Original Article

Trichomoniasis: How do we diagnose in a resource poor setting?

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Abstract

Background: Diagnosis of *Trichomonas vaginalis vaginalis* infection based solely on clinical symptoms and signs is unreliable because the spectrum of infection is broad and other sexually transmitted pathogens cause similar signs and symptoms. **Aims:** Our study was undertaken to study the frequency of *T. vaginalis* infection in women presenting with vaginal discharge, to characterize the clinical features, and to study the sensitivity and specificity of microbiological investigations in the diagnosis of the same. **Materials and Methods:** This was a hospital-based descriptive study done on 400 female patients with vaginal discharge attending the Gynecology out-patient department (OPD) of JIPMER, Puducherry, from May 2010 to July 2011. Women of age between 20 years and 50 years presenting with vaginal discharge irrespective of marital status, were included, and detailed history was elicited and thorough examination was performed. **Results:** In 400 women presenting with vaginal discharge from Gynecology out-patient department (OPD) included in the study, *T. vaginalis* infection was found in 27 (6.75%) women. The risk factors for trichomoniasis included history of pre- or extramarital sexual contact in the woman or her partner, symptomatic partner, and alcohol consumption. A positive association with pelvic inflammatory disease was also observed. The most frequent symptoms included lower abdominal pain, dysuria, and dyspareunia. Combining of Whiff test, pH > 4.5, and pus cells in Gram-stained smear, the specificity in diagnosing the infection (97.3%) approached that of the reference standard, i.e., culture. On combining wet mount with Papanicolaou smear, the sensitivity increased to 92.6%, which was higher than that individually done. **Conclusion:** To conclude, diagnosis of *T. vaginalis* infection based solely on clinical symptoms and signs is unreliable, and combination of simple laboratory tests increases the diagnostic performance close to the reference standard (culture), especially in resource poor settings.

Key words: Culture, Papanicolaou smear, *Trichomonas vaginalis,* vaginal discharge, wet mount

INTRODUCTION

Trichomoniasis, caused by parasitic protozoan *Trichomonas vaginalis*, is the most common non-viral sexually transmitted infection (STI). Diagnosis of *T. vaginalis* infection based solely on clinical symptoms and signs is unreliable because the spectrum of infection is broad and other sexually transmitted pathogens cause similar signs and symptoms.[1] Therefore, the laboratory plays a key role in the diagnosis of this infection; and more so, it is compelling to identify *T. vaginalis* infection reliably in resource poor settings with the readily available tests. The present study was proposed to address these issues. Our study was undertaken to study the frequency of *T. vaginalis* infection in women presenting with vaginal

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discharge, to characterize the clinical features, and to study the sensitivity and specificity of microbiological investigations in the diagnosis of the same.

MATERIALS AND METHODS

This was a hospital-based descriptive study done on 400 female patients with vaginal discharge attending the Gynecology out-patient department (OPD) of JIPMER, Puducherry, from May 2010 to July 2011. The Institute Ethics Committee clearance was obtained and informed consent was taken from the recruited women. Women of age between 20 years and 50 years presenting with vaginal discharge, irrespective of marital status, were included, and detailed history was elicited and thorough examination was performed. Women who had used antibiotics or vaginal medication in the previous 14 days and pregnant women were excluded from the study.

The vaginal pH was measured and a sterile cotton swab was used to collect five samples of vaginal discharge from the posterior fornix under direct vision. In virgins, the specimen was obtained from the introitus. The Papanicolaou smears (Pap smears) were taken and a bimanual examination was done in all, except in virgins, to look for adnexal tenderness. The first swab was subjected immediately to wet mount microscopy to observe for motile trichomonads under 100× and 400× magnifications. With the second sample, a 10% potassium hydroxide (KOH) mount was prepared and Whiff test was done and the same was examined for the presence of budding yeast cells under 100× and 400× magnifications. The third sample was immediately inoculated directly and swirled into Diamond medium (Himedia labs, Mumbai, India) for *T. vaginalis* culture. The culture tubes with 5 ml of the broth were incubated in an anaerobic atmosphere at 35°C. The fourth sample was inoculated on to Sabouraud's dextrose agar medium for candidia culture. The fifth sample was streaked onto a microscopic slide for Gram staining to check for the presence of clue cells, yeast cells, and pus cells under 1000× magnification. Women with high-risk sexual behavior were counseled and tested for HIV using enzyme-linked immunosorbent assay (ELISA).

