Original Article

Prevalence of high-risk human papilloma virus types and cervical smear abnormalities in female sex workers in Chandigarh, India

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Abstract

Purpose: Cervical cancer is the most common cancer among women in developing nations. Nearly 90% of the cases have been linked to the presence of high-risk human papillomavirus (hrHPV) types 16 and 18. The risk of cervical cancer may be high in female sex workers (FSWs) due to multiple sexual partners. This study aimed to determine the prevalence of cytological abnormalities and hrHPV types 16 and 18 in FSWs in Chandigarh, North India using the liquid-based cytology (LBC) approach. **Materials and Methods:** The cervical brush samples were collected from 120 FSW and 98 age-matched healthy controls (HCs). These were subjected to pap smear using conventional method, LBC and the detection of hrHPV types 16 and 18 was carried out using polymerase chain reaction. **Results:** The LBC samples showed better cytological details and also reduced the number of unsatisfactory smears from 11% in Pap to 1.5% in the LBC. A significantly higher number of inflammatory smears were reported in FSWs (51.7% vs. 34.7%, $P = 0.01$). The hrHPV types 16/18 were detected in 33/120 (27.5%) FSW versus 23/98 (23.5%) HCs. The risk of acquiring hrHPV was higher in FSWs, who had age at first sex ≤25 years, higher income and the habit of smoking. **Conclusion:** The high prevalence of hrHPV among FSWs and HCs suggests the need for the implementation of effective National Screening Programme for early detection of hrHPV types to decrease the burden of cervical cancer, especially in high-risk population.

Key words: *Cervical cancer, female sex workers, human papillomavirus, liquid based cytology*

Introduction

Cervical cancer is the fourth most common cause of cancer in women worldwide, and nearly 528,000 new cases and 266,000 deaths are reported each year.[1] Nearly 70% of the burden is in developing countries, and more than one-fifth of all new cases have been diagnosed in India alone in 2012.[1] The infection with human papillomavirus (HPV) plays a major role in the development of cervical cancer.[2] This virus belongs to the family *Papillomaviridae* and has more than 100 known genotypes[3] of which more than 40 genotypes can cause genital infections.[4] These are further categorised into high-risk HPV (hrHPV) and low-risk HPV types. Among the hrHPV types, HPV 16 is highly oncogenic and implicated in the causation of nearly 50–60% of high-grade squamous intraepithelial lesions (HSILs) and cervical cancer.[5] This is followed by HPV 18 which accounts for 10–20% of all cervical cancers. Infection with HPV is usually acquired at the onset of sexual activity, but most of the infections resolve with time. However, persistent infections can progress to cervical intraepithelial neoplasia and invasive cancer.[2,6]

A wide variation in the prevalence of HPV positivity ranging from 6% to 38% has been reported in the general population from different geographical regions.[5,7-14] The risk of acquiring HPV infection directly correlates with the number of sexual partners and may be higher in female sex workers (FSWs) as compared to the general population.[15-17] Globally, HPV positivity in FSW ranges from 2.3% to 100% (median 42.7%). This difference in positivity may be due to the difference in various diagnostic modalities

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or may be attributed to the different sexual practices of a country *per se*.[15] The FSWs may be at a higher risk of HPV infection due to early onset of sexual activity, multiple partners, frequent change of partners, smoking, higher incidence of sexually transmitted infections (STIs), concomitant human immunodeficiency virus infection and use of oral contraceptives. The literature on the HPV positivity in FSWs from India is scanty and calls for the attention of researchers.[5]

Since HPV plays an important role in carcinogenesis, this study postulates that FSWs may be at a higher risk of cervical cancer and aims to determine the prevalence of cervical abnormalities in FSWs and to correlate it with detection of hrHPV types in this population. The conventional screening for cervical cancer relies on the Pap smear examination which has various limitations. Recently, liquid-based cytology (LBC) has come up with various anticipated advantages such as reduction of unsatisfactory smear, reduced screening time and also residual material can be used for HPV-DNA testing.[18] This study, therefore, used an LBC approach for the screening of epithelial abnormalities, cytology and the molecular detection of hrHPV16 and 18 in a vulnerable population of FSWs.

