

Original Research Article

Prevalence of Trichomonas Vaginalis Infection among Three Groups of Females with Different Sexual Behaviour in Hyderabad

S. Kiranmai¹, K. Raghuram Prasad²

¹Assistant Professor, Department of Microbiology, MediCiti Institute of Medical Sciences, Ghanpur, Hyderabad.

²Consultant Plastic Surgeon, Abhinav Plastic and Cosmetic surgery centre, Dwaraka Nagar, Kadapa.

Corresponding Author: S. Kiranmai

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ABSTRACT

Introduction: Trichomoniasis is the most common curable sexually transmitted infection worldwide. The incidence of Trichomoniasis is highest in women with multiple partners and in groups with high prevalence of other STD's (Sexually Transmitted Diseases). There is growing evidence that Trichomonas vaginalis (T. vaginalis) infection of the vagina is associated with an increased risk of acquisition and transmission of HIV infection. Since no symptom alone or in combination is sufficient to diagnose T. vaginalis reliably, laboratory diagnosis is necessary. In this study, we assessed the prevalence of Trichomonas vaginalis infection in three groups of female population with different sexual behaviour by using three different diagnostic modalities from vaginal swab samples.

Methods: A cross sectional study was conducted among total 200 females, 100 female sexual workers attending two FSW (Female Sex Workers) clinics in highly concentrated areas of them in Hyderabad and 50 symptomatic females attending Gynaecology OPD and other 50 females attending family planning OPD in Gandhi hospital, Hyderabad. Three vaginal swabs were collected from each female and performed wetmount, culture and PCR respectively for the diagnosis of Trichomonas vaginalis.

Results: The prevalence of vaginal infection with T. vaginalis was 53% in FSWs, 10% in symptomatic females attending Gynaecology OPD and 0% in asymptomatic females attending family planning OPD. The number of sexual partners were high in FSWs (>6) and the personal hygiene is poorly maintained among them (98% had poor personal hygiene). Most of the FSWs were illiterates (44% compared to 34% and 12% in other groups). The safe sexual practices like condom usage was high among the FSWs (98%) but the incidence of Trichomonas was high among the FSW who had irregular condom usage (62%).

Conclusion: We detected the high prevalence rates of Trichomonas vaginalis infection among the FSWs in comparison with the other females.

Key words: Trichomonas vaginalis, Female sex workers (FSW), Trichomoniasis, Prevalence.

INTRODUCTION

Trichomoniasis is the most common curable Sexually Transmitted Infection (STI) world-wide. [1] Trichomoniasis is the infection of genital tract in both females and males; although in females it is greater. The infection is frequently asymptomatic (10-50%) in both

men and women. [2,3] When symptomatic, it can cause vaginitis, urethritis and cervicitis in women and urethritis in men. [4,5] The incidence of Trichomoniasis is highest in women with multiple partners and in groups with high prevalence of other Sexually transmitted diseases. [6] Whether Trichomoniasis is a risk factor for

HIV transmission or just a marker of high risk heterosexual activity remains unclear.^[7] However, since the incidence of *T. vaginalis* infection is highest for groups with a high prevalence of other STDs, this latter hypothesis remains to be confirmed.^[8]

There is growing evidence that *Trichomonas vaginalis* infection of the vagina is associated with an increased risk of acquisition and transmission of HIV infection.^[7,9-14] Moreover trichomoniasis has been implicated in the adverse pregnancy outcome.^[15] Trichomoniasis is associated with complications like poor outcomes of pregnancy such as premature rupture of membranes, preterm labour, premature delivery, low birth weight baby and other conditions like post abortion and post hysterectomy infections. According to recent studies it is also a risk factor for cervical cancer.^[16]

Traditionally physicians make the diagnosis based on clinical grounds, but in women, the characteristics of the vaginal discharge, including colour and odour, are poor predictors of *T. Vaginalis*.^[17,18] since no symptom alone or in combination is sufficient to diagnose *T. vaginalis* infection reliably, laboratory diagnosis is necessary.^[3]

Inoculation studies conducted in the 1940's concluded that trichomonads are body site specific and cannot survive outside the natural habitat.^[19,20] Clinical research on *T. vaginalis* has not received the same attention as other sexually transmitted infection such as Gonococci and Syphilis.^[21] Published data on the prevalence and incidence of trichomoniasis are limited, but it appears that there are large variations in prevalence between different groups of people.

The aim of the current study was to assess the prevalence of *Trichomonas vaginalis* by different diagnostic modalities from vaginal swab samples.

MATERIALS AND METHODS

Study Design: We included in the study the female sex workers attending two FSW clinics in highly concentrated areas of them in Hyderabad and symptomatic females attending Gynaecology OPD and also asymptomatic females attending family planning OPD in Gandhi hospital, Hyderabad. All women were in the age group of 18-40yrs.

They took part in the study after giving informed consent, and provided they did not menstruate at the time of sample collection. The subjects receiving/received antibiotics in the last two weeks were excluded from the study.

