RESEARCH COMMUNICATION

Prevalence of Human Papillomavirus and Co-Existent Sexually Transmitted Infections among Female Sex Workers, Men having Sex with Men and Injectable Drug abusers from Eastern India

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Abstract

Background: Human papillomavirus (HPV) is a very common sexually transmitted disease affecting both men and women and is responsible for different ano-genital cancers in either sex. Co-existing sexually transmitted infections (STI) including HIV have been considered as important co-factors for carcinogenesis induced by HPV. The purpose of this study was to determine the prevalence of any HPV, HPV 16 and HPV 18 and also concomitant STIs among female sex workers (FSW), men having sex with men (MSM) and injectable drug users (IDU). Material and Method: This cross-sectional study was conducted among 45 FSWs, 26 MSMs and 58 IDUs who attended the STI or de-addiction clinics. Genital scrape samples collected from glans penis and coronal sulcus in males and cervical squamo-columnar junction in females were tested for HPV DNA by PCR using HPV L1 consensus primer. Type specific PCR to detect HPV 16 and 18 was done on the samples positive on consensus PCR. All participants were tested for associated STIs including HIV and hepatitis B and cervical cytology was done on all females. Results: Among the FSWs, HPV was detected in 73.3% and HPV 16 and 18 was detected in 25.7%. Though the HPV prevalence was similarly high among MSMs (69.2%) and IDUs (72.4%), the prevalence of HPV 16 and 18 was much lower in these groups compared to the FSWs. Prevalence of cervico-vaginal infection with Trichomonas vaginalis and syphilis was significantly higher in the HPV positive women compared to the HPV negative women. There was no statistically significant difference in the prevalence of other STIs among HPV positive and negative women and men. Conclusion: HPV infection is highly prevalent among FSW, MSM and IDUs. *Trichomonas vaginalis* infection is more frequent in HPV positive women

Keywords: Human papillomavirus - co-existing STIs - female sex workers - men having sex with men - drug users

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Introduction

Human papillomavirus (HPV) is the most common sexually transmitted viral infection and studies estimate that globally 50-80% of ever sexually active men and women are infected with the virus at least once during their lifetimes (Koutsky, 1997). HPVs have been classified into low and high risk types depending on their oncogenic potentials. Anogenital infections with high-risk HPV types (types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70) predispose men and women to the development of preinvasive and invasive disease in cervix, vulva, anus and penis. Besides HPV, genital tract infections from other organisms like *Trichomonas vaginalis* (TV), *Chlamydia trachomatis and Herpes simplex virus* (HSV) type 2 have also been implicated in cervical cancers in women. Longitudinal studies have observed that TV infection is associated with high relative risk of both preinvasive and invasive cervical cancer (Viikki et al., 2000). Chlamydia, the obligate intracellular bacterium is frequently found in association with benign proliferative, pre-neoplastic and malignant changes in cervical epithelium (Bułhak et al., 2007). There is evidence that chlamydia infection may contribute to neoplastic changes in the transformation zone of uterine cervix independent of high risk HPV infection (Schlott et al., 2005). Although prospective studies based on serology did not find any independent association between HSV and cervical carcinogenesis, there is evidence that HSV 2 as a cofactor can lead to cervical cancer in presence of high risk HPV (Szostek et al., 2009). Human Immunodeficiency virus (HIV) positive women have significantly higher prevalence of genital squamous intraepithelial lesions and of multifocal HPV related diseases (Palefsky et al., 1999). If cervical cancer

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does develop in HIV positive women, it may be more aggressive in nature and less responsive to treatment.

The female sex workers (FSW), the men having sex with men (MSM) and the injectable drug abusers (IDU) registered with the STI and de-addiction clinics are at high risk of contacting various sexually transmitted infections (STI) due to their high risk sexual behaviours. Co-existence of HPV and other sexually transmitted infections can increase their predisposition to different ano-genital cancers. Association between different STIs is frequent, but there are few statistics on the prevalence of co-infections specially in the high risk groups we have included in our study. The present study aims to evaluate the magnitude of HPV infection in FSWs, MSMs and IDUs and to assess the risks of concomitant STIs in the HPV infected sub-populations.

