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ORIGINAL ARTICLE

Detection of HPV by PCR—A Novel Step in the Prevention of Cancer Cervix

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Abstract
Objectives (1) To compare the efficacy of Pap smear and HPV PCR for detection of CIN; and (2) To study the distribution of HPV genotypes.
Methods One hundred women presenting at the female Outpatient Department with unhealthy cervices were subjected to a detailed history, clinical examination, Pap smear, HPV DNA PCR test, and colposcopic-directed biopsy (where indicated).
Results This study has shown that there is a strong association of HPV infection with higher grades of CIN (100 % in patients with CIN 2, CIN 3, and CIS). The detection of CIN by HPV PCR was more accurate than by Pap smear. The most prevalent HPV genotype found in our study was HPV 16.
Conclusion The advent of HPV testing has opened the doors for more accurate cervical cancer surveillance strategies than Pap smear. Early detection and treatment of CIN will considerably reduce the incidence of cervical cancer.

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Introduction

Globally, cervical cancer is the second most common cancer in women and the third most frequent cause of cancer death (IARC, WHO 2002) [1]. Cancer of cervix is preventable, yet approximately 493,100 new cases and more than 273,000 deaths occur each year among women worldwide [1]. India, which accounts for the one-sixth of the world's population also bears the one-fifth of the world's burden of cervical cancer [2]. Cervical cancer is a leading form of cancer among women living in low resource regions of the world and often kills women at young age when they are still raising families.

Human Papillomavirus (HPV) is considered the primary driving force behind malignant transformation of cervical cells, with certain high-risk HPV types now labeled as the first-ever identified, indisputable, solely infectious cause of a human cancer [3].

With the above mentioned background, the present study was undertaken with the following objectives: (1) to evaluate cervical epithelial abnormalities by traditional Pap smear cytology and HPV DNA testing by PCR; (2) to compare the efficacy of Pap smear and HPV PCR for detection of CIN; (3) to correlate colposcopic-guided biopsy with cytological findings and HPV positivity; (4) to

evaluate the role of HPV DNA testing in primary screening of cervical cancer; and (5) to study the distribution of HPV genotypes in the study group.

Methods

The present study was carried out on 100 patients presenting at the female Outpatient Department (FOPD) from July 2006 to June 2008. It was carried out in collaboration with Departments of Pathology and Microbiology, IMS, BHU, and Reliance Life Sciences (Mumbai) at Sir Sunder Lal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi. One hundred females in the reproductive age group attending FOPD and showing unhealthy cervices on per speculum examination were subjected to a detailed history, clinical examination, cervical cytology (Pap smear), HPV DNA Testing by Polymerase Chain Reaction (Reliance Life Sciences), and colposcopic-directed biopsy in 78 cases with abnormal clinical findings or Pap smear abnormalities. The collected data was analyzed to show the inter-relationship between Pap smear results, HPV infection, and histopathology.

Results

Table 1 shows that after Pap smear cytology, 22 % patients had normal smears, 50 % had inflammatory smears, 6 % had ASCUS, 6 % had koilocytosis, 6 % had LSIL, 4 % had HSIL, and 6 % had atrophic smears. Of these, four patients (8 %) were HPV DNA positive in inflammatory smear group, two (33.3 %) were positive in koilocytosis group, four (66.6 %) were positive in LSIL group, and four (100 %) were positive in HSIL group.

Tables 2 and 3 show that all the HPV-positive patients in the LSIL group were less than 30 years of age, and all the HPV-positive patients in the HSIL group were more than 30 years of age.

Table 1 Relation between cervical cytology (by conventional Pap smear) and HPV positivity (by PCR)

Cytological class	No. of cases	Percentage	No. of HPV-positive cases	Percentage
Normal	22	22.00	0	0.00
Inflammatory	50	50.00	04	8.00
ASCUS	06	6.00	0	0.00
Koilocytosis	06	6.00	02	33.33
LSIL	06	6.00	04	66.66
HSIL	04	4.00	04	100.00
Atrophic	06	6.00	0	0.00

Table 2 Correlation of age and HPV positivity in patients having LSIL in cytology ($n = 6$)

Age (in years)	No. of cases	Percentage	No. of HPV-positive cases	Percentage
<30	04	66.66	04	100.00
≥30	02	33.33	0	0.00
Total	06	100.00	04	100.00

Table 3 Correlation of age and HPV positivity in patients having HSIL in cytology ($n = 4$)

Age (in years)	No. of cases	Percentage	No. of HPV-positive cases	Percentage
<30	0	0.00	0	0.00
≥30	04	100.00	04	100.00
Total	04	100.00	04	100.00

From Table 4, it is evident that the patients who showed lower grades of intraepithelial lesions on Pap smear cytology actually had higher grades of intraepithelial neoplasia on histopathology (colposcopic-directed biopsy).

Table 5 shows that HPV DNA was positive in 4 % patients with chronic cervicitis; 33.3 % patients with condyloma acuminata; 25 % patients with CIN 1; and 100 % patients with CIN 2, CIN 3, and carcinoma in situ.

Table 6 reveals that out of 100 patients under study, three were positive for low-risk HPV genotypes (types 6, 11, and 43). Eleven were positive for high-risk HPV genotypes (type 16, 18, 33, and 59). Maximum patients (five) harbored HPV type 16 infection The distribution of HPV genotypes in various histopathological categories was as follows: two patients of chronic cervicitis had HPV type 16 infection, two patients of condyloma acuminata had types 6 and 11 infection, three patients of CIN 1 had type 16 infection, one patient of CIN 1 had type 43 infection, two patients with CIN 2 had types 18 and 59 infection, two patients with CIN 3 had HPV type 18 infection; and two patients of CIS had HPV types 18 and 33 infection.

