

RESEARCH COMMUNICATION

Type-Specific Incidence and Persistence of HPV Infection among Young Women: A Prospective Study in North India

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Abstract

Background: Infections with human papillomavirus (HPV) are highly prevalent among sexually active young women in India. However, not much is known about the incidence of type-specific human papillomavirus (HPV) infections and their patterns of persistence, especially in the Indian context. **Objective:** The objective of this study was to evaluate the rate of acquisition and persistence of HPV types in young women. **Methods:** Women residing in an urban slum in Delhi (n=1300) were followed for 24 months at 6 monthly intervals. Exfoliated cervical cells collected at each visit were tested for the presence of HPV DNA. Genotyping was performed using the reverse line blot assay. **Results:** The incidence rate for any HPV type was calculated to be 5 per 1000 women-months. Among high risk HPV types, HPV16 had the highest incidence rate followed by HPV59, HPV52 and HPV18, i.e., 3.0, 0.58, 0.41 and 0.35 women per 1000 women-months respectively. The persistence rate was higher for high-risk than low-risk HPV types. Among low-risk types, HPV42, HPV62, HPV84 and HPV89 were found to persist. Whereas almost all high risk types showed persistence, the highest rate was found in women with HPV types 16, 45, 67, 31, 51 and 59. The persistence rate for HPV16 infection was 45 per 1000 women-months. **Conclusion:** Incident HPV infections and high risk HPV type-specific persistence were found to be high in our study population of young married women. Understanding the patterns of HPV infection may help plan appropriate strategies for prevention programs including vaccination and screening.

Keywords: Human papillomavirus - acquisition - persistence - north India

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Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses (Walboomers et al., 1999; Bosch et al., 2002; Zur Hausen 2002). Although there is conclusive evidence that HPV infection is a necessary cause of cervical cancer, the discrepancy between the high frequency of HPV infections in young sexually active women and the relatively low occurrence of cervical lesions in the same population suggests that HPV is not a sufficient cause for cervical neoplasia (Moscicki et al., 2001). Most of these HPV infections are found to be transient and only women who harbor persistent high risk HPV infection are at risk of developing cervical lesions. High risk HPV infections (hrHPV) seem to persist longer than infections with low risk HPVs (Franco et al., 1999; Woodman et al., 2001; Giuliano et al., 2002; Richardson et al., 2003; Munoz et al., 2004; Brown et al., 2005). To effectively design cervical cancer prevention strategies, information regarding the incidence, prevalence and clearance of HPV infections in a variety of populations is needed. However, little is known about the type-specific

incidence and persistence rates of HPV infection in the population of young women in India. Therefore we carried out a prospective follow-up study to investigate the acquisition and persistence of HPV type-specific infections.

Materials and Methods

Study design

A community based study was carried out at Govindpuri, a slum cluster in New Delhi with a large migrant population from different parts of North India. Married women in the age group of 16-24 years were invited to participate. Door to door surveys of the area and enumeration of the population were done by health workers. Meetings were arranged to inform women about the significance and plan of the study. Exclusion criteria were: pregnancy, obvious cervical tumors and previous surgical procedures of the cervix or hysterectomy. Informed written consent was obtained from all participants. The study was approved by the Institute ethical review committee.

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Sample collection, storage, processing

Cervical samples of exfoliated cells were collected for HPV DNA testing using a Digene® cervical brush sampler which was introduced inside the endocervix with the lowermost bristles touching the ectocervix. The brush was rotated 3-5 times in the counter-clockwise direction, placed in the specimen transport medium (STM, Qiagen Gaithersburg Inc., USA) and transported to the laboratory. Samples were vortexed, aliquotted into storage vials and stored at -80°C until testing. Similar samples were collected every six months during the follow up period of two years.

For DNA extraction, the method of Gravitt et al. (1998) was followed: In brief, 150 µl of the sample was digested with 15 µl of 10X digestion buffer (containing 700 µl Tris-EDTA buffer, 100 µl 10% Tween-20 and 200 µl of 20 mg/ml proteinase K) at 65°C for 1 hour; followed by heat inactivation at 95°C for 10 minutes (Gravitt et al., 1998). The DNA was precipitated with 600 µl of absolute ethanol containing ammonium acetate, overnight at -20°C. The precipitated DNA was centrifuged for 30 min at 13,000g at 4°C, the supernatant immediately removed, the DNA pellet dried at room temperature, resuspended in 50 µl of TE (10 mM Tris, 1 mM EDTA, pH 8.0) and the DNA stored at -20°C until amplification for HPV testing.

