FIRST REPORT ON FREQUENCY OF SEXUALLY TRANSMITTED INFECTIONS IN TRIPURA, NORTH EAST INDIA- TERTIARY REFERRAL CENTER STUDY

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ABSTRACT

BACKGROUND

Sexually transmitted infections are among the top five disease categories affecting adults in the developing world. In the past two decades, North East India has been known to experience an explosion of frequencies of human immunodeficiency virus (HIV) infections and other sexually transmitted infections (STIs). However, no study on the frequency of STIs has ever been reported from Tripura. We wanted to estimate the frequency of STIs in the state of Tripura.

METHODS

Informed consent and data on socio-demographic status and high-risk behavior were obtained. Clinical specimens in the form of swabs, aspirated material and blood were collected. STIs were diagnosed by clinical features and cultured or confirmed in the laboratory by standard techniques. Level of significance as compared between uninfected and STI infected subjects was determined through chi-square analysis at a significance level of $p < 0.001$.

RESULTS

The overall STI frequency rate was 57%. The male infection rate was 42% with highest infections being of gonorrhea at 21%. In females, the infection rate was 63% with highest infections of vulvovaginal candidiasis at 29%. In males, 91% of the infections were found in the age group of 20-39.

CONCLUSIONS

Male subjects harbored infections which were more likely to be transmitted through sexual routes, such as gonorrhea and chlamydia. Female subjects suffered mostly from vaginitis. HIV co-infection rates were not very high. The high-risk groups were petty businessmen, transport workers, and housewives. People were barely in the habit of using barrier protection or practicing proper hygiene.

KEY WORDS

Sexually Transmitted Infections, Human Immunodeficiency Virus, North East India, Socio-Demographic Data

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BACKGROUND

As per Center for Disease Control and prevention surveillance report,¹ sexually transmitted infections (STIs) are among the top five disease categories affecting adults in developing world and second in list for maternal diseases, infertility and death. Every day more than one million STIs are acquired.² India is a vast country with diverse cultures, lifestyles, ethnicity and STI frequencies vary largely from one place to another. No authentic community-based statistics considering the entire of India is available. Most of such studies are performed on

high risk groups such as commercial sex workers, truck drivers, hotel workers and drug abusers. This proves as a hindrance for formulating control strategies and treatment guidelines. The survey report of 2007 from National Family Health Survey³ states that Tripura is one of the six states which have STI rates higher than 15% among women. It also stands second (10%) in STI prevalence rate among male.³ Despite such high STI rates based on survey, there is no published report which has looked in details into the frequency of STIs in Tripura. Also, only a handful of studies on frequency of STIs are available from North East India.^{4,5,6,7,8} In the light of this, the current trend of STIs in Tripura was a very relevant and urgent topic to look into. Hence with this aim we formulated the following study comprising of a detailed look into the most commonly occurring STIs as well as the socio demographic profile of the attending subjects.

METHODS

Setting

The cross-sectional study was done in a tertiary hospital in Tripura between August 2012 and July 2015 after obtaining

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clearance from institutional ethical committee. Considering importance and technical feasibility, frequency of STIs were looked for comprised of- gonorrhoea, non-gonococcal urethritis/cervicitis, chlamydial infection, syphilis, trichomoniasis (TV), vulvovaginal candidiasis (VVC), bacterial vaginosis (BV) and chancroid. In addition, HIV seropositivity among these included subjects was also looked into.

Subjects and Enrolment Criteria

A total of 500 male and female cases with active symptoms of vaginal/cervical/urethral/anorectal discharge or swelling, genital ulcers/warts, vaginal itching, inguinal/scrotal swelling, inguinal/vaginal bubo, lower abdominal pain and ectopic pregnancy were included in the study after clinical evaluation. The subjects also needed to be in the reproductive age group of 15-49 years as per World Health Organization criteria.⁹ Excluded cases from the study were those on concurrent antibiotic use, beyond the reproductive age group, menstruating, pregnant or asymptomatic.

