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ORIGINAL PAPER

Community Prevalence of Human Papillomavirus by Self-Collected Samples in South India

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Abstract

Background Cervical cancer is still the most common malignancy in Indian women with 127,000 new cases and 67,000 deaths each year. The aim of this study was to determine the community prevalence of HPV infection and the feasibility of self-collected vaginal swabs for HPV testing.

Methods The study design was cross-sectional, and the location was Kaniyambadi block of Vellore district in India. A computer-generated list of women less than 50 years was prepared and women randomly invited to participate in screening for cervical neoplasia. Community health workers trained in visual inspection with acetic acid, obtained self-collected vaginal swabs from the participants and brought them to the cancer screening clinic where cervical samples for cytology and HPV testing were taken by a clinician. HPV testing was done by PCR and genotyping by the line blot assay. Quality control for the HPV testing was determined by sending samples to Johns Hopkins University, Baltimore. Data analysis consisted of descriptive statistics such as proportions.

Results There were 809 women in the study of whom 2 % were nulliparous, 34 % were illiterate, 0.7 % had multiple sexual partners and only 3 % had used oral contraception. Low-risk HPV was seen in 22 (2.7 %) and high-risk HPV in 48 (5.9 %) self-collected vaginal samples. Among the clinician-collected cervical samples, low-risk HPV was seen in 25 (3 %) and high-risk HPV in 51 (6.3 %). Low-grade cervical intraepithelial neoplasia (CIN) was found in 26 (3.2 %) and high-grade CIN in 10 (1.2 %). There was 96 % concordance between the laboratories at Vellore and Baltimore for HPV detection. Women said that self-collection was convenient (88 %) and painless (87 %). Most women (71 %) said that they preferred self-collection to samples being taken by a health worker or a doctor. Compliance to self-collection was 100 % among those who participated.

Conclusion This study showed that self-collected vaginal samples for HPV testing are feasible and acceptable to women in India. The prevalence of high-risk HPV was low in this community while the prevalence of high-grade cervical intraepithelial neoplasia was 1.2 %.

Keywords Self-collection · Community · Human papillomavirus · Prevalence · CIN

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Introduction

The use of HPV testing as a means of screening for cervical pre-cancer and also the introduction of HPV vaccination to prevent cervical cancer have made it imperative to understand the epidemiology of human papillomavirus (HPV) infection.

The oncogenic or high-risk HPV genotypes are a necessary but not a sufficient cause of cervical cancer. The HPV vaccine is now available in India; however, little is known about the epidemiology of HPV infections in Indian communities. There may be geographical variations in the prevalence of HPV infection as well as variations in the relative importance of the different HPV genotypes. Some studies have looked at HPV genotypes in cervical neoplasia and immunosuppressed women. Other studies have looked at HPV in women attending a health facility but without cervical neoplasia. There have also been studies evaluating HPV DNA testing as a means of screening for cervical neoplasia. Moreover, most studies have taken convenience samples and not looked at population level prevalence.

The aim of this study was to determine the community prevalence of HPV in the Vellore area of Tamil Nadu state in south India. A secondary aim was to determine the feasibility of HPV testing of self-collected vaginal swabs.

Methods

This study was part of a cervical cancer screening project done in 2004–2005. Approval from the Institutional Review Board was obtained to carry out the study. The site of the study was Kaniyambadi block in Vellore district of Tamil Nadu state in south India. The entire population of this area had already been enumerated and listed on a computer database. Two rural and two semi-urban panchayats (unit of local governance) were chosen. A random list of women, 50 years or less, from these areas was selected and invited to take part in this study. Only women who had ever been married were included. Written informed consent was obtained from participants after explaining the study and what it entailed. Women who were menstruating, pregnant, known to have had cervical neoplasia or had undergone hysterectomy were excluded.

Assuming the sensitivity of the test to be 90 % and specificity of 90 %, the number needed to be studied would have to be 985 women in order to show a true prevalence of 10 % with 3 % precision and 95 % confidence.

The consent form and questionnaire were translated into Tamil and then back-translated. All aspects of patient recruitment, interview, examination and collection of specimens were pilot tested. The HPV PCR (PGMY primers) and Line blot assay were standardised in the laboratory (Reagents for line blot assays were provided courtesy, Janet Kornegay, Roche). Four field workers (village health workers) were trained in visual inspection with acetic acid (VIA) and in recruiting women for the study. A social worker was trained in the interview process.

The field workers recruited women and obtained self-collected vaginal specimens that they transported in an ice-bucket to the central laboratory. They also brought the women to the clinic for the interview by the social worker and, examination by a clinician. All women had a Pap smear, clinician-collected cervical swab for HPV testing and colposcopy. A biopsy was taken if any of the tests were abnormal. If HPV PCR was positive, the line blot assay was done to identify the genotypes. HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 were considered high risk.

Fifty DNA samples were sent to Dr Gravitt's laboratory in Baltimore for quality control.