HPV DNA testing by PCR

Cervical samples were screened by PCR with beta-globin gene-specific primers as internal control, to check the quality of the DNA samples. Only beta-globin positive samples were processed further for HPV testing by PCR using consensus PGMY9/11 primers (product size 450 bp). The PCR products were then electrophoresed on 1.5% agarose gel. Samples visually determined to have the presence of a 450 bp band on the agarose gel were considered positive by the PGMY PCR (Gravitt et al., 1998).

HPV DNA testing by Digene Hybrid Capture 2

HPV testing was done using Hybrid Capture 2 (Qiagen Gaithersburg Inc., USA) according to the manufacturer's instructions on an aliquot of 200 µl of sample in STM. The sample was tested for the presence of high-risk HPV (hrHPV) types using probe B which is a pool of full length RNA probes specific for 13 high risk types. The HC2 test is based on the hybridization of HPV DNA with RNA specific probes and the hybrid is detected by a chemiluminescent assay. The values obtained are recorded as relative light units (RLU) per positive control (PC). A sample is considered positive for HPV DNA when the RLU/PC ratio is greater than or equal to one. Thus this is a semi-quantitative test detecting a minimum of 5000 copies of HPV.

HPV-DNA testing by the Reverse Line blot assay (Roche Molecular Systems, USA)

HPV genotyping was performed on cervical samples that were positive by either of the above two techniques (HC2 or PCR). The reverse line blot assay (Roche Molecular Systems, USA) detects 37 HPV types, hybridized on a single probe. A reference overlay

allows determination of the exact type(s) present in each sample. The specimen DNA was amplified using PGMY 09/11 HPV-specific primers that amplify the 450 bp fragment of L1 ORF of genital HPV. Human β -globin target was co-amplified with HPV consensus primers to determine adequacy of the specimen. The PCR products were denatured and hybridized to an immobilized HPV probe array on strips (a kind gift received from Roche Molecular Systems, Alameda, California, USA). Positive hybridization was detected by colour precipitation at the probe site and the type determined by reading from the reference overlay. For purpose of analysis, samples were considered sufficient for HPV determination if the β -globin band was detected.

Follow Up

Regular follow up visits were scheduled every 6 months for a period of two years. In most circumstances, censorships are due to loss to follow up. Although commitment to participation was a prerequisite for inclusion in the study, loss to follow-up was associated with several factors such as high pregnancy rate in this age group, long gaps when women go to the village, lack of motivation among women upon being given negative report of the test conducted and refusal of their spouses to permit further participation. Discussion meetings and camps were organized with the help of the local leaders/social workers. All possible efforts were made to trace the subjects in the category of loss to follow up cases.

Statistical analysis

Data were recorded on the pre designed proforma and managed on a Microsoft Excel spread sheet. Reverse line blot results were recorded for the type-specific HPV infections.

The prevalence of HPV infection was calculated as the total number of women with HPV infection at time of enrollment. Incidence was defined as a measure of the risk of developing a new HPV type-specific infection within a specified period of time, i.e., every 6 months. However, since every woman could not be followed for the entire two year period, the incidence rate for HPV type-specific infection was calculated on the basis of the number of cases in which a given type was detected divided by total woman-months at risk. Women at risk were those negative for that HPV type who returned at least once after enrolment. Those women in whom the event (HPV type-specific infection) did not occur during the study follow up, were considered as censored and such women contributed to the women-months as per the duration for which they were under observation.

We also investigated the persistence of type-specific HPV infection in this population. Persistence rate was calculated on the basis of the results of two consecutive positive tests for a specific HPV type during the study period. Women who were HPV negative or HPV positive at enrollment but did not return for subsequent visits were censored from the study. Women who developed incident HPV type-specific infection for that type at the last visit and who were HPV negative through out the study were excluded from the above calculation. Clearance

of HPV infection was measured from the visit in which a HPV type was detected until the visit at which the same HPV type was not detected. Clearance duration for that particular type was estimated for both incident and prevalent HPV infections using a similar approach. It was assumed that, because of their randomness across visits, the patterns of missing data did not impact the duration of infection. Clearance rate was calculated for each HPV type. For incidence, clearance and persistence rates, 95% confidence interval was also reported. STATA (9.0) (STATA Corporation, College Station Road, Houston, Texas) was used for data analysis.