A diagnosis of trichomoniasis was made based if the wet mount showed pear-shaped organisms with characteristic jerky movements or if the Pap smear showed blue or grey pear-shaped organisms with bright-red granules.[2] From the culture, diagnosis was made by performing wet mounts for evidence of motile trichomonads by examining cultures after 24 h of incubation and then daily up to 7 days. Bacterial vaginosis was diagnosed based on Amsel's criteria,[3] and a diagnosis of candidiasis was made based on positive microscopy and/or culture. A diagnosis of pelvic inflammatory disease was made if, in addition to the presenting symptoms of abnormal vaginal discharge and lower abdominal pain, adnexal tenderness was elicited on examination.[4]

The data collected was tabulated in Microsoft Excel worksheet and computer-based analysis was performed using the SPSS 13.0 software (SPSS, Chicago, IL, USA). For comparison of means, unpaired *t*-test and one-way analysis of variance (ANOVA) were used for two and more groups, respectively. For comparison of proportions, Chi-square test was used. In cases where any one of cell value was <5; Fisher's exact test was used.

RESULTS

Among the 400 women enrolled in our study, 27 (6.75%) had infection with *T. vaginalis*, identified by culture technique, the "reference standard," the mean age being 35.37 ± 7.66 years. Eighteen (66.6%) were positive for *T. vaginalis* on screening with the wet mount of vaginal discharge; 19 of 26 (73.1%) available Papanicolaou smear had evidence of trophozoites of *Trichomonas*. Table 1 shows the sensitivity, specificity, and positive and negative predictive values of the abovementioned tests and the ancillary tests in the diagnosis of *T. vaginalis* infection. Of the 27 women with trichomoniasis, 12 (44.4%) had trichomoniasis alone and the other 15 (55.5%) had trichomoniasis with concurrent infections. Bacterial vaginosis was the most common concurrent infection with trichomoniasis with an odds ratio of 1.864 (95% confidence interval: 0.8453-4.112). The demographic and risk behavior characteristics of patients with trichomoniasis are summarized in Table 2. The sensitivity, specificity, and positive predictive and negative predictive values of the various symptoms and signs are presented in Table 3.

DISCUSSION

T. vaginalis, a pathogenic protozoan of urogenital tract causes vaginitis in women, although a considerable proportion of infections are asymptomatic. Prevalence estimates of *T. vaginalis* infection varies considerably in female population around the world, depending on the population studied and techniques employed in diagnosis, ranging from 5% to 74%.[1,5] Our study, which recruited women from the Gynecology OPD revealed that 27 out of the 400 women (6.75%) with vaginal discharge had infection with *T. vaginalis* by culture. Amongst the South-Asian studies, the rate

Table 1: Performance of diagnostics tests for trichomoniasis (individual tests and combinations)

Investigations	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %	Likelihood ratio
Screening tests					
Whiff test	51.8	78.8	15	95.8	2.448
pH	100	41.3	10.9	100	1.703
Pus cells in Gram stain	55.6	67.3	10.9	95.4	1.699
pH>4.5 with positive Whiff test					
Either of the test positive	100	42.9	11.3	100	1.751
Both tests positive	51.8	78.8	15	95.7	2.448
pH>4.5 with pus cells in Gram stain					
Either of the test positive	100	30.3	9.4	100	1.435
Both tests positive	55.6	80.7	17.2	96.2	2.878
pH>4.5 with pus cells in Gram stain with positive Whiff test					
Any of the tests positive	100	29.2	9.2	100	1.413
All three tests positive	25.9	97.3	41.2	94.8	9.670
Diagnostic tests					
Wet mount	66.7	100	100	97.6	-
Papanicolaou stain	76	100	100	98.3	-
Wet mount with Papanicolaou stain					
Either of the test positive	92.6	100	100	99.5	-
Both tests positive	44.4	100	100	96.1	-
Culture	100	100	100	100	-