Socio-Demographic Data Collection

Informed consent and data on socio-demographic status and high risk behaviour were obtained in pre-designed proforma as per National Aids Control Organization (NACO) guidelines.¹⁰ High risk behaviour was defined as practice of unprotected penetrative sex (Anal or vaginal) with new or two or more partners/commercial sex workers/ homosexually active men/a known individual with STI, or past history of STI.¹¹

Clinical Diagnosis of STIs and Collection of Samples

A thorough inspection of vulva, vagina, cervix or urethra was conducted, and any abnormality noted. STIs were diagnosed by clinical features and clinical specimens for microbiological study were collected with swabs from the lateral and posterior fornices of the vagina, endocervix, or urethra. Aspirated material if present was collected from inguinal bubo, and 5 ml clotted blood was drawn for testing syphilis and HIV from all subjects.

Bed Side Investigations

For female subjects, physical characteristics of discharge regarding amount, odour, colour and consistency were observed. The discharge was labeled according to the amount with scanty, moderate or profuse as described previously.¹² Vaginal pH was checked using pH indicator strip and Whiff test was done with 10% potassium hydroxide solution.¹³ Wet mount preparation using 0.9% sodium chloride solution was done for observing *T. vaginalis* and *Candida* spp. Smears were prepared from collected discharges in swabs at the bed side for observing later through Gram and Giemsa staining.¹³

Staining from Direct Specimens of STI Pathogens

Gram staining was done for *Neisseria gonorrhoeae* (Causing gonorrhoea), BV causative anaerobes, *Haemophilus ducreyi* (Causing chancroid), *Candida* spp. (Causing vulvovaginal candidiasis). Gram stained smear was observed for polymorphonuclear leukocytes, clue cells, bacterial morphotypes and fungal cells. In BV clue cells were looked for and scored based on Nugent's¹⁴ criteria, where 7+ to 10+ scores were considered as clinically significant BV, 4+ to 6+ were considered as altered flora, and 0 to 3+ were considered

as normal flora. Giemsa stained smear was observed for *Trichomonas vaginalis*, the causative organisms of Trichomoniasis. Giemsa staining was also done to check for *Chlamydia trachomatis*, the pathogen causing chlamydial infection.

Culturing of STI Pathogens

STIs were confirmed in laboratory by standard techniques.¹³ *N. gonorrhoeae*, was detected through cultures on chocolate agar and blood agar. BV was detected as per Amsel's criteria through physiological tests (pH test >4 and amine tests) followed by culturing in blood agar. For *H. ducreyi* diagnosis was done through culturing in chocolate agar using gonococcal agar base with iso-vitalex, vancomycin and 1.2% hemoglobin powder. *Candida* species were observed through wet mount in 0.9% sodium chloride solution, followed by culturing on Sabouraud's dextrose agar with and without chlorhexidine. Speciation of *Candida* were determined through germ tube test and by looking at the morphology expressed in chromogenic media, HiCrome agar and in Corn-meal agar. *T. vaginalis* was observed through wet mount examination followed by culturing on Whittington media with 8% horse serum and *Trichomonas* selective supplement II. All bacterial and fungal isolates were confirmed through colony characteristics, Gram stain reaction and standard biochemical tests.¹³ *Chlamydia trachomatis* was detected by polymerase chain reaction (PCR) as per previous protocol.¹⁵ The primer pair was selected from a conserved region of the major outer membrane protein (MOMP) gene common to all serotypes of *C. trachomatis* and the housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the amplification control. Primer sequences are as given in Table 1. For testing syphilis through rapid plasma reagin (RPR) test, standard protocol of NACO¹³ was followed. For HIV test positive was declared following strategy III as per NACO guidelines.