Results

Of 1861 women who fulfilled the eligibility criteria, 1511 were recruited: 184 women refused participation, 27 were excluded because of pregnancy. Thus 1300 women were enrolled. They were followed for two years at intervals of 6 months. At analysis there were a total of 2259 completed visits. The mean number of visits (+ SD) calculated was 1.73+1.3.

Cervical samples from all women were screened by PCR and HC2 testing. The prevalence calculated was 91/1300 (7%) HPV positive by gel visualization of PCR

consensus primer products, while 110/1300 (8.4%) were HPV positive on HC2 testing. HPV genotyping was done on 145 samples that were HPV positive by either PCR or HC2, of which 104 (71.7%) were found to be positive on the reverse line blot assay. HPV16 was the commonest high-risk type (3%) followed by HPV52 (1.2%) and HPV51 (0.8%). Among low-risk types, HPV62 was the commonest (0.8%), followed by HPV84 and HPV89 (0.5% each). Multiple infections were found in 3% of the samples. The baseline pattern of HPV distribution has been previously published (Datta et al., 2010).

Incidence of HPV infections

Type-specific incidence and persistence rates were calculated based on the reverse line blot results. Table 1 shows the incidence rates of type-specific HPV infections during the follow up period. For any HPV type the incidence rate was 5 per 1000 women-months. Among type-specific HPV infections, HPV16 showed the highest incidence rate followed by HPV59, HPV52 and HPV18 i.e. 3, 0.58, 0.41 and 0.35 women per 1000 women-months respectively.

Clearance of HPV infections

The clearance rate was higher for low-risk than for

Table 1. Type Specific Incidence Rate

Types	Incident infections, No of women	Follow up Duration, women-months	Incidence rate/1000 women months (95% CI)
HR-HPV types:			
16	51	16556	3.08(2.2-4)
18	6	17124	0.35(0.12-0.76)
31	9	17172	0.52(0.23-0.99)
33	3	17136	0.17(0.03-0.51)
35	2	17214	0.11(0.01-0.41)
39	6	17094	0.35(0.12-0.76)
45	2	17214	0.11(0.01-0.41)
51	5	17106	0.29(0.09-0.68)
52	7	16994	0.41(0.16-0.84)
53	3	17250	0.17(0.03-0.5)
56	6	17124	0.35(0.12-0.76)
58	3	17184	0.17(0.03-0.51)
59	10	17118	0.58(0.28-1)
66	7	17190	0.4(0.16-0.83)
67	1	17244	0.05(0.001-0.3)
68	3	17184	0.17(0.03-0.51)
69	2	17250	0.11(0.01-0.41)
70	1	17226	0.05(0.001-0.32)
82	3	17256	0.17(0.03-0.05)
LR-HPV types:			
6	3	17250	0.17(0.03-0.5)
11	1	17274	0.05(0.001-0.32)
42	6	17172	0.03(0.12-0.76)
54	2	17256	0.11(0.01-0.41)
55	2	17190	0.11(0.01-0.41)
61	1	17232	0.05(0.001-0.32)
62	3	17190	0.17(0.03-0.5)
72	1	17256	0.05(0.001-0.32)
81	6	17220	0.34(0.12-0.75)
84	5	17154	0.29(0.09-0.68)
89	5	17148	0.29(0.09-0.68)
Any HPV type	79	15552	5(4-6.3)

Table 2. Type Specific Clearance Rate

Types	No of women who cleared infections	Follow up Duration, women-months	Clearance rate/1000 women months (95% CI)
HR-HPV types:			
16	35	444	78.8(55.5-107.9)
18	7	78	89.7(36.8-176.2)
31	9	126	71.4(33.1-131.2)
33	3	84	35.7(7.4-100.8)
35	4	24	166.6(47.3-373.8)
39	9	66	136.3(64.2-243.1)
45	2	24	83.3(10.2-269.9)
51	5	96	52(17.1-117.3)
52	13	162	80.2(43.4-133.3)
53	1	12	83.3(2.1-384.7)
56	9	84	107.1(50.1-193.6)
58	3	54	55.5(11.6-153.8)
59	8	102	78.4(34.4-148.7)
66	9	66	136.3(64.2-243.1)
67	2	18	111.1(13.7-347.1)
68	4	42	95.2(26.5-226.2)
69	2	18	111.1(13.7-347.1)
70	2	18	111.1(13.7-347.1)
73	1	6	166.6(4.2-641.2)
82	1	18	55.5(1.4-272.9)
LR-HPV types:			
6	2	12	166.6(20.8-484.1)
11	3	18	166.6(35.7-414.1)
42	5	72	69.4(22.9-154.6)
54	1	6	166.6(4.2-641.2)
55	4	36	111.1(31.1-260.6)
61	2	12	166.6(20.8-484.1)
62	5	48	104.1(34.6-226.5)
72	1	6	166.6(4.2-641.2)
81	3	24	125(26.5-323.6)
83	2	24	83.3(10.2-269.9)
84	8	48	166.6(74.8-302.2)
89	7	60	116.6(48.2-225.7)