Materials and Methods

Study subjects

This study was conducted from July 2011 to June 2012 in a group of 120 FSWs aged 18–45 years and 98 age matched controls from the general population. The general population was later labelled as 'healthy controls' (HCs) for analysis. The FSWs were defined as females who had sex for money and lived with or without a partner. The samples were collected from FSWs who were registered under the target intervention project at run by the NGO in the Sec 45 and Manimajra region of Chandigarh, India. The participants were counselled about the nature of the sample to be collected and only those participants who provided written informed consent were included in the study. The pregnant women at the time of sample collection were excluded from the study. The study was initiated after obtaining ethical clearance from National AIDS Control Organization (NACO), New Delhi, India and Institute Ethics Committee as per National guidelines. A pretest interview schedule was used to collect the information on sociodemographic characteristics such as age, education, smoking, drinking and the use of condom during the last sexual act by the participants.

Sample collection and processing

The cervical samples were collected from participants by trained personnel using Cervex-Brush® (Rover's Medical Devices BV, The Netherlands). The 'Split Cervix Samples' obtained were used for conventional Pap smear (CPS) and then the brush was dropped in 10 ml of PreservCyt solution and transported to the laboratory for further processing as Surepath™ and stained with PREPSTAIN™. The cervical cytology was reported in a blinded manner by a cytopathologist in accordance with 'The Bethesda System 2001'[19] and classified as - Normal Smear, Inflammatory Smear and Epithelial Abnormalities. Cervical sample reporting was done by cytopathologist who was unaware of the subject from whom the sample was collected. Inflammatory smears included smears with bacterial vaginosis (BV), Candida, actinomycetes like organisms (ALO), *Trichomonas vaginalis* (TV) and herpes simplex virus (HSV), and epithelial abnormalities included atypical squamous cells of undetermined significance (ASC-US), low grade squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC).

Detection of high-risk human papillomavirus types

The residual concentrated material in the SurePath concentration tube was subjected to DNA extraction followed by detection of hrHPV types 16 and 18 by conventional multiplex polymerase chain reaction (PCR). The DNA extractions were carried out using commercially available kit as per the manufacturer's instructions (Qiagen, Germany). The DNA was eluted in 50 µl of elution buffer and integrity of the extracted DNA was checked by amplifying housekeeping gene β-globin. Multiplex PCR was carried out using primers targeting upstream regulatory region for HPV16 and E6 region for HPV18.[20] Three microlitre of eluted DNA was used for PCR amplification with 1X PCR buffer (10 mM Tris with 15 mM $MgCl_2$), 200 µM of dNTPs (Fermentas, MD, USA), 1 µM of each forward and reverse primer and 1 unit of Taq polymerase (Genei, Bangalore, India). The reaction condition of the thermocycler were standardised to initial denaturation at 94°C for 5 min, followed by 30 cycles with 94°C for 30 s, 55°C for 30 s, 72°C for 1 min and final extension at 72°C for 5 min. The band size of 216 bp for HPV16 and 100 bp for HPV18 were visualised in agarose gel stained with ethidium bromide. The positive controls for HPV 16 and HPV 18 were received as a kind gift from Dr. EM Villiers, DKFZ Institute, Germany. Positive and negative controls were included in each run and all precautions were taken to prevent carry over contamination.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 (free demo version (Graphpad Software Inc, USA)). The normality of the data was checked using Kolmogorov–Smirnov test. To find out the association in categorical variables, Chi-square test was performed. For continuous variables, the data were represented as mean ± standard deviation (SD) and Mann–Whitney U-test was performed.

For assessing the risk factors of acquiring HPV among FSWs odds ratio (OR) were calculated by performing multivariate analysis. $P < 0.05$ was considered statistically significant.

Results

Sociodemographic characteristics

The mean age of FSWs and HCs were 34.48 years and 34.22 years (SD ±8.0 and ±7.9), respectively. The sociodemographic and behavioural characteristic of FSWs and HCs are given in Table 1. The literacy level was higher in HCs as compared to FSWs. Nearly 60.8% of FSWs were illiterate as compared to 32.7% of HCs, also level of secondary and higher education in FSWs (10%) was significantly lower than HCs (27.6%) ($P < 0.001$). The high-risk behaviour in the form of smoking and drinking was significantly higher in FSWs (25% and 27.5%) than in HCs (5.1% and 4.1%) ($P < 0.001$). A higher percentage

Table 1: Demographic and behavioural characteristics of female sex workers and individuals from general population