Table 2: Demographic and risk behavior characteristics of patients with *Trichomonas vaginalis* infection as compared to those without infection

Parameters	No of women without trichomoniasis (%) *N*=373	No of women with trichomoniasis (%) *N*=27	*P* value	Odd's ratio (95% confidence interval)
Demographic parameters				
Age (years)				
≤25	49 (13.1)	0	0.8441	1.105 (0.5034-2.425)
26-34	126 (33.8)	12 (44.4)		
≥35	198 (53.1)	15 (55.6)		
Age at onset Mean±SD (years)	34.19±8.66	34.48±7.93	0.7086	
Duration Mean±SD (months)	14.2±27.1	12.2±20.2	0.8659	
Socioeconomic status				
Upper lower and lower	337 (90.3)	26 (96.3)	0.4942	2.777 (0.3658-21.088)
Upper and lower middle	36 (9.6)	1 (3.7)		
Marital status				
Married	334 (89.5)	24 (88.8)	0.7188	1.347 (0.3837-4.7 28)
Widow	21 (5.6)	1 (3.7)		
Separated	10 (2.6)	2 (7.4)		
Risk behavior				
Pre/extramarital contact	10 (2.6)	5 (18.5)	0.0018	8.250 (2.594-26.234)
No condom use by partner	368 (98.6)	26 (96.3)	0.3443	0.353 (0.0397-3.138)
Husband having extramarital contact	38/365*(10.4)	11 (40.7)	0.0001	5.916 (2.559-13.679)
Alcohol use	0	3 (11.1)	0.0003	106.71 (5.356-2126.3)
Symptoms in partner	9/366# (2.4)	4 (14.8)	0.0083	6.899 (1.974-24.110)
STI and PID				
HIV	7 (11.8)	0	0.0134	2.857 (1.270-6.424)
PID	71/366# (19.4)	11 (40.7)		

*Eight of them were unmarried, so excluded; #Value in the denominator excludes unmarried women, except one, who had a premarital contact; PID=Pelvic inflammatory disease; STI=Sexually transmitted infection

observed in our study was comparable with the population-based study on community prevalence of STIs and HIV infections in rural south India done by Thomas *et al.*,[6] which reported a prevalence of 5.25%

Table 3: Diagnostic value of clinical features associated with *Trichomonas vaginalis* infection (*N*=27)

Parameter (*n*, %)	Sn (%)	Sp (%)	PPV (%)	NPV (%)	OR (95% CI)
Symptoms					
Lower abdominal pain (22, 81.5)	81.5	27.9	7.5	95.4	1.701 (0.6275-4.612)
Dysuria (13, 48.1)	48.1	49.8	6.5	93.0	0.9236 (0.4226-2.019)
Dyspareunia (8, 29.6)	29.6	88.4	12.1	94.2	2.236 (0.9343-5.351)
Itching (6, 22.2)	22.2	61.6	4.02	91.63	0.4595 (0.1811-1.166)
Post-coital bleeding (1, 3.7)	3.7	99.4	33.3	93.3	7.000 (0.6139-79.816)
Signs					
Mucopurulent/purulent discharge (25, 92.6)	92.6	68.4	17.5	99.2	27.013 (6.292-115.98)
Profuse vaginal discharge (14, 51.8)	51.8	80.4	16.1	95.8	4.426 (1.994-9.823)
Adnexal tenderness (11, 40.7)	40.7	80.6	13.4	94.9	2.857 (1.270-6.424)
Malodorous discharge (9, 33.3)	33.3	87.7	16.4	94.8	3.554 (1.507-8.381)
Frothy discharge (7, 25.9)	25.9	100	100	94.9	273.29 (15.071-4955.7)
Vaginal erythema (4, 14.8)	14.8	93.3	13.8	93.8	2.421 (0.7766-7.547)
Cervical erythema (2, 7.4)	7.4	96.9	1.5	93.4	2.582 (0.5422-12.294)

