Potential contribution of human papillomavirus testing to cervical cancer screening

R. PÓKA (*) - J. CZEGLÉDY (**) - L. GERGELY (**) - L. LAMPÉ (*)

Summary: A prospective study was carried out to assess the clinical value of HPV DNA identification in terms of cases missed by either cytology or combined cytology and colposcopy screening methods, 231 exfoliative cytology specimens and forty-one cervical tissue samples were analysed for the presence of HPV 6, 11, 16 and 18 DNA sequences using filter in situ and Southern blot hybridisation methods, 36% of cytology specimens examined by filter in situ hybridisation method were found to carry HPV DNA sequence. Forty-nine (27%) out of 184 cases without cytological evidence of neoplasia had a positive HPV test. Simultaneous colposcopic examination of these patients showed no abnormality in 17 cases. The relevance of HPV investigations was based on the characteristic HPV prevalence in preneoplastic and normal cervical tissue samples. The results suggest that traditional cervical screening may be improved by simultaneous HPV testing. According to the presented data, only a very small portion of a random patient population can be expected to carry HPVs without cytologic or colposcopic abnormalities.

Key words: Cervix; Neoplasia; Human Papillomavirus.

INTRODUCTION

As a result of wide-spread use of cervical cytology and colposcopy, more cases of cervical carcinoma are being diagnosed in early stages. In Hungary more than 90% of cervix carcinoma cases are diagnosed in early stages in spite of the fact that a relatively low portion (50%) of the female population makes use of the opportunity of being screened. The incidence of intraepithelial neoplasia is much higher than that of invasive cervix carcinoma.

The role of sexually transmissible factors in cervical carcinogenesis has long been recognised. Beside the chronic nonspecific inflammations much attention had been paid to the genital Herpesviruses during the 1970's ([1]). Their role has remained unclear, but their contribution as a cofactor cannot be disputed. During the last decade much data have been accumulated on the presence of Human Papillomavirus in genital lesions. Numerous studies have indicated that HPVs can frequently be identified in preneoplastic and invasive cervical lesions ([2-4]). Development of gene-cloning and DNA-hybridisation

(*) Department of Obstetrics and Gynaecology
(**) Microbiology, University Medical School of Debrecen
Hungary

techniques has resulted in identification of more than fifty different HPV types ([5]).

This prospective study aimed at assessing the clinical importance of HPV DNA testing in terms of HPV-positive cases that are negative by either cytology or combined cytology plus colposcopy screening methods.

PATIENTS AND METHODS

Exfoliated cervical cells from 231 randomly selected patients were collected by cotton swabs under colposcopic guidance for cytological examination and filter in situ hybridisation (FISH), 2×10^5 cells of each sample were transferred to nitrocellulose filter (Amersham), Following prehybridisation, (α^{-32p}) dATP-labelled HPV 6, 11, 16 and 18 plasmids (Specific activity $3\text{-}5\times10^8$ cpm/μg DNA) were used for hybridisation at Tm −38 °C in presence of 50% formamid ([3]). Filters were washed at Tm −15 °C and autoradiographs were evaluated after 10 days exposure ([6]).

Forty-one cervical tissue samples were obtained by excision biopsy or by cone biopsy, After DNA-extraction, Southern blot hybridisation was carried out using HPV type 6, 11, 16 and 18 test DNAs, 5-10μg DNA was extracted from each specimen, DNA samples were digested by restriction enzymes (Bam HI, Eco RI, Pst I), separated in 1.2% agarose gel then transferred to nylon filter (Hybond, Amersham). Hybridisation was carried out under high stringency (Tm - 18 °C, 5×SSC, 50% formamid) using plasmid delivered radioactive probes ([4]). The filters were washed under high stringency (1×SSC, 0.1×SSC 65 °C). Two autoradiographic films of each specimen were evaluated.

RESULTS

Using filter in situ hybridisation method, eighty-three cases (36%) out of 231 exfoliative cytology specimens were identified as carrying HPV DNA sequences (Table 1). Simultaneous cytological assessment showed dyskaryosis in thirty-four of the 83 HPV-positive cases. 72.3% of patients with a positive smear (47 cases) carried HPV-DNA sequences. In contrast, only 26.6% of patients without cytological evidence of neoplasia had a positive HPV test, and 8.8% of 148 HPV-negative cases had abnormal smears.