All women were interviewed about their literacy, sexual behaviour, safe sex practices like condom usage, genital hygiene practices and previous history of STI's. After interview, three vaginal swabs were collected from each of them prior to application of antiseptic/disinfectant by touching the fornices and walls of vagina. After collection, the first swab was immediately used for wet mount preparation; second one for culture in Whittington's medium and the third was placed in 0.5ml of 2SP solution for PCR and stored at -20°C.

LABORATORY METHODS:

Wet mount preparation: The swab inoculated with vaginal discharge for each patient was gently agitated in one drop of normal saline on a clean slide and then covered with a cover slip. The wet mount was examined with × 40 objectives and the presence of motile *T. vaginalis* was detected by the characteristic twitching motility.

T. vaginalis culture: To prepare Whittington medium, trichomonas agar base is added to distilled water and sterilized by autoclave for 15min at 15lbs pressure (121°C). It is cooled to 50°C and aseptically horse serum is added. To produce the axenic isolates the medium is supplemented with the antibiotics (Penicillin and Streptomycin). The culture

medium is dispersed in a screw capped tubes in the volume of 5ml each and stored at 4°C.

Cultivation: Before inoculation of medium, the culture tubes were warmed up to 37°C for 15min. The vaginal swabs were placed into the medium and left to incubate at 37°C for 7days. The cultures were examined microscopically on days 2, 5 and 7 after inoculation. A positive result is defined as the presence of motile *T. vaginalis* at any time; a negative result was defined as absence of motile *T. vaginalis* at all readings.

Trichomonas vaginalis Polymerase chain reaction (TVPCR):

(Rosche NG/CT kit modification)

DNA Extraction: 2ml screw cap tube is centrifuged for 10min after transferring 250µl of sample suspension after vortexing thoroughly for 20 seconds. 250µl LYS is added to the supernatant and mixed by vortexing and incubated for 10min. 250µl DIL is added to each tube and mixed by vortexing and incubated for 10min. processed specimens are kept at room temperature for up to 2 hrs before transferring aliquots to the PCR reaction tubes.

PCR primers: The primers based on *T. vaginalis* β-tubulin gene for PCR identification were used. The sequences of primers were as follows:

β-tubulin 9/2 primer 1:- Biotin- 5' GCA TGT TGT GCC GGA CAT AAC CAT.

β-tubulin 9/2 primer 2:- Biotin- 5' CAT TGA TAA CGA AGC TCT TTA CGAT.

PCR protocol: PCR reactions were performed with an automated thermocycler. The total volume of PCR reactions was 50µl of the master mix and 50µl of the DNA extracted were added to the PCR tube. The amplification was performed in the PCR tubes and the procedure is as follows:

Hold Program: 50°C- 2 min

Hold Program: 95°C- 5 min

CYCLE:

Denaturation: 95°C- 45sec

Annealing: 62-52°C- 45sec 35 cycles

Amplification: 72°C- 60sec

Hold Program: 72°C- 7 min

Hold Program: 72°C- Not to exceed 24 hours

Detection: All reagents and samples were brought to room temperature. Working wash solution is prepared. 25µl of denatured amplicon is pipetted to micro well plate and incubated for 1hr at 37°C. After washing the plate 5 times AV-HRP and SUB-A were added as given in the insert of the ROCHE NG/CT kit. At last stop solution is added and read under ELISA reader.

The results are interpreted as shown in table 1.

Table 1: Interpretation of results

Result A ₄₅₀	Interpretation
< 0.3	DNA not detected. Negative.
≥ 0.8	DNA detected. Specimen is presumptive positive for <i>T.vaginalis</i> .
≥ 0.3, < 0.8	Equivocal. Results are inconclusive. Repeat PCR testing on specimen in duplicate. Two of three results over 0.5 shall report the specimen as positive.

RESULTS

A total of 200 cases, 100 FSWs, 50 symptomatic females in Gynaecology OPD, 50 asymptomatic females in family planning OPD were included in the study after obtaining consent from them. All the subjects were in the age group of 18-40yrs.

The maximum number were in the age group of 21-30 yrs.(57.5%). Laboratory tests were done for all the patients.

T. vaginalis was found in vaginal specimens of 53% in FSW, 10% in symptomatic females and 0% in asymptomatic females. Prevalence was

higher in female sex workers than in other females and this difference is statistically significant. Table 2 summarizes the prevalence of *Trichomonas vaginalis* by wet mount, culture and PCR in the three groups

Table.2. Trichomonas positivity in the study groups by different diagnostic modalities

Diagnostic modalities	FSW (n=100)	Gynaecology OPD (n=50)	Family Planning OPD (n=50)
Wet mount Positive	10(10%)	01(2%)	00
Culture Positive	27(27%)	3(6%)	00
PCR positive	45(45%)	5(10%)	00
Total positive	53(53%)	5(10%)	00

PCR could detect more number of positives for Trichomoniasis (45%), compared to other tests i.e., wet mount and culture (10% & 27%) in female sex workers and 10% compared to 2% and 6% in females attending Gynaecology OPD.

