

Periodic prevalence of various STDs and its correlation with cervical dysplasia

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Abstract

Introduction: Sexually transmitted infections (STIs) constitute an important public health problem worldwide and contribute to increasing morbidity and mortality due to their association with Human Immunodeficiency virus and for predisposing to cervical dysplasias. STIs can be caused by transmission of bacteria, virus, fungi or protozoa and most of these can be controlled by early detection, appropriate medication and health education. **Materials and Methods:** In a Regional Reference STD laboratory in West Bengal, samples from 2143 female patients with chief complain of vaginal discharge, were tested for detection of three STIs namely Trichomonas vaginalis, Candida species and Treponema pallidum. Concomitantly, study of cervical cytology on Papanicolaou stained smears was carried out in all cases. The study period extended over four years. Trichomoniasis was identified by wet mount and culture, Candida by culture and Gram staining, Syphilis by Venereal Disease Research Laboratory (VDRRL) and Treponema Pallidum Haemagglutination (TPHA) test. The cytological findings were interpreted according to Bethesda 2001 classification. **Results:** The majority of the patients belonged to the age group of 25 to 44 years and Hindus outnumbered Muslim. The percentage positivity of Syphilis has decreased over the last four years whereas Candida and Trichomonas infections demonstrated a rising trend. Candida and Trichomonas infection were frequently associated with cervical dysplasia which was not observed in case of Syphilis. **Conclusion:** Screening programmes incorporating cervical cytological study and tests for detection of different STIs will help facilitate early and effective intervention necessary for preventing progression of STI and development of cervical dysplasia.

Keywords: Cervical cytology, sexually transmitted infections, trends.

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bacteria, virus or protozoa that are passed from one person to other through sexual intercourse. Most of these infections can be easily cured by using antibiotics or other drugs while few are difficult to cure. Based on World Health Organization (WHO) report, approximately 340 million new cases of four main curable sexually transmitted infections (STIs), viz., gonorrhoea, syphilis, chlamydial infection, and trichomoniasis occur every year, nearly 80% of which occur in developing countries¹. Since 1999/2000, CDC estimates that 19 million new infections arise each year, almost half of them among young people aged 15 to 24². The incidence of syphilis decreased dramatically during the 1980s, stabilized in the 1990s, and has recently increased since 1999/2000³. Some factors including population growth, rural to urban migration and poverty create exceptional vulnerability to these types of infection from high risk behavior^{4,5,6}. An

INTRODUCTION

Epidemiological studies show that Sexually Transmitted Infections (STIs) are not uncommon in the world as well as in India. In recent era, STIs are a major public health problem worldwide. STI are caused by transmission of

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increasing trend of opportunistic infection of HIV, in turn increases STI cases tremendously in different parts of the country. STDs are classified into ulcerative and nonulcerative groups; the prevalence of HIV was much higher in the group with ulcerative STDs (17.1%) than those with nonulcerative STDs (9.5%)². Trichomoniasis is one type of STI and the causative agent is *Trichomonas vaginalis* (*T. vaginalis*). Epidemiological studies showed that globally 170 to 190 million *T. vaginalis* cases are reported annually and in India hospital-based studies showed that in women attending STD clinic incidence of *T. vaginalis* is as high as 61.7% and as low as 6.7% among women with reproductive tract infections⁷. Episodic outbreaks of syphilis occur in certain high-risk populations, such as men who have sex with men (MSM), commercial sex workers and drug users. A proper understanding of the patterns of STDs prevailing in different geographic regions of a country is necessary for proper planning and implementation of STD control strategies. Beside trichomoniasis, syphilis and candidiasis, Human Papilloma Virus is one of the most common sexually transmitted viral infection and opportunistic infections in HIV infected patients. The highest levels of this infection have been reported in the United Kingdom (80-120/100000) and Ireland (100/100000) in 2000. World-wide the percentage of sexually active men and women infected with this virus are 50-60%⁸. There are indications that HPV infection may contribute to dysplasia on cervical cytology as well as invasive genital cancer. India contributes about one fifth of the world cervical cancer trouble alone⁹ and several records have recognized that oncogenic HPV is related with more or less all cases of cervical cancer¹⁰. Primary infection of this virus often regresses artlessly, but delayed, constant infection, especially with HPV types showing a strong association with malignant lesions, leads to viral assimilation with the host cell genome and expression of the viral oncogenes¹¹. More recent experimental studies indicate that protozoan like *Trichomonas vaginalis* might be responsible for the induction of changes in human cervical mucosa¹². Genital infections like Trichomoniasis, candidiasis also promotes HPV infection persistence, aggravating disease pathogenesis.