Discussion

In the present study, when a correlation of cervical cytology and HPV positivity was made, it was observed that 100 % patients who had HSIL were positive for HPV infection, 66.6 % of LSIL were HPV positive, 33.3 % of koilocytosis were HPV positive, and 8.0 % patients with inflammatory smears were HPV positive (Table 1). Our findings are in close conformity with the data given by the Advanced laboratory (June 2001); University of Pittsburgh.

Table 4 Correlation of histopathology (colposcopic-directed biopsy) and cervical cytology

Histopathology	Conventional Pap smear cytology					
	Inflammatory	ASCUS	Koilocytosis	LSIL	HSIL	Atrophic
Chronic cervicitis ($n = 50$)	40	04	0	0	0	06
Condyloma acuminata ($n = 6$)	02	0	04	0	0	0
CIN I ($n = 16$)	08	02	02	04	0	0
CIN 2 ($n = 2$)	0	0	0	02	0	0
CIN 3 ($n = 2$)	0	0	0	0	02	0
CIS ($n = 2$)	0	0	0	0	02	0
Total	50	06	06	06	04	06

Table 5 Correlation of histopathology (colposcopic-directed biopsy) and HPV DNA positivity by PCR

Histopathology	No. of cases ($n = 78$)	Percentage	No. of HPV-positive cases ($n = 14$)	Percentage
Chronic cervicitis	50	64.10	02	4.00
Condyloma acuminata	06	7.69	02	33.33
CIN 1	16	20.51	04	25.00
CIN 2	02	2.56	02	100.00
CIN 3	02	2.56	02	100.00
CIS	02	2.56	02	100.00

Table 6 Correlation of histopathology and HPV genotype

Histopathology	No. of HPV-positive cases	Percentage	HPV genotype
Chronic cervicitis ($n = 50$)	02	4.00	Type 16, Type 16
Condyloma acuminata ($n = 6$)	02	33.33	Type 6, Type 11
CIN 1 ($n = 16$)	04	25.00	Type 16, Type 16, Type 16, Type 43
CIN 2 ($n = 2$)	02	100.00	Type 18, Type 59
CIN 3 ($n = 2$)	02	100.00	Type 18, Type 18
CIS ($n = 2$)	02	100.00	Type18, Type 33

According to them, HPV is present in more than 95 % cases of invasive carcinoma, 79–90 % cases of HSIL, 50–75 % cases of LSIL, and in 2–5 % of normal smears.

All the HPV-positive patients in the LSIL group were less than 30 years of age, and all the HPV-positive patients in the HSIL group were more than 30 years of age (Tables 2, 3). The International Journal of Clinical Pathology, 2002 [4] mentions that women in the second and third decades who are newly sexually active and have an immature transformation zone actively undergoing squamous metaplasia are very efficient at acquiring a high-risk HPV infection. On the other hand, these women have a very low chance of developing cervical cancer. Women in their thirties and older, with more mature stable transformation zone, are at lower risk of acquiring a new HPV infection. Those in this older age group who test positive for high-risk HPV are likely to be manifesting a long standing persistent infection that has not been immunologically cleared. Therefore, HPV DNA testing may be used along with cervical cytology as an acceptable screening modality in women over age 30 years [5]. The combined tests' most powerful parameter is the negative predictive value [6], i.e., a negative Pap, and a negative HPV DNA test confers virtually 100 % assurance of not having CIN 2 or 3 or cancer.

It is evident from the results of this study that some of the higher grades of CIN which were underdiagnosed on cervical cytological examination were detected by histopathology of the biopsy specimen obtained after colposcopic examination. These higher grades of CIN were positive for high-risk HPV types by PCR testing. Therefore it can be concluded that the detection of CIN by PCR test was more accurate than by Pap smear cytology (considering histopathology as gold standard for diagnosis) (Table 4).

From this study, it is evident that there is a strong association of HPV positivity with higher grades of CIN (Table 5). Similar results have been obtained by Sowjanya et al. [7] in their study in Andhra Pradesh. High-risk HPV prevalence was found to be 87.8 % in their sample of invasive squamous cell carcinomas.

The ICMR annual report 2002–2003 mentions that various studies conducted at ICPO (Institute of Cytology and Preventive Oncology, New Delhi) have shown that HPV DNA screening has 75 % sensitivity for detection of CIN 1 lesions and nearly 100 % sensitivity for detection of high grade (CIN 2, 3) lesions with a specificity of about 83 %. Though the positive predictive value was rather low (6.7 %), a very high negative predictive value of 99.2 % makes it an ideal tool for Indian situation where frequent screening, as is being done in western countries, is not possible. Hence, a potentially attractive option might be "once-in-a lifetime" screening for high-risk HPV at 35 years of age.

Also a joint statement issued by the World Health Organization and the European Research Organization on Genital Infection and Neoplasia in the year 2000 mentioned that HPV testing showed 95–100 % sensitivity for high grade cancer precursors compared with 40–85 % for traditional cervical cytology (Pap test). In fact, the statement strongly suggested that HPV testing should be adopted as the primary screening method for women over age 30.

In this study, the most prevalent HPV genotype was found to be HPV type 16 followed by type 18 (Table 6). The results of this study are in agreement with those of Sowjanya et al. [7] who have reported that the most prevalent HPV types found in invasive cervical cancer cases in Andhra Pradesh were HPV 16 followed by HPV 18. The evaluation of the distribution and prevalence of major HPV types has special importance for the development of HPV vaccines. To maximize the cost effectiveness of the HPV vaccination programs in India, it is important to understand the distribution of the major HPV types in various geographical regions [7]. More comprehensive genotyping of cervical cancer tissues from various parts of India will be needed to justify a single national vaccine strategy for the Indian subcontinent.

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