Sn=Sensitivity; Sp=Specificity; PPV=Positive predictive value; NPV=Negative predictive value; OR=Odd's ratio; CI=Confidence interval

for trichomoniasis by direct wet mount of vaginal swabs. Among mixed infections occurring with *T. vaginalis*, concurrent bacterial vaginosis was the most common as observed by Bhalla *et al.*,[7] probably because *T. vaginalis* alters the vaginal ecology and facilitates the development of bacterial vaginosis or women with bacterial vaginosis have lost the natural protection against genital tract infection, leading to the acquisition of STIs like *T. vaginalis* infection.[8]

Unlike chlamydia and gonorrhea, the prevalence of *T. vaginalis* has been shown to increase with age.[5] The increased prevalence of trichomonal infection in older women suggests longer duration of infectiousness and the predominant asymptomatic nature of infection.[9] This was evident from our study as the mean age at presentation for women with *T. vaginalis* infection was 35.37 years. This was comparable with the prospective study from Srilanka by Fernando *et al.*,[10] who reported a mean age of 33 years.

Infection with *T. vaginalis* can be a marker for high-risk behavior, and the predominant asymptomatic nature of the infection underscores the need for identification of risk factors associated with the infection. Significant risk factors associated with *T. vaginalis* infection in our study were a history of pre/extramarital sexual contact in the women (OR = 8.250, 95% CI 2.594-26.234), husband having extramarital contact (OR = 5.916, 95% CI 2.559-13.679), a history of symptomatic partner in the form of dysuria and urethral discharge suggestive of trichomoniasis in men (OR = 6.899, 95% CI 1.974-24.110), and alcohol consumption by women (OR = 106.71, 95% CI 5.356-2126.3). Kaur *et al.*,[11] in their study in north India to assess the prevalence of *T. vaginalis* infection in symptomatic women as well as in women with carcinoma cervix and HIV infection, observed that being a housewife, belonging to the middle socioeconomic status, and non-use of contraception were significantly associated with trichomoniasis.

The signs and symptoms associated with the infection have a relatively low-positive predictive values for trichomoniasis because of the frequent occurrence of these signs and symptoms among women with other infections.[12,13] Also, Fouts *et al.*,[14] in their study on women attending a STI clinic in Georgia demonstrated that, if the clinical features alone were used to diagnose trichomoniasis, 88% of the infected women would be missed and 29% would be falsely indicated as having infection. In our study on women presenting with vaginal discharge, the reported symptoms had low-positive predictive values for trichomoniasis. Among the clinical signs in women with vaginal discharge, having a profuse vaginal discharge (OR = 4.426, 95% CI 1.994-9.823), malodorous discharge (OR = 3.554, 95% CI 1.507-8.381), frothy discharge (OR = 273.29, 95% CI 15.071-4955.7), and mucopurulent or purulent discharge (OR = 27.013, 95% CI 6.292-115.98) were significantly associated with trichomoniasis. Frothy discharge was observed in only 25.9% women in our study, but this was the most specific sign with a positive predictive value of 100%. Colpitis macularis was not observed in any of the women by naked eye examination in our study as colposcopy was not done on a routine basis. Women with *T. vaginalis* infection have a significantly higher risk of pelvic inflammatory disease (PID) than women without trichomoniasis.[15] Adnexal tenderness, as an evidence of PID was present in 40.7% patients, which was significantly higher than that seen in women without trichomoniasis.