Statistical Analysis

Level of significance as compared between uninfected and STI infected subjects were determined through chi-square analysis at a significance level of $p < 0.001$ and most affected subjects were determined.

RESULTS

Age-Sex Distribution Among STI Infected Subjects

Subjects were grouped age wise into four groups: 15 -19, 20-29, 30-39, and 40-49 (Fig. 1). Distribution of positive cases amongst the male attendees was highest in the 20-29 and 30-39 age groups comprising of 40% (29/72) and 47% (27/58) respectively. Within the female attendees too, the frequency decreased with increase in age groups with 77% (68/88) cases for 20 -29 age group, 60% (77/129) cases for 30 -39 age group, and 57% (71/124) cases for 40 -49 age group.

Sociodemographic Profile

As summarized in table 2, as per grouping based on characteristics, the most infected male subjects were people who migrated from elsewhere, people who lived away from their partners for more than six months, had education of 11th grade or higher, were professionally truck drivers or local

transport workers, were not in the habit of using any birth control measures, or had more than two partners till date. Among the female infected subjects (Table 3) most affected were women residing in urban areas, were widowed or separated, or were not educated beyond 5th standard. It is however noteworthy that among the male attendees, out of ten Border Security Force (BSF)/Central Reserve Police Force (CRPF) personals eight were found to be infected and all the 18 women who were housewives of BSF/CRPF personals were infected. Of the women educated below 5th standard, most were illiterate.

STI Overall Picture

The overall frequency of STIs found in the study was 57% (285/500). As shown in fig. 2, male attendance was 29% (147/500) and female attendance was 71% (353/500). Among male participants 42% (62/147) and among female participants 63% (223/353) were found to harbour one or more of the STI pathogens. Amongst the positive female cases 61% (136/223) had attended the obstetrics and gynaecology (O & G) clinic and 39% (87/223) had attended the STI clinic. All the male positive cases had attended the STI clinic.

Frequency of Various STIs Among the Male Subjects

The STI frequency is as listed in table 4 and fig. 3. Among the male subjects the highest infection rates were of gonorrhoea constituting of 21% (31/147) cases. PCR screening for Chlamydia trachomatis was detected in 9% (13/147) cases. Other infections apart from those caused by N. gonorrhoeae and C. trachomatis, resulting in non-specific urethritis (NSU) was found amongst 7% (11/147) male cases. Syphilis based on RPR screening results was found in 3% (5/147) male subjects. Chancroid was found in a single male subject as was candidal balanitis.

Frequency of Vaginitis Among the Female Subjects

Vaginitis cases were most common among the female subjects with VVC, trichomoniasis and clinically significant BV making up 56% (197/353) of the female infected cases (Table 4). Amongst the female subjects, occurrence of VVC was highest numbering to 29% (103/353) cases, of which it occurred as single infection in 20% (72/353) subjects. Trichomoniasis was found in 10% (37/353) of female subjects of which it occurred as single infection in 5% (19/353) subjects. Altered vaginal flora (Nugent scoring system 4+ - 6+) was found in 14% (49/353) subjects. Clinically significant BV (Nugent scoring system 7+ - 10+) was found in 16% (57/353) subjects of whom BV occurred in 9% (32/353) subjects as single infection. VVC trichomoniasis co-infection was found in 3% (11/353) subjects, VVC BV co-infection in 5% (18/353) subjects, and BV trichomoniasis co-infection in 1% (5/353) subjects. 0.4% (2/353) subjects had co-infection of all the three diseases. The frequencies are clearly depicted through a Venn diagram in fig. 4.

