the *Trichomonas*-positive women and *Trichomonas*-negative women in our study were tubectomized. However, 10.3% of *Trichomonas*-positive women gave a history of at least one episode of abortion when compared to only 2% of *Trichomonas*-negative women with a history of abortion, which is significant. Six women in the symptomatic group and two women in the asymptomatic group gave a history of previous infection in the form of vaginal discharge, and two patients in the symptomatic group had a history of PID, which was not significant when compared to *Trichomonas*-negative cases. Only two women among the *Trichomonas*-positive cases in the asymptomatic group had a history of alcohol abuse, which was not significant, and no woman gave a history of smoking in our study. About 45.2% of *Trichomonas*-positive cases in the symptomatic group and 50% of women in the asymptomatic group gave a history of premarital and extramarital contact, when compared with only 8.5% of women in the *Trichomonas*-negative group had a history of pre- and extramarital contact, which was statistically significant. Seven women among the *Trichomonas*-positive cases were indulged in prostitution as compared to four women among the *Trichomonas*-negative cases. About 22.6% of trichomoniasis-positive women in the symptomatic group gave a history of their husband having extramarital contact when compared to only 1.25% of women among the negative cases, which was statistically significant. A history suggestive of symptoms of urethritis and balanitis was noted in 7.7%, and 5.1% of men had a history of dysuria and pruritus. The risk factors associated with trichomoniasis infection in our study were women with low socioeconomic status, with a history of pre- or extramarital sexual contact, their husband having extramarital contact, a history of a symptomatic partner with, for instance, dysuria, and urethral discharge, which was suggestive of trichomoniasis in men. Kaur et al. [20], in a study done in North India to assess the prevalence rate of *Trichomonas vaginalis* infection in the symptomatic women and in women diagnosed with carcinoma cervix and HIV, observed that women who were housewives, from low to middle socioeconomic status and non-users of contraception were significantly associated with trichomoniasis. Klinger et al. [18] in their study to determine the predictors and risk factors associated with *Trichomonas* infection in women in Moshi, Tanzania, observed that having a partner with infection was the strongest risk factor in women. Other risk factors observed in their study were daily alcohol consumption, being separated, and having a partner with extramarital contact. Sutton et al. [21] observed that factors associated with an increased risk of *T. vaginalis* infection in women in the U.S. were belonging to a non-Hispanic black race, having more sex partners, being elderly, having low education level, and belonging to a low socioeconomic status.

Trichomonas vaginalis is known to cause persistently untreated cases [22]. In this study, the mean duration of the onset of symptoms in patients diagnosed with trichomoniasis was thirty years, and the mean duration of vaginal discharge in positive patients was 4.8 months (149 days). Signs of infection in symptomatic women include vaginal discharge (92.5%), frothy, foul-smelling discharge (50%), and vaginal wall erythema (10%) [23]. The characteristic features of the infection are present in only about 40% to 50% of patients [24]. Women with *T. vaginalis* may have abdominal pain due to salpingitis or endometritis and postcoital bleeding due to cervicitis [19]. The clinical features associated with trichomoniasis infection have a relatively low positive predictive value because of the frequent occurrence of similar signs and symptoms among women with other STI infections [25]. Fouts et al. [26] in their study conducted on women attending the STI clinic in Georgia found that, if only the clinical features alone were used to diagnose trichomoniasis, about 88% of the infected women would be missed, and 29% women would be falsely diagnosed as having an infection. In our study, vaginal discharge was the most frequently reported symptom in trichomoniasis-positive cases (sensitivity: 81.5 %), followed by vulval itching, lower abdominal pain, and dysuria. However, these symptoms had low positive predictive values for trichomoniasis. Women with concurrent trichomoniasis and other infections were more symptomatic with a higher frequency of reported itching and lower abdominal pain as compared to those with trichomoniasis alone, although this was not statistically significant. Among the clinical signs documented, women presenting with profuse, malodorous, frothy vaginal discharge, and mucopurulent discharge were significantly associated with trichomoniasis. Frothy vaginal discharge was observed in 58% of women in our study and was the most specific sign with a positive predictive value of 100%. The mucopurulent discharge was observed among 38.7% of women. Colpitis macularis, which is a specific sign of *Trichomonas* infection, is detected reliably only by colposcopy and rarely by routine examination [24, 25]. The colpitis macularis sign was not seen in any of the women by naked eye examination in our study, as the colposcopy was not done. Wolner-Hanssen et al. [27] in their study on clinical manifestations of trichomoniasis done on women attending an STI clinic observed that frothy discharge was found in only 8% of women with trichomoniasis and had a specificity of 99% and a PPV of 62%. Colpitis macularis was a highly specific sign (99%) and had a high PPV (90%), but was seen without a colposcope in only 2 of the 52 women who had the finding on colposcopy. Purulent vaginal discharge in their study had a specificity of 76% and a PPV of only 30%.