Results

A total of 1060 women were invited to participate but 61 women were excluded as they did not meet study criteria and 190 women refused to consent (refusal rate of 19 %). Finally, 809 ever married women took part in the study.

Table 1 Sociodemographic characteristics

	Number	Percentage (%)
Education		
None	270	34
1–10 years	501	62
Over 10 years	35	4
Marital status		
Currently married	737	91
Widowed	57	7
Separated/divorced	15	2
Pregnancies		
None	16	2
1–3	480	60
Over 3	310	38
Number of partners		
One	801	99
Two or more	6	1
Husband circumcised		
No	745	98
Yes	13	2
Used condoms		
No	777	96
Yes	32	4
Used OCPs		
No	776	97
Yes	30	3
Ever had Pap smear		
No	769	95
Yes	40	5

Table 2 Prevalence of HPV infection

Type of infection	Cervix		Vagina	
Any HPV type	76	9.4 %	70	8.7 %
Low-risk HPV types	25	3.1 %	22	2.7 %
Probable high risk	5	0.6 %	7	0.9 %
High-risk HPV types	47	5.8 %	39	4.8 %
Multiple infections	15	1.9 %	11	1.4 %

Table 3 Number of HPV infections per person

Number of HPV types	Cervix		Vagina	
	n	%	*n*	%
0	743	91.9	737	91.1
1	55	6.8	57	7.1
2	8	1.0	9	1.1
3	2	0.2	6	0.7
4	1	0.1	0	0

The sociodemographic characteristics are shown in Table 1. The mean age of the women was 38.9 (SD 5.3), and the mean age at marriage was 18.1 (SD 2.9). The median parity was 3 (range 0–8). Forty-three percentage of women began sexual activity before age 18. Only 3 % gave a history of oral contraceptive use and 4 % had used condoms. Less than 1 % admitted to more than one sexual partner. Most women were currently married (91 %). About one-third of women had not attended school at all.

Seventy (8.7 %) women tested positive for HPV by PCR on vaginal samples. Low-risk HPV was seen in 22 (2.7 %) and high or probably high-risk HPV in 48 (5.9 %) of self-collected vaginal samples. The prevalence of HPV in the community is shown in Table 2. Single infections were seen in 6.8 % of cervical specimens and 7.1 % of vaginal specimens as shown in Table 3. About 1 % had infection by two types and less than 1 % had 3 or more types. The prevalences of individual HPV types are shown in Table 4. The commonest infection was by HPV 16. The distribution across age groups is also shown in Table 4. The highest prevalence was seen in women >45 years and the lowest in women 35–45 years.

Among the clinician-collected cervical samples, HPV was present in 76 (9.4 %) women. Low-risk HPV was seen in 25 (3.1 %) and high or probably high-risk HPV in 51 (6.3 %). The distribution of genotypes in the cervix and vagina is shown in Fig. 1.

There was 96 % overall concordance in HPV DNA test results between Vellore and Baltimore. All positives from Vellore were also positive in Baltimore. Two out 37 negatives from Vellore were positive in Baltimore. Low-grade cervical intraepithelial neoplasia (CIN) was found in 26 (3.2 %) and high-grade CIN in 10 (1.2 %).

Discussion

There are very few studies on the epidemiology of HPV infection in India. To understand and control HPV-related cancers, the distribution and determinants of HPV infection would have to be known. The prevalence of high-risk HPV was 6 % in a previously unscreened population in a pooled analysis of three studies in east India [1]. A study from north India looked at HPV prevalence in married women aged 16–24 years and found an overall prevalence (low- and high-risk types) of 8.4 % [2]. Studies from western and central India have shown a prevalence of 10.3 % [3, 4]. A review of existing studies from India was collated with an average HPV prevalence of 7.9 and 84 % of cervical cancers were attributed to HPV types 16 and 18 [5].

As expected, HPV prevalence is higher in high-risk populations. HPV infection in immunosuppressed women is 3–4 times higher than in the general population. We had reported 37 % of HIV-infected women to have HPV DNA in their cervical swabs [6]. The prevalence of high-risk HPV is also high in renal transplant recipients [7]. The prevalence of HPV was 73 % in female sex workers, 72 % among IV drug users and 69 % in men who have sex with men [8].

HPV infection is a necessary cause of cervical cancer. We found high-risk HPV in 95 % of invasive cancers and 91 % of cervical intraepithelial neoplasia [9]. Regional variations in HPV genotypes could not be demonstrated in India [10].

Unlike in most western studies, reports from India suggest that high-risk HPV prevalence increases with age or at least does not decrease significantly [1, 3, 11]. Differences in sexual mores and lower clearance of infection may account for this finding. This finding has also been reported from Africa [12].