In the present study, most of the females attending the family planning OPD were found to be maintaining good personal hygiene (40%) compared to the

females attending gynaecology OPD (18%) and FSW clinics (4%). Table 3 shows the number of TV PCR positive in relation to the personal hygiene. Trichomoniasis is more in females who did not maintain the personal hygiene (45.83% & 12.19%) compared to the women with good maintenance (25% & 0%).

In this study, literacy rate was low (56%) among the female sex workers and was high (88%) among the females attending Family Planning OPD. Fig.1. depicts the literacy rate among the study groups.

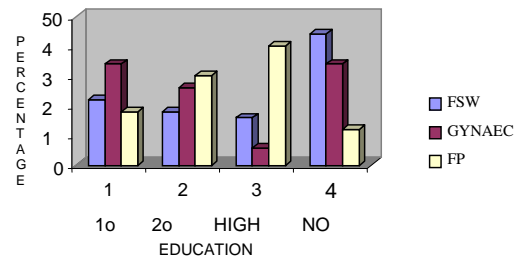


Fig.1. Literacy rate among the study Groups:

Table 3: Trichomonas positivity in relation to personal hygiene

Personal Hygiene	FSW (N=100)	Pcr Positive (N=45)	Gynaecology OPD (N=50)	Pcr Positive (N=5)
Maintained	04	01(25%)	09	00(0%)
Not Maintained	96	44(45.83%)	41	05(12.19%)

Table 4: Incidence of Trichomonas with safe sex practice in study groups:

	FSW (n=98)	GYNAEC (n=2)	FP (n=2)
Condom Usage	(n=53)	(n=5)	(n=0)
Trichomonas Positive	31(62%)	02(100%)	00
Trichomonas positive with Irregular condom usage	20(38%)	00(00)	00
Trichomonas positive with regular condom usage	02(100%)	03(67%)	00
Trichomonas positive without condom usage			

Table.5: Trichomoniasis positivity in relation to past history of STI's

Previous STI	FSW (n=100)	Positive (n=53)	Gynaecology OPD (n=50)	Positive (n=5)
Present	92	50(48%)	48	5(10.41%)
Absent	08	03(37.5%)	02	0(0%)

In this study, the incidence of positives for *Trichomonas* is high (56.82% & 17.65%) in females with no formal education than in literate females.

Safe sexual practices like condom usage was more among the FSWs (98%) compared to the (4%) general population, but *Trichomonas vaginalis* was common in

the FSWs with irregular condom usage (62%). (Table 4)

The number of sexual partners as known was more in the FSWs with majority (80%) of them had ≥ 6 number of partners. All the 53 FSWs with *Trichomonas* positive belonged to this

group. Whereas females attending the gynaecology OPD had ≤ 2 partners.

Table.5. shows the prevalence of Trichomonas in relation to the previous history of STI's. Trichomoniasis is more in females with previous history of STI's (48% & 10.41%) than in females with uneventful previous history (37.5% & 0%).

DISCUSSION

Using PCR assay, we found high prevalence rate of Trichomonas vaginalis in FSW among the three groups studied in Hyderabad with different levels of sexual activity.

The present study has demonstrated that Trichomoniasis is prevalent in Age group 21-30years (46.15% FSW & 13.79% Females attending Gynaecology OPD), Illiterate population (56.82% & 17.65%), Irregular condom usage (62% & 100%), bad personal hygiene (45.83% & 12.19%) and cases with past history of STIs (48% & 10.41%).

In 2002, Jane R Schwebke et al, conducted a study on the prevalence of Trichomonas in women with other STIs and found positives by PCR- 52 %. [22] This almost correlates with the present study as 48% positives are seen in females with previous history of STIs.

In a multicentre study, on factors determining the differential spread of HIV, which was conducted in 1997-98, the prevalence of Trichomoniasis was 42% among commercial sex workers compared to 53% in this study. [23] In a study on the prevalence of Trichomonas in Adolescent girls, pregnant women and commercial sex workers, the prevalence of Trichomonas was high (33.2%) in commercial health workers compared to others, (21) similar results are seen in the present study.

A decrease in the prevalence of Trichomonas vaginalis in Commercial sex workers was also observed. These changes in prevalence may be attributed to changes

in sexual behaviour of the study populations, in particular increase in condom use, and improved STI case management. [24]

In the study group attending Gynaecology in the present study, the PCR positivity was 10%, this compares well with the A. Pillay et al (2004)- 12.6% of females are positive by PCR for Trichomoniasis and Barbar Van Der Pol (2006)- PCR 17% in general population. [25,26]

CONCLUSION

Accurate diagnosis of Trichomoniasis in sexually active women is extremely important since T. vaginalis may be the cause of high morbidity and, in common with other non-ulcerative sexually transmitted diseases, may be regarded as a risk factor for contraction of HIV infection.

We confirmed that the prevalence of T. vaginalis is very high among the Female Sex Workers. The high rates of T. vaginalis infection found in this study are worrisome considering the association of Trichomonas with increased risk of HIV acquisition. Screening and treatment of these groups of females is essential to restrict the spread of sexually transmitted infections and HIV in the community.

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