Investigation

Wet mount: Wet mount using normal saline for the demonstration of trichomoniasis had low sensitivity (65%) and high specificity (100%), PPV 86.7%, NPV 97%, and LR 74.75%. The wet mount was positive in 21 patients and negative in 10 patients in the symptomatic group, positive in 5 patients and negative in 4 patients in the asymptomatic group. All wet-mount negative patients were positive by culture P-value < 0.05 and were significant among both the symptomatic and asymptomatic patients.

Culture of Diamond's medium: Culture proved diagnosis in all 40 cases of trichomoniasis with 100% sensitivity and specificity. The culture had 100% sensitivity and specificity when examined within three days of inoculation. On the 5th day and 7th days, when the sensitivity levels decreased to 97.5% and 77.5%, respectively, the specificity remained at 100%. In a study done by Mohmoud et al. [12] on 450 cases, culture identified 35 (100%) cases whereas wet-mount and PAP smear diagnosed 34.2% and 60% of cases, respectively, which is similar to our study. Another study, done by Cevahir et al. [13], showed that culture was positive in all 40 cases of trichomoniasis (100%), whereas wet mount was positive in only 20 (50%) cases.

COINFECTION

The majority of *Trichomonas*-positive patients in our study were coinfected with bacterial vaginosis (64.3%) in the symptomatic group and about 35.7% in the asymptomatic group with a p-value of 0.285, which was not significant. Mucopurulent cervicitis ($p = 0.821$) and candidiasis ($p = 0.999$) were not statistically significant in our study.

CONCLUSION

The risk factors associated with trichomoniasis in our study were women belonging to low socioeconomic status, who were single, separated from their husbands, having multiple sexual partners, and women whose husbands had extramarital contact. Diagnosis by the culture method is the gold standard investigation for trichomoniasis, with a 100% sensitivity and specificity. The wet mount test had low sensitivity and high

specificity. Also, wet mount failed to identify 14 cases that were positive by culture ($p < 0.05$). Routine use of wet mounts solely for the diagnosis of trichomoniasis may lead to false-negative results, hence culture must be routinely done whenever wet mount is negative, trichomoniasis is strongly suspected, and in all high-risk groups. The clinical characteristics of symptomatic patients are moderate to profuse foul-smelling frothy, mucopurulent vaginal discharge when compared to scanty to moderate odorless mucoid discharge of asymptomatic patients. The relatively high prevalence in the asymptomatic group also indicates the need for routine screening of women in their reproductive age group and include counseling and behavioral changes to reduce the risks of acquiring other STIs.

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Study Limitations

The limitation of this study was the lack of the use of molecular diagnostic methods such as PCR and NAAT as diagnostic methods, which have higher sensitivity and specificity.

ABBREVIATIONS

OPD: Outpatient department
STIs: Sexually transmitted infections
PID: Pelvic inflammatory disease
STD: Sexually transmitted disease
VDRL: Venereal Disease Research Laboratory
PPV: Positive predictive value
NPV: Negative predictive value
LR: Likelihood-ratio test
PCR: Polymerase chain reaction
NAAT: Nucleic Acid Amplification Test
TV: Trichomonas vaginalis
BV: Bacterial vaginosis

Statement of Human and Animal Rights

All the procedures followed were in accordance with the ethical standards of the committee responsible on human experimentation (institutional and national) and with the 2008 revision of the Declaration of Helsinki of 1975.

Statement of Informed Consent

Informed consent for participation in this study was obtained from all patients.

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