Several studies have been done in India screening for cervical neoplasia using VIA, cytology and HPV DNA testing; however, still there is no national screening programme in place. Most women find a visit to the gynaecologist and a speculum examination uncomfortable. There are issues of inadequate access due to poor roads, infrequent transportation and non-functional health facilities. Thus, there is an inherent appeal for self-collected samples for cancer screening. Self-collection of vaginal swabs for HPV detection has been tried in several parts of the world. In India, a high agreement between self-collected vaginal specimens and physician collected specimens has been reported from Hyderabad [13] and Delhi [14]. A low cost,

Table 4 Prevalence (percentage) of HPV by age in cervix (C) and vagina (V)

HPV type	<35 years		35–39.9 years		40–44.5 years		>45 years		All ages	
	$n = 180$		$n = 275$		$n = 202$		$n = 152$		$n = 809$	
	C	V	C	V	C	V	C	V	C	V
16	1.7	0.2	2.5	2.2	3.0	2.0	5.3	4.0	3.0	2.5
18	0.6	0	0	0.4	0.5	0	0.7	0	0.4	0.1
31	0.6	0.6	0	0	0	0	0.7	0	0.3	0.3
33	0.6	0.6	0	0	0	0	0	0	0.3	0.1
39	0	0	0.4	0.4	0.5	0.5	0	0.7	0.3	0.4
40	0	0	0	0	0.5	0.5	0	0	0.1	0.1
42	0	0	1.1	0.7	0	0	0	0	0.4	0.3
45	0	0	0	0	0.5	0.5	1.3	1.3	0.4	0.4
52	0.6	1.1	0.7	1.1	1.0	0.5	0	0	0.6	0.7
53	0.6	0	0	0.7	0	1.0	0.7	0.7	0.3	0.6
54	0.6	0.6	0	0	0.5	0	0	0	0.3	0.1
56	0.6	0.6	0.4	0.4	0.5	0	0.7	0.7	0.5	0.4
59	0	0	0.4	0	0	0	0	0	0.1	0.1
61	0	0	1.1	1.5	0.5	0.5	0	0	0.5	0.6
62	0	0	0	0	0	0	0.7	0	0.1	0
66	0	0	0	0.4	0	0	0	0	0	0.1
70	0	0	0	0.4	0	0	0	0	0.1	0.1
72	0.6	0.6	0	0	0.5	0	0	0	0.3	0.1
81	0.6	0.6	0	0	0.5	0.5	0.7	1.3	0.4	0.5
83	0	0	0	0	0	0	0.7	0.7	0.1	0.1
84	0.6	0	0.4	0.4	1.5	1.0	0.7	0.7	0.7	0.5
89	2.2	2.2	0	0	0	0	0	0	0.5	0.5
Any type	9.4	8.9	7.6	8.7	9.9	7.4	11.8	9.9	9.4	8.7
None	90.6	91.1	92.4	91.3	90.1	92.6	88.2	90.1	90.6	91.3

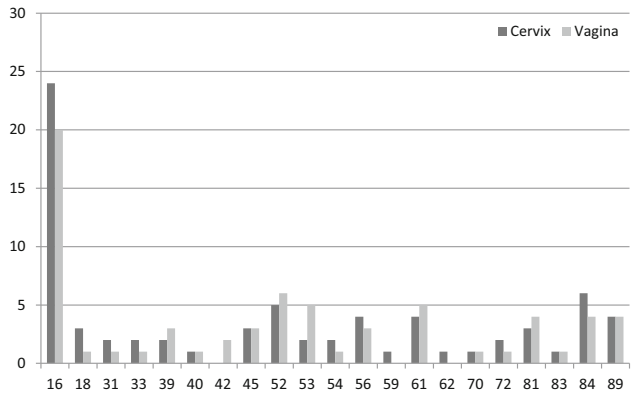

Fig. 1 HPV genotypes in cervix and vagina (number of women)

objective test that is easily implemented for a whole district or state would go a long way in secondary prevention of cervical cancer.

Our study population had good access to health care. The community health department has been working in partnership with the government agencies in this area for over 50 years. The study area was semi-urban in the south of India. There could be some regional variations, and the prevalence in this study would not represent all of India.

In this study, the HPV detection method used was polymerase chain reaction (PCR) and line blot assay. This is a sensitive approach useful for epidemiological studies that enabled us to identify the exact HPV genotypes within the financial constraints. Other tests like the Hybrid Capture are clinically more useful as they do not detect low copy numbers even though they do not provide the exact genotype. A positive Hybrid Capture result would correlate well with CIN.

The limitations of this study are that it was part of a larger screening project and HPV results were not correlated with the biopsy results. The samples were collected 10 years ago, and the HPV epidemiology could change over time. However, we do not believe that the epidemiology would have changed significantly in a decade. The strength of the study is that it was a random sample of the community and is representative of the Vellore area. The methods were robust and all women had VIA, colposcopy and necessary treatment if their biopsy was abnormal. The

study also showed that self-sampling was acceptable and feasible. Although this study was done primarily to evaluate different screening strategies, it provides useful epidemiological information.

Understanding the epidemiology of HPV infections would also facilitate a primary prevention programme with vaccines. The nonavalent vaccine would prevent over 90 % of cervical cancers [15]. It is hoped that India will soon make its own affordable HPV vaccine. Self-sampling could potentially be useful as a screening method to bring women to the clinic for treatment.

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