Gene	Sequence (5' → 3')	Product Size (bp)	Annealing Temp (°C)
MOMP	F: GCCGCTTTGAGTCTGCTTCC R: GTCGAAACAAAGTCACCATAGTA	180	52
GAPDH	F: CCATTCATTGACCTCCACTACA R: CGTTGCTGACAATCTTGAGAGA	199	60

Table 1. Chlamydia Trachomatis (MOMP) Gene and Housekeeping (GAPDH) Gene Primers used for PCR in the Study

*F, Forward primer; R, Reverse primer

Characteristics	Uninfected Male Subjects (%) (N = 85)	STI Infected Male Subjects (%) (N = 62)	Total Male Subjects (N = 147)
Place of residence (significant; p < 0.001)			
Rural	59 (84)	11 (16)	70
Urban	24 (40)	36 (60)	60
Mobile*	2 (12)	15 (88)	17
Educational qualification (significant; p < 0.001)			
Till 5 th	26 (76)	8 (24)	34
6 th -10 th	49 (68)	23 (32)	72
11 th - Post graduation*	10 (24)	31 (76)	41
Profession (significant; p < 0.001)			
Self employed	33 (77)	10 (24)	43
Truck driver/Local transport worker*	3 (15)	17 (85)	20
Border Security Force/Central Reserve Police Force	2 (20)	8 (80)	10
Day labourer	30 (81)	7 (19)	37
Service (Government/Private)	9 (45)	11 (55)	20
College student	9 (53)	8 (47)	17
Marital status			
Married	47 (59)	33 (41)	80
Never married	36 (57)	27 (43)	63
Separated/Widowed	2 (50)	2 (50)	4
No. of partners till date (significant; p < 0.001)			
0-1	73 (76)	23 (24)	96
≥ 2*	12 (24)	39 (76)	51
Birth control measure (significant; p < 0.001)			
Using barrier protection	10 (83)	2 (17)	12
Not using barrier protection	47 (77)	14 (23)	61
Not using any protection*	28 (58)	46 (42)	74
Residence of partner (not applicable in case of never married/widowed/separated): Uninfected subjects (N = 47), STI infected subjects (N = 33) Total male subjects (N = 80) (significant; p < 0.001)			
Living together	43 (64)	24 (36)	67
Migrant population (more than 6 months)*	4 (31)	9 (69)	13

Table 2. Socio Demographic Status of Male Subjects

*Most affected subjects as determined through chi-square test at significance level (p < 0.001) after comparing between uninfected and STI infected subjects
†Numbers in parenthesis indicate percentage of total subjects under the particular criteria.

Characteristics	Uninfected Female Subjects (%) (N = 130)	STI Infected Female Subjects (%) (N = 223)	Total Female Subjects (N = 353)
Place of residence (significant; p < 0.001)			
Rural	106 (48)	115 (52)	221
Urban*	15 (13)	102 (87)	117
Mobile	9 (60)	6 (40)	15
Educational Qualification (significant; p < 0.001)			
Till 5 th *	25 (17)	119 (83)	144
6 th -10 th	90 (53)	81 (47)	171
11 th -Post graduation	15 (39)	23 (61)	38
Profession			
Housewife	113 (39)	176 (61)	289
Self employed	8 (32)	17 (68)	25
Service (Government/Private)	9 (23)	30 (77)	39
Marital Status (significant; p < 0.001)			
Married	127 (42)	173 (58)	300
Never married	0	4 (100)	4
Separated/Widowed*	3 (6)	46 (94)	49
No. of Partners till Date			
0-1	128 (37)	214 (63)	342
≥ 2	2 (18)	9 (82)	11
Birth Control Measure			
Using barrier protection	6 (55)	5 (45)	11
Not using barrier protection	60 (35)	112 (65)	172
Not using any protection	64 (38)	106 (62)	170
Daily Hygiene/Menstrual Care			
Hygienic practices	12 (43)	16 (57)	28
Unhygienic practices	118 (36)	207 (64)	325
Residence of partner (not applicable in case of never married/widowed/separated): Uninfected subjects (N = 127), STI infected subjects (N = 173), Total female subjects (N = 300)			
Living together	121 (42)	168 (58)	289
Migrant population (more than 6 months)	6 (55)	5 (45)	11

Table 3. Socio Demographic Status of Female Subjects

*Most affected subjects as determined through chi-square test at significance level (p < 0.001) after comparing between uninfected and STI infected subjects. †Numbers in parenthesis indicate percentage of total subjects under the particular criteria.