Table 3. Type Specific Persistence Rate

Types	No of women who cleared infections	Follow up Duration, women-months	Persistence rate/1000 women months (95% CI)
HR-HPV types:			
16	20	438	45.6 (28.1-69.6)
18	3	78	38.4(8-108.3)
31	7	96	72.9(29.8-144.4)
33	5	66	75.7 (25-168)
35	1	18	55.5(14-272.9)
39	2	60	33.3(4-115.2)
45	2	24	83.3(10.2-269.9)
51	6	84	71.4(26.6-149)
52	9	138	65.2 (30.2-120.1)
56	2	72	27.7(3.3-96.7)
58	3	48	62.5(13-171.9)
59	6	90	66.6(24.8-139.4)
66	1	66	15.1(0.3-81.5)
67	1	12	83.3(2.1-384.7)
68	2	36	55.5(6.8-186.6)
82	1	18	55.5(1.4-272.9)
LR-HPV types:			
42	2	72	27.7(33.8-96.7)
62	3	48	62.5(13-171.9)
84	1	42	23.8(0.6-125.6)
89	3	42	71.4(14.9-194.8)

high-risk HPV types (Table 2). Among the low-risk types the clearance rate was high for HPV11, HPV61, HPV72 and HPV84. Among the high-risk types clearance for HPV35, HPV73, HPV39 and HPV66 was high. The clearance rate for HPV16 was comparatively low.

Persistence of HPV infections

The persistence rate was higher for high-risk than low risk types (Table 3). Among the low-risk types, women infected with HPV types 42, 62, 84 and 89 were found to have. Almost all high-risk types showed persistence, the highest rate being found for women having HPV45, HPV67, HPV31, HPV51 and HPV59. For HPV16 infection the persistence rate was 45 cases per 1000 women-months.

Discussion

Estimation of the prevalence and incidence of HPV infection and analysis of the dynamics of HPV clearance versus persistence helps us in determining/ predicting whether a squamous intraepithelial lesion (SIL) will regress spontaneously or will persist and increase the likelihood of developing into a frank malignant lesion.

Acquisition of new HPV infection is common, particularly among sexually active young women, and the incidence appears to be higher for oncogenic than for non-oncogenic types (Giuliano et al., 2002; Richardson et al., 2003). In Canadian university students, the incidence rate was 1.9% per month, and the cumulative rate was 18.0% at one year and 36.4% at two year follow-up (Richardson et al., 2003). In another study of Canadian women, the overall infection incidence was 11.1% per year, with the highest rate (25.0%) in the 15-19 year age group (Sellors et al., 2003). The rate of new HPV infections in young women in the United States was 2.9% per month (Giuliano

et al., 2002), and the three-year cumulative incidence was over 40% (Winer et al., 2003). In the present study, the rate of acquisition of new infections for any HPV type per 1000 woman-months was estimated 5 (95% CI: 4.0-6.3). The incidence rate was relatively low compared to other studies, e.g., incidence rates for the female university students in Montreal, were 14.0 cases/1000 women months (95% confidence interval (CI), 11.4-16.3) and 12.4 cases/1000 women-months (95% CI, 10.4-14.8) for acquiring high and low risk HPV infections, respectively (Richardson et al., 2003). In a prospective study done in Ludwig-McGill cohort by Trottier et al., in 2008 the incidence rate for any type was 9.5 women per 1000 women months in women in the 18-60 years age group. In their analysis in the wide age range, the incidence rates for hrHPV and lrHPV infections were 6.1 and 4.9 episodes per 1000 women-months, respectively. Incidence of HPV16 was the highest, followed by HPV53 and HPV51. In our study too the incidence of HPV16 was the highest, but the next most common were HPV59 and HPV31, unlike the prevalence of other types at the study entry. The incidence rate for the low-risk types was found to be lower than the high-risk types. Similar results were also found in the cohort studied by Munoz et al., in 2004 which included women 15-85 years old who were monitored on an average for 14.1 years. They determined the age specific incidence rates for various HPV types and found that the highest level for incidence of high-risk types was seen in young women less than 20 years of age which was followed by sharp decrease and then was increased again in postmenopausal women.