Materials and Methods

The cross-sectional study was conducted on 45 female sex workers (FSW), 26 men having sex with men (MSM) and 58 male injectable drug abusers (IDU) registered with some of the clinics of National AIDS Prevention and Control Organization at West Bengal, a province in eastern India. The patterns of high risk behaviour (FSW, MSM or IDU) with their HIV infection status were obtained from the existing medical records maintained in the respective clinics. All the potential subjects were counselled by the investigators in presence of a trained counsellor of AIDS Control Society about the objectives and methodology of the study. Those who provided written informed consent were enrolled in the study. Ethical clearance was obtained from Institutional Ethics Committee, Medical College, Kolkata.

The subjects were interviewed to fill up a structured questionnaire on socio-demographic characteristics. Their high risk behaviour patterns were reconfirmed during the interview. Scrape samples were collected with Cervex brushes® (Rovers Medical Devices B. V., Netherlands) from ectocervix and endocervix in females and from glans penis and coronal sulcus in males for HPV testing. The brush was swirled and the tip was detached into sterile cold PBS solution. The vials containing the samples were transported to the laboratory maintaining cold chain. An Ayre's spatula was used to obtain cervical scraping for Pap smear from each FSW. Multiple cervical and vaginal swabs were obtained from the FSWs for Chlamydia direct antigen detection test, Candida culture in Saborauds Dextrose Agar and TV culture in Kupferburgs media. Blood samples were collected from all subjects for serological tests like Venereal Disease Research Laboratory test (VDRL), *Treponema pallidum* Haem-agglutination test (TPHA), hepatitis B surface antigen (HBsAg) detection test, HSV 2 IgM and HIV antibody test. Patients whose samples showed VDRL reactivity of ≥1:8 and TPHA positivity were considered to be positive for syphilis.

DNA extraction from genital scrape samples was done by the standard phenol chloroform extraction method. PCR was performed using GP5+/GP6+ L1 consensus primers. PGMY09/11 L1 consensus primers were used to confirm negative cases and detect cases with low levels of HPV. Amplification of β-globin DNA was performed as a positive control for the presence of amplifiable DNA in the specimen. Type-specific PCR using HPV 16, 18 specific primers was done to detect these two genotypes in samples positive on HPV L1 consensus PCR.

Slides obtained for cervical cytology were stained by Papanicolaou method and interpreted using Bethesda 2001 classification (Solomon et al., 2002).

For Chlamydia detection, the Clearview Chlamydia MF Kit, manufactured by Inverness Medical Professional Diagnostics, UK was used. Chlamydial antigen was extracted from the swab by heating at 80 °C with extraction reagent. The extract was added to the absorbent pad in the sample window. The test strip contained a region of immobilized monoclonal anti – chlamydial antibody in the result window. Positive result was denoted by the appearance of a line in the result window.

For candida detection, cream coloured colonies on Saborauds Dextrose Agar were picked up, smears were prepared and stained by Grams method to visualize budding yeast cells.

Wet mount examination for *trichomonas vaginalis* was done on samples from Kupferburgs media daily for seven

Table 1. Socio-demographic Parameters of the Study Groups

	FSW (N=45)	MSM (N=26)	IDU (N=58)
Age Distribution			
<20 years	3 (6.7%)	7 (26.9%)	6 (10.3%)
20-29 years	28 (62.2%)	7 (26.9%)	31 (53.4%)
30-39 years	10 (22.2%)	1 (3.8%)	17 (29.3%)
>40 years	3 (6.7%)	0	3 (5.2%)
Unknown	1 (2.2%)	11 (42.3%)	1 (1.7%)
Religion			
Hindu	28 (62.2%)	23 (88.5%)	48 (82.8%)
Muslim	17(37.8 %)	2 (7.7%)	6 (10.3%)
Others	0	0	2 (3.4%)
Unknown	0	1 (3.8%)	2 (3.4%)
Literacy			
Illiterate	33 (73.3%)	2 (7.7%)	11 (18.9%)
Primary & Middle	5 (11.1%)	9 (34.6%)	19 (32.8%)
High school & above	4 (8.9%)	14 (53.8%)	27 (46.6%)
Unknown	3 (6.7%)	1 (3.8%)	1 (1.7%)
Duration of Risk Behaviour			
<1 year	10 (22.2%)	1 (3.8%)	4 (6.9%)
2-5 years	21 (46.7%)	5 (19.2%)	40 (69%)
6-10 years	4 (8.9 %)	4 (15.4%)	9 (15.5%)
>10 years	9 (20%)	1 (3.8%)	3 (5.2%)
Unknown	1 (2.2%)	15 (57.7%)	2 (3.4%)