	Clinical BV\$	Candidiasis	Trichomoniasis	Gonorrhoea	NSU/NSC	Chlamydia	Chancroid	Syphilis	Total STI Positive	HIV (among the STI positive cases)
Male (N = 147)	0	1 (0.68)	0	31 (21)	11 (7)	13 (9)	1 (0.68)	5 (3)	62 (42)	5 (3)
Female (N = 353)	57† (16)	103† (29)	37† (10)	1 (0.28)	0	17 (5)	1 (0.28)	7 (2)	223 (63)	5 (1)
Overall (N = 500)	57 (11)	104 (21)	37 (7)	32 (6)	11 (2)	30 (6)	2 (0.4)	12 (2)	285 (57)	10 (2)

Table 4. Frequency of Various STIs Among Subjects

*BV, Bacterial vaginosis; Candidiasis, Vulvovaginal candidiasis in women, candidal balanitis/balanoposthitis in men; NSU, Non-Specific Urethritis; NSC, Non-Specific Cervicitis.

†For each disease, total occurrence was calculated considering single and concomitant infections.

#Numbers in parenthesis denote percentage.

\$Nugent score 7*-10*

Frequency of Other STIs Among the Female Subjects

As seen in table 4. And fig. 5, gonorrhoea was found in a single female subject who was a partner of one on the detected male subjects from the study. Vaginal chlamydial infection as detected through PCR screening was found in 5% (17/353)

female attendees. RPR seropositivity was detected among 2% (7/353) female subjects. Chancroid was found in a single female subject. Non-specific cervicitis (NSC) was not found among any of the female subjects.

DISCUSSION

STI infections if detected early are easier to treat. However multi-drug resistance compounded with asymptomatic infections and increased chance of HIV co-infectivity makes this group of diseases rank high in morbidity and mortality. In resource limited settings therefore prior knowledge of the frequency of STIs would help in empirical treatment, educating patients and decision making regarding STI screening to limit the infectivity of both HIV and STIs.

Tripura, a small hilly state in North East India is bordered by Bangladesh, Assam and Mizoram. It also has large mobile or migrated population from Manipur, Nagaland and Meghalaya. In the past two decades North East has been known to experience an explosion of incidences of HIV and other STIs especially in the states of Manipur and Nagaland.⁴ In this scenario, the current trend of STIs in Tripura was a very relevant topic to look into. More than half of the attended subjects (57%) were detected with STIs, showing the importance of this study.

In the present study, the attendance of female cases (71%) was found to be much higher than male cases (29%) in the clinics. The reason for lower attendance of male participants could be because of their preference and means of visiting private doctors rather than attending the hospital in order to keep their privacy intact. Female attendees being majorly from the rural areas (63%) and housewives (77%) were not monetarily so independent for private treatments. The other factor was lack of awareness regarding STIs, 61% visited O & G clinic as opposed to 39% who visited the STI clinic. Stigma attached to STIs could also be a reason.

Socio-behavioural patterns of male subjects had higher likeliness of spreading STIs than female subjects. Where 35% males reported to having two or more partners in their lifetime, females reported only 3%. Notably, while 54% of the attended male subjects were married, only 8% of the male participants reported using barrier protections. The BSF/CRPF males who also mostly belonged to migrated population were particularly susceptible to spreading infections which could be seen since all 18 housewives of BSF/CRPF men were found to be infected. 80% (8/10) men themselves from the BSF/CRPF were found to harbour at least one of the tested STIs. The most susceptible characteristics among males were living away from their partners for more than six months, professions such as truck driving or working as local transport workers, not using any birth control measures, or having more than two partners till date, thus indicating the infections to be exclusively of STI nature. The most susceptible characteristics as found among female subjects were place of residence to be the slum urban areas where susceptibility to get infected is higher. Widowed or separated women were also the most affected as were women who were illiterate or educated below 5th standard, indicating that infections could be of the nature that can be an STI or can be due to lack of hygiene. The data showing that 92% of the female subjects were not in proper knowledge of daily hygienic practices or menstrual care also further points towards this possibility.