MATERIAL AND METHODS

The Regional STI Reference Research Teaching and Training Centre at Institute of Serology, Kolkata receives samples from STI patients from different regions of West Bengal and conducts diagnostic tests for various STIs thus contributing to early detection and facilitating control of STI. A total of 7752 patients attending the Department of Dermatology, Venereology and Leprology

of Kolkata Medical College and Hospital and also of R.G. Kar Medical College and Hospital, Kolkata, came up with various types of complaints involving genital organs for a period of 4 years from 1st January, 2010 to 31st December 2013. Of these 2403 were males and 5349 were female patients. Approvals from the institutional ethical committees of both the institutions were obtained prior to initiation of the study. All the experiments were carried out in Institute of Serology, Kolkata, India. After proper counseling and obtaining informed consent extensive clinical examination was made in 2143 symptomatic female patient with complaints of STDs, this included speculum examination of all the patients. The diagnosis was based on clinical features supported by laboratory confirmation following standard techniques¹³. The nature of ulcers and discharges indicated the type of STIs. VDRL testing was carried out for all the patients; for this purpose blood was drawn from every patient from which, serum was separated and tested for VDRL positivity with the help of VDRL antigen manufactured at Institute of Serology, Kolkata. Subsequently Treponema pallidum haemagglutination assay with TPHA Test Kit (New Market Laboratories Ltd, Kentford, CB8 7PN, UK) was performed in VDRL positive cases. All genital lesions were cleaned with sterile saline, adherent mucosa layer was gently removed without causing bleeding and samples were collected from vagina and cervix for diagnosis of STIs. For *T. vaginalis* detection, one vaginal swab was used to make a thin smear in one drop of normal saline over a clean glass slide and observed microscopically under 40X within 5-8 minutes of collection. Another swab was inoculated in Kupferberg medium, incubated at 37⁰C and observed for motility up to 7 days. Kupferberg medium was prepared in following manner 23.50 gm Kupferberg trichomonas broth base (Himedia, India) was mixed with 950ml of distilled water and autoclaved, to which was added 50ml sterile beef serum and distributed in 10 ml amount. To detect infection by *Candida* spp. vaginal swabs were taken, one for microscopy (by KOH wet mount) and the other for cultivation in specific Sabouraud Dextrose Agar (SDA; Himedia, India) plate. The SDA plate was incubated at 25⁰C for 24-48 hours for appearance of typical cream coloured large colonies which was followed by Gram staining on smear from colonies for morphological identification of *Candida* spp. Such colonies were inoculated on to HiCrome medium (Himedia), incubated at 25⁰C for appearance of colonies of different colours for species identification¹⁴. Sabouraud's dextrose agar was distributed in 5 ml amounts in large test tubes, autoclaved and allowed to solidify. For studying cervical cytology, an Ayres spatula was to obtain samples from ectocervix and cytobrush to collect endocervical samples. These

were stained by papanicolaou (Pap) method, observed microscopically and interpreted according to Bethesda 2001 classification¹⁵. Cytological observation was graded as: Negative for Intraepithelial Lesion or Malignancy (NILM), Atypical Squamous cells of Undetermined Significance (ASCUS), Atypical Squamous cells, cannot exclude High Grade Lesions (ASC-H), Low grade Squamous Intraepithelial Lesion (LSIL), High Grade Squamous Intraepithelial Lesion (HSIL), squamous cell carcinoma and adenocarcinoma. Chi square test was performed for comparison of significant value of the differences in percentages.

OBSERVATIONS AND RESULTS

In a referral laboratory in Eastern India over last four years, a total of 7752 STD patients including 2403 males and 5349 female attendees were introduced in this study. Among these attendees, 2143 were symptomatic female patients whose samples were screened for sexually transmitted infections and cervical dysplasia. Based on age distribution, most of the STI attendees were in the 25-44 year age group ranging from 58.13 to 74.13 per cent and in <19 year age group ranging from 5.29 to 8.13 per cent with a significant difference in the percentage of STI cases amongst the four study years. During these study period difference Hindu population was high than Muslim pollution for symptomatic female attendees (the difference was not significant). High number of females with STIs reported to the clinic. The profile of syphilis amongst all symptomatic female STI cases in respective years is shown on Table 2. There was a significant decrease in the number of the positivity of syphilis cases in females. Both VDRL and TPHA tests and trends of positivity were reduced in successive years (Table 2). As shown in Table 4, VDRL per cent positivity was less than TPHA per cent positivity rate in mentioned years without any significant difference. This was because TPHA detects both old and new infection while VDRL detects only recent infection. The percent positivity rate of