Table 2. Prevalence of Any HPV and of HPV Types 16 & 18 in the Study Groups

	FSW (n=45)	MSM (n=26)	IDU (n=58)
Any HPV	35 (73.3%)	18 (69.2%)	42 (72.4%)
HPV 16	7 (15.6%)	0	2 (5.2%)
HPV 18	2 (4.4%)	0	0
Both HPV 16 & 18	2 (5.7%)	0	0

Table 3. Prevalence of Various STIs by Genital HPV Infection Status in the Study Groups

	FSW (N=45)			MSM (N=26)			IDU (N=58)		
	HPV +ve (N=35)	HPV –ve (N=10)	p	HPV +ve (N=18)	HPV –ve (N=8)	p	HPV +ve (N=42)	HPV –ve (N=16)	p
Candida +ve	31 (88.6%)	9 (90.0%)	0.69	Not done	Not done		Not done	Not done	
T Vaginalis +ve	8 (22.9%)	0	0.04	Not done	Not done		Not done	Not done	
Chlamydia +ve	5 (14.3%)	2 (20.0%)	0.8	Not done	Not done		Not done	Not done	
HBsAg +ve	0	0		0	1 (12.5%)	0.31	1 (2.4%)	1 (6.2%)	0.47
HSV 2 +ve	12 (34.3%)	1 (10.0%)	0.13	3 (16.7%)	1 (12.5%)	0.64	1 (2.4%)	1 (6.2%)	0.47
Syphilis +ve	13 (37.1%)	0	0.02	0	2 (7.7%)		0	0	
HIV +ve	6 (17.1%)	1 (10.0%)	0.5	1 (5.6%)	0	0.69	2 (4.8%)	0	0.52

Table 4. Cytology Diagnosis by Genital HPV Infection Status in the FSWs (X^2=4.87; p=0.18)

	HPV + ve (n=35)	HPV – ve (n=10)
Negative	15 (42.9%)	7 (70%)
ASCUS	3 (8.6%)	-
LSIL	16 (45.7%)	2 (20%)
HSIL	-	-
Inadequate	1 (2.8%)	1 (10%)

days to detect the motile organisms.

The serological tests were performed using standard kits available and following the manufacturer's instructions. VDRL was performed using kits manufactured by Institute of Serology, India. For TPHA kits manufactured by New Market Laboratories, UK were used. HBsAg was detected by ELISA using kits manufactured by Qualpro Diagnostics, India. HSV 2 detection was by ELISA techniques using kits manufactured by Calbiotech, USA. HIV rapid detection was done by a membrane based flow-through immunoassay for detection of antibodies to HIV 1 and HIV 2 in serum using Retroquick HIV Kit manufactured by Qualpro Diagnostics, India.

Results

The socio-demographic parameters and the duration of risk behaviours of the three study groups are given in Table 1. All the drug abusers were males. The prevalence of HPV (any type) was uniformly high in all the groups; 73.3% in FSWs, 69.2% in MSMs and 72.4% in IDUs. Highest proportion of HPV infected cases were observed in men and women aged 20-29 years. HPV infection was most common in female sex workers who reported to be in the trade for 2-5 years. Significantly higher prevalence of HPV types 16 and 18 was observed among the FSWs (24.4%) compared to the MSMs (0%) and the IDUs (5.2%) (Table 2). The frequencies of concomitant sexually transmitted infections among the HPV infected and non-infected study populations are shown in Table 3. The prevalence of TV (22.9%) and syphilis (37.1%) was significantly higher in the HPV positive FSWs compared to the HPV negative ones (0% and 0% respectively). None of the women with TV infection was HPV 16 or 18 positive. HSV 2 and HIV infections were observed more frequently among the HPV positive women though the difference between HPV positive and negative women was not statistically significant.

