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Short Communication

Detection of human papillomavirus in women attending Pap cervical screening camp at a peripheral hospital of North-Eastern India

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ABSTRACT

Human papillomavirus (HPV) associated cervical cancer is the leading cause of deaths in India. However, cytological/HPV screening may result in early detection of cervical cancer, resulting in early treatment and reduced mortality. Although reports related to general population is available, data on HPV prevalence among women attending AFMS health care facilities is scarce. Cervical samples were collected for cytological staining by Pap test and molecular detection by PCR, genotyping by HPV specific primers and sequencing. Apart from finding of atypical cells of undetermined significance (ASCUS) in one subject, no evidence of malignancy was observed. A high prevalence of HPV was found in this study group, which was intermediate between previous reports from general population and cervical cancer patients. All the subjects had infection of high risk HPV type16. HPV prevalence was found similar between different age groups. Although, none of the study subjects had malignant changes, but due to high prevalence of high risk HPV infection and other associated risk factors, these subjects might be at an elevated risk of developing cervical cancer. Regular follow-up of these patients who were detected HPV positive are required to screen for cervical malignancy.

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Introduction

Worldwide cervical cancer is the second leading cause of uterine cancer in developing countries and epidemiological studies have clearly established HPV as the causal agent.¹ In India it is the primary cause of female mortality.^{1–3} Unlike most other cancers, cervical cancer is preventable and

curable, if detected early. Developed countries have radically reduced cervical cancer incidence through population screening.^{1,2} Method such as Pap test and HPV DNA PCR are used to screen large populations.^{2–4} HPV is a double-stranded DNA virus (family-Papillomaviridae), comprising more than 100

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genotypes, some of which have high oncogenic potential (“high-risk genotypes” such as HPV 16 and HPV 18).² HPV genotyping thus enables identification of patients at risk of developing cervical cancer.

In India, inadequate HPV screening programs have contributed to enormous cervical cancer burden.^{1,2} Some data on HPV screening among women attending general health care facilities is available from our country. However, there is a scarcity of data on HPV among women, who access health care facilities provided by AFMS. We thus aimed to assess the prevalence of HPV high-risk genotypes (16 & 18) within this group of women.

Material and methods

Cervical samples analyzed in this study were collected from women, attending a Pap test camp (as a part of welfare measure), organized by a peripheral hospital under AFMS (situated in North-Eastern India). Total 112 women were interviewed based on a standard questionnaire and informed consent was obtained before sampling. Besides basic demographic data, marital status, number of pregnancies, parity and other risk factors associated with HPV or cervical cancer was also collected. Subjects with ongoing pregnancy, menstrual bleeding, and unmarried girls were excluded. Cervical examination and sample collection (using Ayre’s Spatula/endocervical cytobrush) was done by trained medical personnel according to the standard procedures. To avoid bias, comparison of the cytological results & molecular detection results were done independent of each other.

Cervical scraping was smeared on microscopic glass slides, fixed and Papanicolaou staining was done following standard procedure followed by visualization under a compound microscope (Olympus CH20, Singapore) by a trained pathologist. Observations were recorded as per 2001 Bethesda system terminology for reporting cervical cytology results.⁵ Details of the procedures are available from the corresponding author.

For molecular detection of HPV DNA by Polymerase Chain Reaction (PCR), cervical cells were processed according to modified freeze-thaw and boiling procedure for preparation of DNA extracts.⁶ Extracted DNA was confirmed by performing

PCR of housekeeping gene coding for human interleukin – 1b promoter. HPV PCR was performed by using HPV MY-PCR primers (MY09/MY11) and positive samples were further subjected to HPV type specific PCR for HPV 16 and HPV 18.^{7,8} Details of PCR (reaction mixture composition, primer concentration and thermal profiles) are available from the corresponding author.

Amplicons generated in the type specific HPV PCR were randomly selected, purified, and sequenced (Biolinkk, New Delhi). Sequence electropherograms were checked manually and edited sequences were subjected to similarity search using NCBI BLAST algorithm [<http://blast.ncbi.nlm.nih.gov/Blast.cgi>].

Categorical data were analyzed and P value computed (two tailed) using 2 × 2 contingency table [www.graphpad.com/quickcalcs/contingency1.cfm]. P values less than or equal to 0.05 was considered statistically significant.

Results

Of the 112 study subjects, 94 fulfilled the study criteria and were included in the study. Age of the subjects ranged from 20 to 55 (median 30 years). Parity ranged from 0 to 6 (median 2). For analysis, the subjects were further divided into three age groups: Gr I (20–30 years), Gr II (31–40 years) and Gr III (more than 41 years). Almost 92% subjects comprised the first two groups (48% and 44% respectively), rest comprise the third age-group. Demographical details and cytological findings in these groups are summarized in Table 1.

Negative for Intraepithelial Lesion or Malignancy (NILM) was statistically similar between Gr I and Gr II (58% vs. 46%, $P = 0.119$); between Gr I and Gr III (58% vs. 63%, $P = 0.563$) while significantly lower in Gr II as compared to Gr III (46% vs. 63%, $P = 0.022$). No significant difference was observed in incidence of inflammation (INFLM) between Gr I and Gr III (11% vs. 13%, $P = 0.828$), but was significantly higher in Gr II as compared to both Gr I and Gr III (11% vs. 32%, $P = 0.000$ and 13% vs. 32%, $P = 0.002$, respectively).

Interestingly 54 (57%) of the samples had amplifiable HPV DNA (with HPV primers MY09/MY11). Moreover, all these positive samples were also amplifiable only with HPV

Table 1 – Basic demographic data and cytological findings.