Characteristics	HCs n (%)	FSW n (%)	P
Age (years)			
≤30	36 (36.7)	46 (38.3)	0.85
31-40	46 (46.9)	52 (43.3)	
>40	16 (16.3)	22 (18.3)	
Education			
Illiterate	32 (32.7)	73 (60.8)	<0.001
Till 5th standard	13 (13.3)	12 (10.0)	
6-10th standard	26 (26.5)	23 (19.2)	
Secondary and above	27 (27.6)	12 (10.0)	
Migration status			
Native	37 (37.8)	51 (42.5)	0.48
Migrant	61 (62.2)	69 (57.5)	
Parity			
≤1	16 (16.3)	19 (15.8)	0.92
>1	82 (83.7)	101 (84.2)	
Smoking			
Yes	5 (5.1)	30 (25.0)	<0.001
No	93 (94.9)	90 (75.0)	
Drinking			
Yes	4 (4.1)	33 (27.5)	<0.001
No	94 (95.9)	87 (72.5)	
Condom use during last sexual act			
No	76 (77.5)	34 (28.3)	0.047
Yes	22 (22.4)	86 (71.6)	
HPV			
Negative	75 (76.5)	87 (72.5)	0.49
Positive	23 (23.5)	33 (27.5)	

HPV: Human papillomavirus, FSW: Female sex worker, HCs: Healthy controls

(71.6%) of FSWs reported condom use during the last sexual activity as compared to the HCs (22.4%) ($P = 0.04$).

High-risk human papillomavirus positivity

The percentage positivity of hrHPV DNA was higher in FSWs (27.5%) as compared to HCs (23.5%), but the difference was not statistically significant ($P = 0.49$) [Table 1]. In the FSWs, the HPV-DNA positivity in ≤30 years of age was 26.1% which remained comparable in FSW >40 years of age (22.7%). However, this study observed a decline in hrHPV DNA positivity from 25% to 18.8% in HCs with increasing age [Figure 1].

Association of high risk human papillomavirus positivity with social behaviour

A higher hrHPV positivity was observed in FSWs who were smokers (36.7% vs. 24.4%, OR =4.11, $P = 0.05$) and those who reported age at first sex of ≤25 years (31.3% vs. 5.5%; OR =7.7, $P = 0.02$). Furthermore, hrHPV positivity was higher in FSWs who reported higher income of >Rs. 5000 as compared to those with lower income; although, this was not statistically significant (45.7% vs. 8.3%, OR = 6.418 $P = 0.12$) [Table 2].

Liquid-based cytology results

The rate of unsatisfactory smears reduced from 11% in CPS to 1.5% in LBC. In addition, LBC offered an advantage to detect infections with Candida, BV, ALO, TV and HSV. The positivity based on cytology was 50.8% for BV, 6.6% for Candida, 2.5% for TV, 0.8% for ALO and 0.8% for HSV. FSWs had a higher number of inflammatory smears as compared to HCs (51.7% vs. 34.7%, $P = 0.01$) [Table 3]. Further, HPV positivity in the inflammatory smears were higher but not significant in FSWs vs. HCs (32.3% vs. 23.5%, $P = 0.37$). Among inflammatory smears, the incidence of BV was significantly higher in FSWs versus HCs (50.8% vs. 31.6%, $P = 0.04$). Multiple infections of BV with ALO, TV and HSV were observed in 11 FSWs and in 7 HCs. The concurrent STIs were
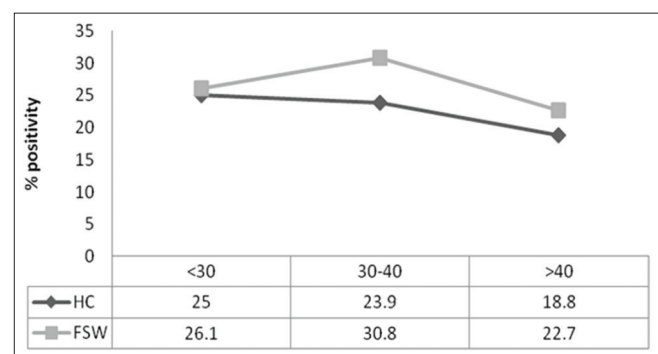

	<30	30-40	>40
HC	25	23.9	18.8
FSW	26.1	30.8	22.7

Figure 1: Human papillomavirus age-wise positivity among female sex workers versus healthy controls

Table 2: Demographic and behavioural characteristics of female sex workers as per their human papillomavirus status