Table 1. – *Presence of human papillomavirus DNAs in normal and dyskaryotic smears.*

HPV test	Cervical smear	
	Negative	Dyskaryosis
Positive	49 (26.6%)	34 (72.3%)
Negative	135 (73.4%)	13 (27.7%)
Total	184	47

Table 2. – *Type-specific HPV prevalences in normal and dyskaryotic cervical smears.*

HPV test	Cervical smear	
	Negative	Dyskaryosis
6	13 (7.0%)	0
11	20 (10.9%)	13 (27.6%)
16	9 (4.9%)	15 (31.9%)
18	2 (1.1%)	4 (8.5%)
11+16	4 (2.2%)	2 (4.3%)
16+18	1 (0.5%)	0
None	135 (73.4%)	13 (27.6%)
Total	184	47

The prevalences of the four tested HPV types are shown on Table 2. Most cases with a positive HPV test carried non-oncogenic HPV types. In contrast, no case with dyskaryosis proved to be positive for type 6 HPV, and they were more likely to carry type 16 HPV DNA sequences (31.9%). Furthermore, incidence of type 18 in this group of patients was similar to that of HPV types 6 and 11 among cases with negative smears.

Colposcopic and cytological diagnoses of HPV positive cases are shown on Table 3. Among HPV positive patients, cytology showed dyskariosis in 47% of cases with colposcopically visible lesions and showed no evidence of neoplasia in 74% of cases with normal colposcopic features.

The HPV prevalence showed characteristic differences between preneoplastic

Table 3. – *Cytological and colposcopic diagnosis of 83 HPV positive cases.*

Colposcopy	Cervical smear		
	Normal	Dyskariosis	Total
CIN I-II	32 (38.5%)	28 (33.7%)	60 (72.3%)
Normal	17 (20.5%)	6 (7.2%)	23 (27.7%)
Total	49 (59.0%)	34 (40.9%)	83 (100%)

Table 4. – *Prevalence of type-specific HPV DNAs in normal and preneoplastic cervical tissue samples.*

Histology	HPV negative	HPV types				
		6	11	16	18	Total
Normal	19	1	1	1	0	22
CIN I-II	4	1	0	2	0	7
CIN III, CIS	4	0	1	4	3	12
Total	27	2	2	7	3	41

and normal cervical tissue samples. HPV test results compared with histological diagnosis of forty-one cervical tissue samples are shown in Table 4. Nineteen specimens (86.4%) without histological evidence of intraepithelial or invasive neoplasia were negative for all tested HPV types. Among the nineteen cases of histologically proven intraepithelial neoplasia eleven (58%) carried HPV-related DNA sequences. Type 16 and 18 HPV DNAs were the most frequently detected. They could be identified in 33% of CIN-III and 25% of in situ carcinoma cases. Altogether 58% of preneoplastic lesions proved to be positive for HPV 16 or 18. This figure indicates the importance of the close follow-up of HPV positive patients. As is show non Table 3, seventee HPV positive cases had neither cytological nor colposcopic evidence of cervical intraepithelial neoplasia. These patients represent a group which requires intensive surveillance with more frequent smear tests.

DISCUSSION

Much attention has been paid recently to the role of human papillomaviruses in cervical carcinogenesis. Numerous studies have indicated that HPVs can frequently be identified in preneoplastic and invasive cervical lesions. Development of genecloning and hybridisation techniques resulted in identification of more than seventy different HPV types. Many studies showed that type 16 and 18 HPVs can be more frequently detected in dysplastic and cervical carcinoma tissues than in normal cervices.

In the present study, eighty-three cases (36%) out of 231 exfoliative cytology specimens were identified by filter in situ hybridisation as carrying HPV DNA sequences (Table 1). Goldberg, using the same methods of sample collection and DNA hybridisation method, reported a 36.5% rate of HPV positive specimens, though in the same patient population he could detect HPV DNAs in 55% of cases when the specimens were collected by way of cervicovaginal lavage ([7]).

41% of our HPV positive samples showed dyskaryosis on smear and 73.4% of cases without cytological evidence of intraepithelial or invasive neoplasia were negative for all tested HPV types. Cases with a negative smear test and a positive HPV test carried mainly the non-oncogenic 6 and 11 types of HPVs. 31.9% of samples with dyskariosis were found to carry type 16 HPV DNA sequences (Table 2).

In seventeen cases with positive HPV test (20.5%), none of the traditional screening methods showed any abnormality (Table 3). Their importance is further emphasized by the fact that positive HPV test results are more frequently found in histologically proven intraepithelial neoplasia (Table 4). Ji reported 37% prevalence of HPVs in histologically proven case sof CIN ([8]). In Mc Cance's series 47% of CIN I-III cases were posi-

tive for HPV type 6 and 11 (9). Type 16 HPV DNA was detected in 86% of cases. Considering these results, close follow-up of HPV positive patients with negative smear and normal colposcopic findings is strongly suggested.

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Address reprints requests to:
R. POKA
St. Bartholomew's Hospital
West Smithfield, London EC1A (UK)