female with Syphilis was considerably reduced due to active surveillance from 2010 to 2013 (Table 2). Among 2143 cases who presented with discharges, 698 samples were *T. vaginalis* positive and *Candida* spp. was positive 422 samples. The occurrence of positivity rate of trichomoniasis and candidiasis were increased in the consecutive study periods. It is noted that the percent positivity rate was high in case of *T. vaginalis* than *Candida* spp. during consecutive years. In both cases, culture was more sensitive than wet mount, KOH mount and Gram stain (Table 2). The increase rates of both the infections are not significant. During the study, prevalence of cervical dysplasia was found to be 13.82%, 11.19%, 8.32% and 16.10% in respective years (Table 3). Based on the Bethesda System, 2001, epithelial cell abnormalities mainly Low-grade Squamous Intraepithelial lesion (LSIL) and above encompass cervical dysplasia. Incidence of *T. vaginalis* or other fungal infections have been correlated with cervical dysplasia. The occurrence of positivity of *T. vaginalis* was most frequently seen among ASCUS, LSIL and HSIL cases (Table 4). From this observation it was noted that positivity rate of *T. vaginalis* was high than *Candida* spp. in case of ≥ASCUS in all respective years. A significant reduction in the percentage of LSIL cases was observed continuously from 2010 (13.82%) to 2012 (8.32%) (P<0.001). In our observation it was noted that correlation of *T. vaginalis* and *Candida* spp. infections with cervical dysplasia was not statistically significant (Table 4). The occurrence of percent positivity rate of syphilis was observed to drop where as *T. vaginalis* and *Candida* spp. showed an increasing trend (Table 5). These results had no statistical significant. From our current studies, high prevalence of cervical dysplasia, *T. vaginalis* infection and candidiasis were observed; on the other hand, prevalence of syphilis infection was reduced. Presence of *T. vaginalis* and *Candida* spp. have a role in the acquisition of cervical dysplasia needs prompt treatment.

Table 1: Changing socio-demographic profile of attendees and age distribution of symptomatic female attendees at the Regional STD Reference Centre, Kolkata from 2010 to 2013

Year Jan-Dec	Total no. of attendees	Male attendees	Female attendees	Symptomatic female	Religion	Age distribution of symptomatic females tested for different STIs			
						Hindu Muslim	<19	20-24	25-44
2010	3190	1106	2084	246	186 60	20	58	143	22
2011	1253	416	837	277	205 72	22	29	202	24
2012	1480	412	1068	661	398 263	35	77	437	112
2013	1829	469	1360	959	674 285	48	148	711	52

Table 2: Year wise positivity trends of syphilis, Trichomonas vaginalis and Candida spp. At the Regional STD Reference Centre, Kolkata from 2010-2013

Year	Symptomatic	Syphilis	Trichomonas vaginalis	Candida spp.
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(Jan-Dec)	discharges	VDRL positive	TPHA positive	Wet mount	Culture	Positivity on culture	KOH mount	Culture	Gram stain	Positivity on culture
2010	246	8	17	49	71	71	21	19	19	19
2011	277	9	19	67	90	90	28	31	31	31
2012	661	21	31	189	246	246	61	81	81	81
2013	959	29	44	243	381	381	159	139	191	191

Table 3: Frequency of cytological changes in cervical dysplasia infected patients (LSIL and above) at the Regional STD Reference centre, Kolkata from 2010 to 2013

Year (Jan-Dec)	Total symptomatic discharges	Within normal limit	ASCUS	LSIL (including koilocytes)	HSIL	Percentage of smear with cervical dysplasia (\geq LSIL)
2010	246	199	8	33	1	13.82%
2011	277	238	8	30	1	11.19%
2012	661	587	19	51	4	8.32%
2013	959	760	66	129	4	16.10%

Table 4: Co-relation of cervical dysplasia with different STIs

Year	Features	Syphilis	<i>T. vaginalis</i>	<i>Candida spp.</i>
	Total positive	8	71	19
2010 (n=246)	Normal	8	51	13
	ASCUS	0	6	2
	LSIL	0	13	4
	HSIL	0	1	0
	Total positive	9	90	31
2011 (n= 277)	Normal	8	67	17
	ASCUS	0	5	2
	LSIL	1	17	12
	HSIL	0	1	0
	Total positive	21	246	81
2012 (n=661)	Normal	19	193	72
	ASCUS	0	12	0
	LSIL	2	38	8
	HSIL	0	3	1
	Total positive	29	71	19
2013 (n= 959)	Normal	27	50	13
	ASCUS	0	6	2
	LSIL	2	14	4
	HSIL	0	1	0

n = no of symptomatic discharges in respective years

Table 4: Prevalence of various sexually transmitted infection among symptomatic women (N=2143) at the Regional STD Reference Centre, Kolkata from 2010-2013

Infection	Specimen	Test performed	Year (Jan-Dec)	% positivity
		VDRL	2010	3.25%
			2011	3.24%
			2012	3.17%
			2013	3.02%
		TPHA	2010	6.91%
Syphilis	Serum		2011	6.85%
			2012	4.68%
			2013	4.58%
		Culture on Kupferberg	2010	28.86%
<i>T. vaginalis</i>	Vaginal swab	Wet mount	2011	32.49%
			2012	37.21%
			2013	37.88%
<i>Candida spp.</i>	Vaginal swab	KOH mount	2010	7.72%