Age group	n	Age median (range)	Parity median (range)	Cytological findings			P values	
				Inadequate cells	Adequate cells			
					NILM (%)	INFLM (%)		ASCUS (%)
Gr I (20–30 years)	45	25 (20–29)	2 (0–6)	14	26 (58) ^{†,‡}	05 (11) ^{⊗,&}	00 (0)	[†] P = 0.119,
Gr II (31–40 years)	41	32 (31–38)	2 (0–6)	08	19 (46) ^{†,#}	13 (32) ^{&,*}	01 (2)	[‡] P = 0.563,
Gr III (41 and more)	08	45 (40–66)	2 (0–5)	02	05 (63) ^{†,#}	01 (13) ^{⊗,*}	00 (0)	[#] P = 0.022 ,
Total	94	30 (20–55)	2 (0–6)	24	50 (53)	19 (20)	01 (1)	[⊗] P = 0.828,
								^{&} P = 0.000 ,
								[*] P = 0.002

NILM – Negative for intraepithelial lesion or malignancy; INFLM – Inflammatory cells; ASCUS – Atypical squamous cells of undetermined significance.

Significant differences are shown in bold underlined P values.

genotype 16 specific primers but not with HPV genotype 18 specific primers (Fig. 1(a) and 1(b)). When HPV DNA positivity was compared based on age groups, HPV DNA was detected in 57.7% (26 of 45) subjects of Gr I, 58.5% (24 of 41) of Gr II, and 50% (4 of 8) of Gr III (no significant difference between groups). When HPV DNA positivity was compared based on cytological findings, irrespective of the age groups, it was found that 60% (30 of 50) of the subjects with NILM and 58% (11 of 19) with INFLM cytology findings were HPV DNA positive ($P = 0.935$). The only subject with ASCUS was also found to be HPV DNA positive.

Discussion

Despite being preventable and curable at an early stage, due to the lack of organized screening and intervention strategies, cervical cancer still causes more than 72,800 deaths annually in India.^{1,2} It is thus extremely imperative to assess the status of HPV infection and genotype for prevention of cervical cancer in India.² However, due to extensive geo-geno-ethnic, and socio-cultural diversity of India, the prevalence, of different infectious agents vary significantly in different parts of India.^{2,9} Same is true for HPV also, as its prevalence varies greatly across India.^{2,4,10} Among asymptomatic population, normally 10–12% to as high as 64% HPV prevalence has been reported from certain parts of India.^{2,11} Moreover, HPV 16 is predominant in India, detectable in 22% of general population, while ranging from ~14 to 88% in cervical cancer population.^{2,4,10–12}

Our finding of HPV 16 among the present study group is concordant with a recent report, in which HPV 16 was solely found among the HPV positive asymptomatic women and no HPV 18 was detectable.⁷ This HPV prevalence is certainly high compared to national average among general population. Notably, our study population is neither representative, nor comparable to general population. All the present study

subjects were married, 85% of them being sexually active and younger than 35 years of age (this age-group is known to have high incidence of transient HPV infection), 84% reported early age of marriage/sexual intercourse (16–22 years, median 19 years), more than 95% had one or more pregnancies (range 1–6, median-2), younger age at child birth, etc., that may influence HPV infection risk significantly.^{2,10,12} We acknowledge that due to the cross-sectional nature of the present study, we could not resolve the issue of transient infection. Nevertheless, considering the risk factors, HPV prevalence differs considerably from previous studies and is not generalizable to other populations.

In the present study, we could not detect any age group specific or clinical stage specific significant trend in HPV prevalence or genotype distribution among the population studied, which is analogous to a previous report.¹² In this study, we could detect evidence of inflammation in only 27% subjects which is quite less, compared to a previous study on similar subjects.¹³ Notably, unlike our study subjects, all the subjects of the previous study were above 35 years of age, a period of life when persistent HPV related changes start to occur.² We admit that the focus of the present study being evaluation the HPV prevalence, we did not verify the other causes in cases where cytological evidence of inflammation was observed. In this study conventional Pap test and HPV DNA PCR was utilized for detecting cytological abnormalities and HPV DNA. Although cytology is a powerful diagnostic technique but it is influenced by low sensitivity, sampling inaccuracy and is prone to observational bias.^{3,14} On the other hand PCR is highly sensitive test for HPV and could identify patients that remain undetectable by cytological techniques.^{4,7,15} However, a combination of both the tests may identify patients at risk of developing abnormalities, that need further therapeutic intervention or regular follow-ups.

In conclusion, the present study gives a snap-shot of prevalence of high risk HPV types among the present study group. We acknowledge that detailed follow up studies are essential to identify a fraction of these women, who might be at an elevated risk of developing cervical cancer, later in their lives. Hence, further studies are essential to obtain more data on HPV persistence and also to evaluate the odds of vaccination to preclude new infections. Moreover, regular Pap tests in combination with HPV testing, wherever possible may be recommended to successfully manage HPV infection and reduce the HPV associated disease burden among these groups of women.

Conflicts of interest

All authors have none to declare.

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Fig. 1 – (a) Agarose gel showing ~334 bp amplicon specific for HPV genotype 16. Ex – extraction control; Nc – negative control; Pc – positive control; Mk – 50 bp DNA Marker (Roche). (b) Agarose gel showing ~154 bp amplicon specific for HPV genotype 18. Ex – extraction control; Nc – negative control; Pc – positive control; Mk – 100 bp DNA ladder (Roche).

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