The sensitivity and specificity of microscopic

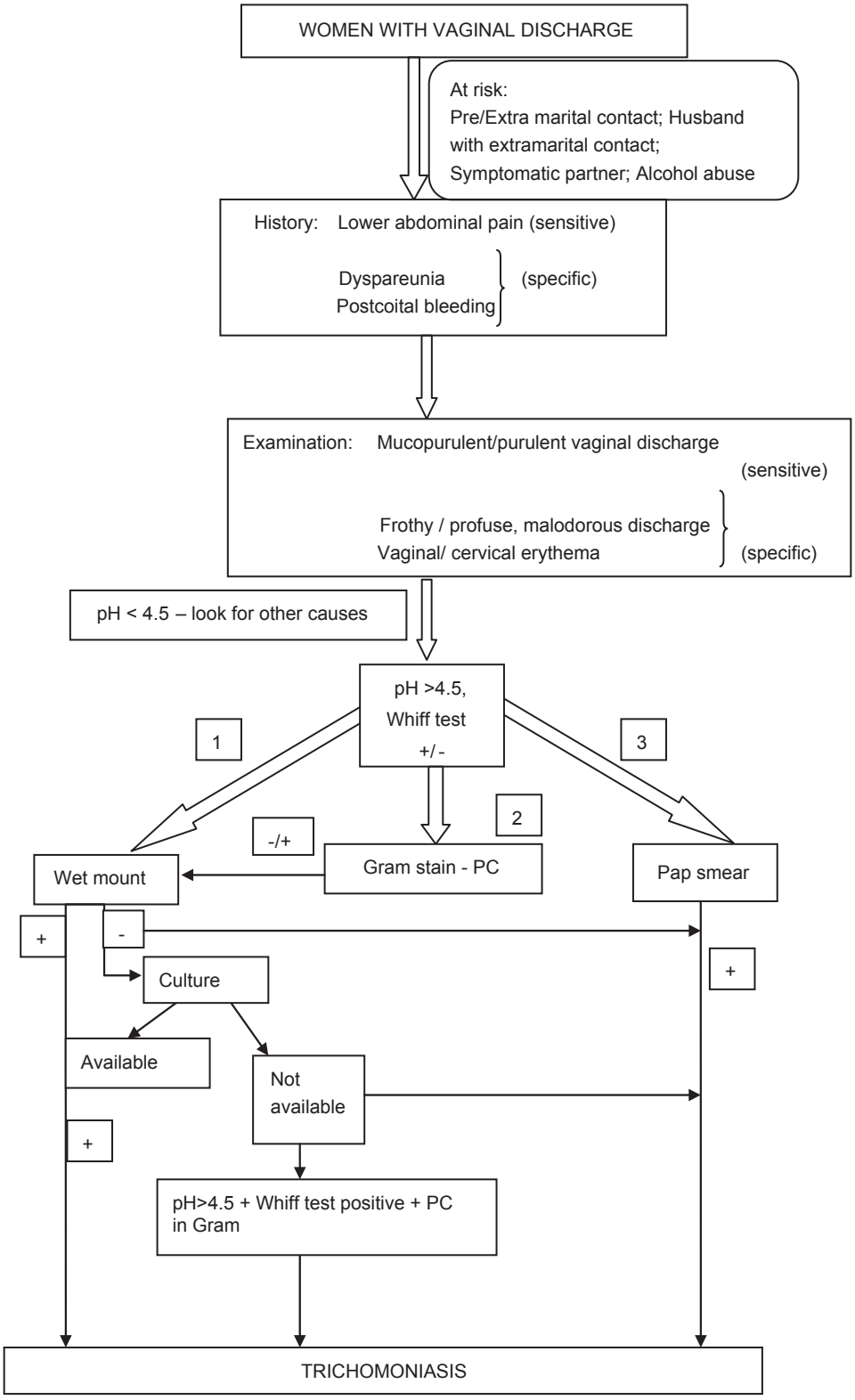

Figure 1: Algorithm for diagnosis of trichomoniasis derived from our study

and cytologic examination were assessed considering culture of *T. vaginalis* as the "reference standard." Whiff test and pH testing are simple tests recommended in the evaluation of vaginal discharge and can be used as screening tools.[16,17] pH testing was reported to be a highly sensitive, but less specific screening tool for diagnosis of trichomoniasis.[17] A similar finding was observed in

our study, where pH of >4.5 had 100% sensitivity, but a low specificity of 41.3% in the diagnosis of trichomoniasis. Whiff test was observed to have low sensitivity (51.8%) and low specificity (78.8%) in predicting *T. vaginalis* infection in our study. Women with trichomoniasis have a significantly higher concentration of white blood cells in the vaginal wet mount than those without trichomoniasis.[12] In our study, 55.6% cases with trichomoniasis had pus cells in Gram-stained smear of vaginal discharge.