Characteristics	FSWs				
	Total	HPV positive n (%)	OR	95% CI	P
Age (in years)					
≤30	46	12 (26.08)	1		
31-40	52	16 (30.7)	0.851	0.254-2.848	0.79
>40	22	5 (22.7)	0.291	0.048-1.756	0.17
Education					
Illiterate	73	21 (28.8)	1		
Till 5th standard	12	4 (33.3)	3.434	0.636-18.523	0.15
6-10th standard	23	6 (26.1)	1.026	0.247-4.266	0.97
Secondary and above	12	2 (16.7)	0.979	0.145-6.622	0.98
Income of FSW (in Rs.)					
<2000	12	1 (8.3)	1		
2000-5000	73	16 (21.9)	2.032	0.192-21.548	0.55
>5000	35	16 (45.7)	6.418	0.593-69.432	0.12
Smoking					
No	90	22 (24.4)	1		
Yes	30	11 (36.7)	4.11	0.949-17.798	0.05
Drinking					
No	87	23 (26.4)	1		
Yes	33	10 (30.3)	0.814	0.216-3.067	0.76
Age at first sex (in years)					
≤25	102	32 (31.3)	7.771	0.990-60.99	0.02
>25	18	1 (5.5)	1		
Sex partners					
1-2	32	8 (25)	0.84	0.333-2.118	0.71
>2	88	25 (28.4)	1		
Parity					
≤1	19	3 (15.8)	1		
>1	101	30 (29.7)	4.46	0.648-30.693	0.13
Duration in sex work (years)					
≤5	4	0	0.276	0.014-5.291	0.21
>5	116	33 (28.4)	1		

HPV: Human papillomavirus, FSWs: Female sex workers, CI: Confidence interval, OR: Odds ratio

Table 3: Human papillomavirus status among the female sex workers and healthy controls as per smear results

Smear result	FSWs (n=120) n (%)	HCs (n=98) n (%)	P
Normal smear	56 (46.7)	62 (63.3)	0.01
HPV +	12 (21.4)	13 (21)	0.95
Epithelial abnormalities	2 (1.7)	2 (2)	
HPV +	1 (50)	2 (100)	
Inflammatory smear	62 (51.7)	34 (34.7)	0.01
HPV +	20 (32.3)	8 (23.5)	0.37
Bacterial vaginosis	61 (50.8)	31 (31.6)	0.04
Multiple infections	11 (9.1)	7 (7.1)	0.3

HPV: Human papillomavirus, FSWs: Female sex workers, HCs: Healthy controls

treated in accordance with NACO guidelines. Epithelial abnormalities like HSIL and SCC were reported in 2 (1.6%) FSWs. The FSWs showing SCC in the LBC smear were positive for hrHPV 16 while the one with HSIL did not show the presence of hrHPV 18. However, in two HCs, epithelial lesions like ASC-US and atypical glandular cells of undetermined significance (AGUS) were observed and both of these were positive for hrHPV 16.

Discussion

HPV which belongs to the family *Papillomaviridae* plays an important role in cervical cancer. This cancer is more frequent in developing countries where population level screening programmes are generally unavailable. According to the World Health Organisation report, globally cervical cancer comprises 12% of all cancers in women and is the leading gynaecological malignancy in the world.[21] India contributes to one-fifth of the total estimated annual global incidence of 500,000 cancers,[22] and it is the

persistent HPV infection which is the most important risk factor for cervical cancer.[23,24]

This study gives an insight into the prevalence of hrHPV and associated risk factors in a vulnerable population of FSWs and self-reported HCs from a low socioeconomic background. A higher monthly income of more than Rs. 5000 (Indian Rupees) was associated with six times higher risk of acquiring HPV infections as compared to an income of less than Rs. 2000, thereby indirectly implying more number of sexual partners. The FSWs debuting an active sexual life at the age of ≤25 years had 7.7 times higher risk of acquiring HPV infection. The result of the present study is in agreement with previous studies which show that early onset of sexual activity, low socioeconomic status, multiple partners and smoking are associated with increased risk of cervical carcinoma. The condom use during last sexual act was reported in significantly higher number of FSWs as compared to the HCs (71.6% vs.22.4%, $P = 0.047$). This may be attributed to the activities of NACO Condom Social networking Programme under which nearly 15.05 crore condoms have been distributed to FSWs till December 2012.[25]

This study documents a percentage positivity of hrHPV of 27.5% and 23.4%, respectively, among the FSWs and HCs living in a low socioeconomic area in and around Chandigarh, India. This is in concordance with hrHPV positivity of 24.4% reported by Ghosh et al.[5] in a small group of FSWs ($n = 45$) from West Bengal. However, these authors failed to include a control group for comparison. The hrHPV prevalence among the HCs in this study is higher (23.5%) than the 7–13% average positivity reported from India.[11,26-28] Recently, Ting et al.[29] have also reported 30% hrHPV mRNA prevalence in a study carried out on 340 FSWs in Kenya. However, Marek et al.[30] have reported significantly higher hrHPV prevalence in sex workers as compared to the control group (59.5% vs. 25% $P < 0.05$). Similar hrHPV positivity in both groups in this study may be due to the lower socioeconomic conditions and the fact that both the FSWs and HCs lived in the high risk area, although this study included only the FSWs registered under the targeted intervention project. Furthermore, the participants were asked sensitive questions regarding their behaviours and thus their responses may have been affected by recall and social desirability biases. The similar HPV positivity in both FSWs and HCs in spite of higher condom use in the former may be because the information as per the study questionnaire was the use of condom in the last sexual act rather than a regular use.