No difference in the prevalence of hepatitis B, HSV 2, syphilis and HIV was observed between the HPV positive and negative men belonging to the MSM or IDU groups.

Cytological abnormalities of cervix (ASCUS or worse) were more frequently seen among the HPV positive female sex workers compared to those who were HPV negative (54.3% vs 20%) (Table 4). However, no woman in the study had high grade abnormalities on cytology.

Discussion

This is the first study from India to report the prevalence of HPV and other concomitant STIs in women and men belonging to the high risk groups (FSWs, MSMs and IDUs). The high prevalence of HPV among female sex workers and HIV infected women has been reported from India earlier (Sarkar et al., 2008:2011). Our study observed almost similar high prevalence not only in FSWs but also in men having sex with men and in men addicted to injectable drugs of abuse. Although the overall HPV prevalence was comparable among the three groups, the significantly higher prevalence of oncogenic HPV types 16 and 18 in FSWs possibly signifies higher risk of neoplasias among the women compared to the men we studied.

The present study has observed higher prevalence of TV, HSV 2, syphilis and HIV among the HPV infected female sex workers. The results do not provide evidence to substantiate the hypothesis that certain STIs and HPV may act synergistically in cervical carcinogenesis. The concomitant appearance of HPV and other STIs in sexually promiscuous women is best explained by the fact that they are co-variables of sexual behaviour. It seems reasonable to consider that these sexually transmitted infections are commonly found in women with HPV because of their promiscuous sexual behaviour. Even then some of the observations in our study indicate higher susceptibility of the HPV infected women to certain STIs which needs further investigations.

In our study the observed prevalence of *Trichomonas vaginalis* and syphilis infection among the HPV infected women was significantly higher compared to the non-infected women. Though it was not possible to derive from the study whether the higher prevalence would increase the risk of neoplasias, a meta-analysis of 24 studies found a significant positive association between TV infection and cervical neoplasia (including both cervical intraepithelial neoplasia and cervical cancer) (Zhang & Begg , 1994). A Finnish prospective study also observed TV infection to be associated with a high relative risk of both preinvasive

and invasive cervical cancer combined (Standardized Incidence Ratio 6.4) (Viikki et al., 2000).

The frequency of *Candida* and *Chlamydia* infection in the HPV positive women was not higher than their HPV negative counterparts in our study. Till date there is no evidence that fungal infection in the vagina is associated with significant increase in the risk of cervical neoplasias either in presence of absence of HPV (Viikki et al., 2000). Though an in vitro study has implicated *Chlamydia* in cervical carcinogenesis (Schlott et al., 2005), a prospective five-year follow-up study of 530 women with cervical HPV infection failed to demonstrate any influence of coexistent cervical infection with *Chlamydia* on the clinical course of HPV lesions (Yliskoski et al., 1992). The rates of persistence of HPV infection or progression to neoplasias were same in the HPV positive women with co-existing *Chlamydia* infection compared to HPV positive women without Chlamydia infection.

The number of subjects in the study was low since most of the eligible men and women we approached did not volunteer to participate in the study due to the fear of being further stigmatized. Most of the FSWs with abnormal Pap smear refused colposcopy when it was explained to them that colposcopy could lead to cervical biopsy for which they would have to abstain from sex for one week. Still the present study reveals some interesting facts about the very high risk but hard to reach population.

In conclusions, HPV infection is highly prevalent in women and men with high risk sexual behavior. Cervico-vaginal infection with *Trichomonas vaginalis* is more frequent in HPV positive women and the natural history of such concomitant infection needs to be studied further.

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