In our study, we attempted to increase the specificity of these simple screening tests in the diagnosis of trichomoniasis by studying the performances of the combinations of these tests [Table 1]. pH > 4.5 had a sensitivity of 100% and a specificity of 41.3%. On combining positive Whiff test with pH > 4.5 and including cases that were positive for both tests, the specificity was increased by two folds (78.8%). Similar finding was observed on combining pus cells in Gram-stained smear with pH > 4.5. When all three (pH > 4.5, Whiff test, pus cells in Gram-stained smear) tests were combined, the specificity (97.3%) approached to that of our reference standard diagnostic test. This offers an advantage in settings where culture is not available and where the patient may be lost to follow-up due to it being time-consuming.

The diagnosis of trichomoniasis in women is most commonly made by wet mount examination; the Papanicolaou smear represents a common, readily available cytological technique for primary screening of trichomoniasis.[18] In our study, the sensitivity of wet mount and Papanicolaou smear was observed to be 66.7% and 76%, respectively; both being 100% specific with 100% positive predictive value in the diagnosis of *T. vaginalis* infection. Thus, culture of direct wet mount positive vaginal specimens may be unnecessary owing to the 100% specificity of the test. Since our study was restricted to women with symptomatic *T. vaginalis* infection, it is probable that these women harbored greater numbers of organisms and thus had a higher rate of positivity for wet mount. On combining wet mount with Papanicolaou smear, the sensitivity increased to 92.6%; this has an implication in resource-limited setting where culture may not be available, combining Papanicolaou smear with wet mount would help identify few more cases of trichomoniasis that would have been missed by wet mount alone. Figure 1 shows an algorithm that can be followed for diagnosis of trichomoniasis.

To conclude, in our study, a moderate prevalence of *T. vaginalis* infection was observed, though the prevalence in the community might be higher as we had included only symptomatic women when majority of the infection is asymptomatic. The majority of the women with trichomoniasis had co-infections with other organisms. We observed a significant association of trichomoniasis with PID, resulting in reproductive morbidity. The poor clinical value of symptoms and signs in the diagnosis of *T. vaginalis* infection could be corrected by appropriate combination of simple tests that increases the performance of these screening tests comparable to that of the diagnostic tests, especially, in the setting of non-availability of culture for *T. vaginalis*.

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"Lactobacillus - Doderlein bacillus"

Lactobacillus is also called doderlein' bacillus. They are Gram-positive facultative anaerobic or microaerophilic rod-shaped bacilli. They are part of normal flora of human vagina, g.i.t. and oropharynx.They are widely distributed in nature i.e.water, sewage, silage and food i.e. dairy products, grains, meats, fish. Over half of end product from glucose fermented into lactic acid so it is called lactobacillus. Lactobacilli are uniformly catalase negative and oxidase negative, do not reduce nitrate, do not produce indole or H_2S, do not liquefy gelatin, and do not form spores.

Normal Physiological Role-Lactobacilli play key role in defense against the development of candida vaginitis, bacterial vaginosis, urinary tract infection and sexually transmitted infections such as gonorrhea. Facultative lactobacilli constituting the predominant normal flora of the healthy vagina produce large amounts of hydrogen peroxide, unlike the anaerobic species which are more often isolated from women with the clinical diagnosis of bacterial vaginosis. Thus facultative organisms may represent a nonspecific antimicrobial defense mechanism for the normal vagina and may prevent the development of bacterial vaginosis and Candida vaginitis.