The rate of STIs was found much higher in the age group of 20-39 (90%) for males. This data agreed with other similar studies from India^{16,17} since sexual activity is at its peak. They are also more likely to have higher number of partners and change partners more often. For female subjects STI cases were also found more in the younger females, except for 19 years or less group which was due to very few volunteers.

STIs transmitted exclusively through sexual route such as gonorrhoea and chlamydial infection were higher amongst the attended male participants. The highest frequency was of gonorrhea with 31 (21%) cases followed by chlamydia (9%). The results were not in accordance with a study from Assam which showed decrease in the trend of gonorrhoea from 17.08% in 1986-1990 to 4.72% in 1995-1999.⁷ Unfortunately, as mentioned earlier, not many studies are available from North East India for comparison.

The female subjects in the study were mostly detected with infections the cause of which could be sexually transmitted or could be due to improper hygiene. Female participants majorly came with the complaint of profuse vaginal discharge and lower abdominal pain, indicative of vaginitis comprising of three main diseases: vulvovaginal candidiasis, bacterial vaginosis and trichomoniasis. In the world scenario too, vaginitis is the commonest reason for gynaecological visits.¹⁸ Among the female subjects the highest frequency was of vulvovaginal candidiasis with 104 (21%) subjects which is higher than the prevalent range of 18% - 20% published in other studies from India^{19,20} but comparable to the study in 2009 from Assam⁵ which showed VVC (21.5%) to be the most common finding amongst STIs. The study found similar occurrence rates of syphilis among males (3%) and female subjects (2%), lower than studies from neighboring Bangladesh²¹ and Nagaland⁶ with 6% and 7% incidences respectively.

STIs breach protective mucosal barrier, recruit immune cells, cause genital bleeding and increase HIV shedding thus increasing risk of HIV infection. In a study trichomoniasis and bacterial vaginosis were found to increase vaginal bleeding by 12 folds.²² In another study syphilis increased HIV RNA load in plasma and decrease CD4 count.²³ From the current study frequencies of HIV and STI co-infection were found to be 3% among male and 1% among female subjects indicating an increase in trend, which was also reported in other studies.²⁴ Our study saw the highest co-infections with syphilis or gonorrhea, similar to trends from around the world as reviewed by Kalichman et al.²⁵ However seropositivity of HIV among the attended subjects was low when compared to other studies from India^{26,27} and was a positive outcome from the study.

CONCLUSIONS

The overall STI frequency rate was 57%. Female attendees outnumbered males in the ratio of 2.4:1. Male STI overall positivity was 42% with highest infections being of gonorrhoea at 21%. In females STI positivity overall was 63% with highest infections of vulvovaginal candidiasis at 29%. In males 91% of the frequencies were found in the age group of 20-39 years, while in females too STI cases occurred more in the younger females. Socio-demography-wise, STIs mostly occurred amongst professions such as truck drivers, local transport workers and BSF or CRPF personals. People were barely in the habit of using barrier protection or practicing

proper hygiene. Limitation of the study was that it was a tertiary care centre-based frequency study and hence the frequency of STIs observed may not match with the actual prevalence rate in the community.

Abbreviations

O & G- Obstetrics and Gynaecology.

STI- Sexually Transmitted Infections.

NSU- Nonspecific urethritis; BV, Bacterial vaginosis.

VVC- Vulvovaginal Candidiasis,

TV- Trichomoniasis

NSC- Non-specific cervicitis

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