Cervical dysplasia	Ecto and endo cervical smear	Culture on SDA	2011	11.19%
		Gram staining	2012	12.25%
			2013	19.91%
			2010	13.82%
		Papanicolaou (Pap) staining method	2011	11.19%
			2012	8.32%
			2013	16.10%

N= Total number of discharge samples

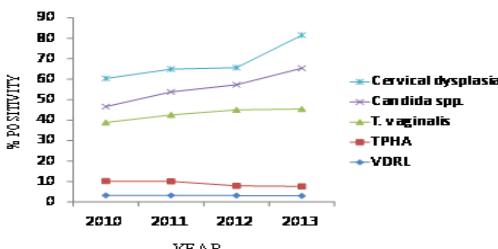


Figure 1. Trends of STIs from 2010 to 2014 in a Referral Laboratory in West Bengal

DISCUSSION

Based on cytological analysis of samples, our observation indicates, there was a gradual increase in the STI patients samples in regional STI referral laboratory, Kolkata. The attendance of female patients in the STI clinic mainly depends on referral from the gynaecology clinic and most of the infections remain asymptomatic¹⁶. In the present study, most of the STI cases belonged to 25-44 year age group and they were symptomatic¹⁷, patients of the Hindu population were high than Muslim population in all the respective years. Some studies observed that trichomoniasis was most the commonest STI, followed by candidiasis and syphilis¹⁸. Previous decades, up to 2006, have shown an increase in the prevalence of syphilis as a sexually transmitted infection followed by gonorrhea, candidiasis, herpes progenitalis and chancroid, which were prevalence in men¹⁷. Mutualistic association of HIV seropositivity with syphilis was highest as in earlier studies¹⁹. Based on our findings we concluded that the recent trends of prevalence of percent positivity of syphilis based on VDRL and TPHA tests was reduced in respective years. Previously some studies have shown that few species of *Candida* are pathogenic and induce hyphal and pseudohyphal formation and enhance the colonization and induced mucosal swelling which established to candidiasis infection^{20,21}. These characters facilitate *Candida* spp. to enter the mucosal surface and trigger many signal pathways¹⁶ causing infection. Simultaneously, *T. vaginalis* infection played a role for developing cervical abnormalities²². Some byproducts of *T. vaginalis* can damage the vaginal epithelium, degrade cervical mucus and cleave immunoglobulin^{23,24}. Previously it was reported that the double-stranded RNA

of *T. vaginalis* was associated with differential alteration and function of enzymes which increase the virulence factor²⁵. Effect of this enzyme expression and increasing virulence, *T. vaginalis* could alter the various STIs inducing cervical abnormalities. Studies in literature showed that women with positive cytological results for *T. vaginalis* are at significant risk of acquiring HR-HPV infection²⁶. As *T. vaginalis* infection was a STI cases, it has correlation with both abnormal cervical cytology and with HPV infection. In a previous study it has been reported that cervical dysplasia has been more frequently women with significant positive cytological results for trichomoniasis and candidiasis^{27,28}. Chakrabarti *et al*, 1992²⁹ reported that Trichomoniasis and Candidiasis had no co-relation with cervical dysplasia. From our observed data it was concluded that percent positivity rate of *T. vaginalis* infection was most frequent than *Candida* spp. in consecutive years. Our study showed an increasing trend of trichomoniasis and Candidiasis, but percentage of syphilis was decreased over last four years. A previous study reported that the concomitant appearance of cervical dysplasia with higher prevalence of *T. vaginalis*, syphilis and *Candida* spp.³⁰. Epidemiological report supported that the cofactors such as *T. vaginalis* infection increase cervical neoplasia³¹. In the present study *T. vaginalis* and *Candida* spp. were found to be common etiological agents of vaginal discharge. This study clearly shows that women with positive cytological results for cervical dysplasia are likely to co-infected with *T. vaginalis* and *Candida* spp.

CONCLUSION

In conclusion, *T. vaginalis* and *Candida* spp. infections were frequently observed with cervical dysplasia and percent positivity of both the infections showed increasing trends in consecutive year whereas a distinct varying trend was observed in syphilis cases in which both positives of VDRL and TPHA tests were considerably reduced in consecutive years. It is observed that both trichomoniasis and vaginal candidiasis were more common with any cervical cytological abnormalities in all the symptomatic women, but syphilis was not associated with cervical cytological abnormalities. It could be concluded that the high prevalence of STIs would increase the risk of cervical dysplasia. Cervical cytology screening program and testing for concomitant infection are to be strengthened as soon as possible in high risk area. This is necessary for improvement of STIs treatment; need to develop modification of the STI management guidelines throughout the country and enhancing the active surveillance systems and case detection.

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