We found a considerable difference in persistence of high-risk and low-risk HPV types as persistence was seen more in high-risk than low-risk types. Among high-risk types, the mean duration of persistence due to HPV16 alone was found to be 12.5 months, highest for both incident and prevalent infections (data not shown). In a study by Richardson et al., in 2003, the mean duration for HPV16 was found to be most persistent, i.e., 18.3 months, followed by HPV53 (mean duration 14.8 months) and HPV31 (mean duration 14.6 months). We did not observe any appreciable differences in the risks between those women who had persistent infection for two years and those who were negative throughout the study. Among those who tested positive twice for carcinogenic HPV, all subsequent diagnoses of cervical intraepithelial neoplasia were linked to persistence of high risk HPV genotypes. In our analysis, however, women who first had positive results on testing and then had negative results seemed to have a level of risk that was similar to those who tested negative twice.

Most of the low-risk types detected in our study were found to have been cleared while high-risk types had high persistence rate. This is in concurrence with the study by Moscicki et al. (1998) in which high-risk HPV types were less likely to regress than low-risk types. It is the persistence of hrHPV infections that is associated with cervical dysplasia and cervical cancer. According to Brown et al. (2005) the unique ability of hrHPV types to persist longer than low risk types could be a partly responsible for the differential propensity of hrHPV vs.

hrHPV to contribute to the development of cervical cancer.

Longer duration of hrHPV persistence in younger women, similar to those enrolled in our study, was also observed by others (Ahdieh et al., 2001; Trottier et al., 2008;). However, some studies have shown that HPV persistence is more common among older women (Castle et al., 2005; Goodman et al., 2008). Differences in study design and analysis may partially explain the discrepancies as most studies of this relationship have used data on prevalent HPV infection. Measurement of HPV persistence was influenced by our ability to identify the onset of infection.

HPV infection is common, but it is highly transient. In our population, type-specific clearance for both high- and low-risk was calculated. Clearance rate for HPV35, HPV39 and HPV66 was higher compared to HPV16 and HPV33. The relatively low clearance rate of HPV16 was also reported in earlier studies (Ho et al., 1998; Molano et al., 2003; Richardson et al., 2003). This observation is consistent with other natural history studies of cervical HPV infection (Franco et al., 1999; Moscicki et al., 2006; Trottier et al., 2008). The clearance of hrHPV infections was slower than lrHPV infections which is in accordance with most other investigators (Giuliano et al., 2002; Molano et al., 2003; Richardson et al., 2003; Brown et al., 2005) who have also reported a longer duration of persistence of infection caused by oncogenic rather than non-oncogenic HPV types.

Our current study has some limitations. An important caveat in a study of natural history of women past their onset of sexual activity is that one cannot measure the true incidence of new infections of those at enrollment; this is particularly important to distinguish a new infection from a re-occurrence or reactivation of a previously latent infection which indicates a risk different from that associated with a newly acquired infection. This distinction is difficult to make because of the limitation of molecular sensitivity of HPV detection methods. For this reason we can only claim to have measured "presumed" incidence rate of new type for those negative at enrollment and might have affected the clearance and persistence rates. Another drawback of our study was the loss to follow up. Unfortunately, despite repeated efforts, traceability and compliance among these women was low, partly because of social factors and partly because many of these were migrants who returned to their home states during the study.

There were majority of women who reported to be monogamous but it is possible that there were sexually active women in the unmarried age group but Indian social taboos around premarital sexuality do not permit us to get accurate information in this regard, nor can we suggest including them in community based studies at the present time. Thus affecting the prevalence and incidence rates reported in our study.

In conclusion, our study is the first to report data on the incidence and persistence of type -specific HPV infection among the young women in India. This study shows that acquisition of new HPV infection among these women was high and clearance was also frequent. Testing for HPV persistence might be clinically useful

for assessing the risk of cervical intraepithelial neoplasia among women who test positive for carcinogenic HPV. Furthermore repeat testing (versus one time tests) might further divide the population into low risk and high risk for incipient precancerous lesions. Detection of HPV 16 and might be particularly useful to identify women at risk for cervical precancer. The present study provides further evidence that, using a standardized approach to HPV detection, the use of HPV persistence as a clinical end point, the detection of HPV DNA on cervical samples taken 6 months apart provides useful information on the role of vaccination after onset of sexual activity.

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