Further, a declining trend in HPV positivity from 25% to 18.8% in HCs and 26.1% to 22.7% in FSWs with age ≤30 years and >40 years, respectively, was observed. This may be due to spontaneous clearance of infection or development of protective immunity. However, the decline in FSWs was less as compared to HCs. This could be because the HCs who were farther from their sexual debut (>40 years) may have cleared their infections before study enrolment. On the other hand, a sustained high risk of exposure of FSWs to HPV resulted in higher HPV percentage positivity in >40 years of age.

In spite of the fact that India contributes to the maximum number of cervical cancer cases and deaths due to the same, there is a lack of National screening programme. In a resource poor country like India, the women from low socioeconomic areas get only once in a lifetime opportunity to undergo Pap smear examination. In many instances, this is reported as obscure due to blood or unsatisfactory smears. Fluid-based thin layer processing of samples is a recently developed upcoming screening technique[31] which reduces sampling error, makes cytological abnormalities clear and can detect multiple infections. However, cost is an important limiting factor if LBC has to be used at a large scale for screening cervical cancer in developing countries. In the present study, the use of LBC reduced the unsatisfactory smear rate from 11% in CPS to 1.5% in LBC, apart from the advantage of using the residual material for molecular detection of HPV. In this study, a significantly higher prevalence of BV was observed in FSWs as compared to HCs. Since BV is not an STI in the true sense, it may be attributed to the poor hygiene practices of FSWs.[32] Previous studies have shown that STIs are more common in women in whom cervical vaginal microbiota is not dominated by lactobacilli.[32] However, no association was observed between candida or TV positivity with HPV positivity as previously reported by Chakrabarti et al.[33] The LBC could detect multiple STIs in one go, all these patients were treated symptomatically as per NACO guidelines.[25,34]

The epithelial abnormalities in the form of HSIL and SCC were observed in 2 (1.6%) FSW, both of them were confirmed by biopsy, the results were communicated to the health department, and the enrolled patients were treated at our medical centre. The women with SCC showed the presence of hrHPV 16. Among HCs, ASC-US and AGUS one each was reported in two females and both of them were positive for HPV 16. Thus, clinically significant lesions were observed in FSWs as compared to HCs, thus highlighting the need for periodic screening in this group of workers to identify early cervical abnormalities. Further, the HPV DNA positive, cytologically normal smears were retrospectively reviewed but did not show any evidence of koilocytosis.

The major drawback of the present study was that only hrHPV types 16 and 18 were detected, this is because these hrHPV types have been implicated in the development of cervical carcinoma in more than 80% of the cases. Another drawback was the lack of follow-up of hrHPV-positive subjects and subsequent cytological testing.

This would have been pertinent as previous studies have shown that nearly 25% of women 40 years or older who are cytologically negative with a concurrent positive HPV DNA test develop cytologic abnormalities within 5 years and after 10 years, more than 35% develop abnormal pap smears.[35] Although Indian studies have shown that once a lifetime testing for HPV DNA may substantially reduce the burden of cervical cancer,[36] but this testing may not be economically feasible in a resource-limited setting.

The major strengths of this study include the use of LBC samples for the detection of HPV DNA in a large number of FSWs and the inclusion of matched control groups. In another study, our group has reported the presence of hrHPV types 16 and 18 in 37.1% of women whose smears were screened by LBC technique.[37] There is only one study from India[31] wherein the authors have reported better results of LBC as compared to CPS in females with gynaecological problems. This study was carried out in a tertiary care hospital of North India and large numbers of women present to the gynaecology clinic, but the controls were recruited from the same locality to match the socioeconomic conditions and hygiene practices which have a significant bearing on the HPV positivity.

Conclusion

This study reports high prevalence of hrHPV types among FSWs and HCs and suggests the need for the implementation of effective National Screening Programme for early detection of hrHPV types to decrease the burden of cervical cancer, especially in high-risk population.

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Conflicts of interest

There are no conflicts of interest.

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