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Detection of Human Papillomavirus Type 16 in Sexual Partners of Patients Having Cervical Cancer by Polymerase Chain Reaction

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The polymerase chain reaction (PCR) was employed to detect human papillomavirus (HPV) 16 and 18 in cytological samples from the uterine cervix and in urine samples from the male consorts. HPV 16 was detected in 2 (25%) of 8 males whose wives were positive for HPV 16, while it was detected in only one (7%) of 14 consorts whose wives were negative for HPV 16 and 18. This is the first report of the detection of HPV 16 in urine. Viral detection in urine samples by the PCR method is a non-invasive, convenient and useful tool for large-scale epidemiologic studies and investigations of the mechanism of virus transmission.

Key words: Human papillomavirus — Sexual partner — Polymerase chain reaction — Cervical cancer — Sexually transmitted disease

Carcinoma of the uterine cervix has been considered to be a sexually transmissible disease for the last three decades. This idea is based on the clear-cut association between the development of this cancer and the sexual habits of the patients and their male partners. Early age at first intercourse, multiple sexual partners and promiscuous male partners all increase the risk of cervical carcinomas.[1] At the present time, viral agents, particularly human papillomavirus (HPV), are considered as the most likely causative factor.[2] In fact, several types of HPV have often been identified in cervical carcinoma tissues by DNA hybridization techniques,[2-4] and specific regions of HPV have been shown to be associated with malignant transformation of normal cultured cells.[2] Although the natural history of HPV infection of the female genital tract is becoming well characterized, less is known about the sexual transmission of HPV and the productive lesions in men responsible for the transmission to women because of sampling problems.[5,6] Therefore, screening of couples (females and their male consorts) for HPV in routine clinical samples is very important for understanding HPV infection as a sexually transmitted disease (STD) and for prevention of cervical neoplasms.

The recently described polymerase chain reaction (PCR) enables detection of specific DNA sequences rapidly, sensitively and specifically in routinely processed clinical materials.[7] With this method, we have previously detected HPV 16 and 18 in samples of exfoliated cervical cells.[8] However, there is no report concerning the detection of HPV 16 or 18, which are key agents in carcinogenesis of the cervix, in the urine of the male, although one report dealt with detection of HPV 6 and 11 in the urine.[9] This is the first report of detection of HPV types 16 by the PCR method in urine samples from male consorts.

Samples of exfoliated cervical cells were obtained from the uterine ectocervix of 23 patients who underwent hysterectomy because of cervical neoplasms at the Department of Obstetrics and Gynecology, Osaka University Medical School. Diagnosis of these patients was performed by routine histological examination of the surgical specimens according to the histological typing system of the World Health Organization (WHO). They consisted of 15 cervical squamous cell carcinomas *in situ* (CIS), six cervical squamous cell carcinomas of the large cell type, and two cervical adenocarcinomas. Exfoliated cells obtained with a cotton-tipped swab from the ectocervix were resuspended in 2 ml of phosphate-buffered saline (PBS), pH 7.4, and stored at −20°C until DNA extraction.

Urine samples were collected early in the morning from each male consort of the female patients. One hundred ml of each urine sample was centrifuged at 2,000 rpm for 30 min. The sediment was suspended in 2 ml of PBS and divided into two parts. One portion (1.9 ml) was stored at −20°C until DNA extraction. The other portion (0.1 ml) was suspended in 2 ml of Sternheimer Malbin solution for cytopathological analysis. Most of the obtained epithelial cells consisted of transitional cells and squamous cells, amounting to at least 3×10^4 cells per sample.

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DNA extraction from samples was carried out as follows. Samples were digested overnight with 100 μg/ml of proteinase K in a 50 m*M* Tris-HCl buffer (pH 8.2) containing 10 m*M* EDTA and 2% sodium dodecyl sulfate at room temperature. Cellular DNA was collected by precipitation with ethanol after three extractions using phenol and chloroform/isoamyl alcohol (24:1). The amounts of cellular DNA obtained from cervical exfoliated cells and urine were 3.1–21.4 μg/sample and 1.3–10.5 μg/sample, respectively.

The PCR was performed to generate amplified fragments surrounding the E6 open reading frames of both HPV 16 and 18.[10] The primers used were 5′ATTAGTGAGTATAGACATTA3′ (H1), 5′GGCTTTTGACAGTTAATACA3′ (H2) and 5′GGTTTCTGGCACCGCAGGCA3′ (H3). H1 and H2 correspond to the nucleotide positions (np) 320–339 and 410–429 of HPV 16, respectively. H1 and H3 correspond to np 328–347 and 418–437 of HPV 18, respectively. The reaction mixture containing 1 μg of sample DNA, 1 μg of each primer, and 200 μ*M* each of dATP, dGTP, dCTP and dTTP in 100 m*M* Tris-HCl buffer (pH 8.2) was denatured for 5 min at 95°C, followed by addition of 2.5 U of Taq polymerase. One cycle of PCR consisted of denaturation at 94°C for 1 min, reannealing at 55°C for 2 min and polymerization at 72°C for 1 min and 30 s. After 25 cycles of PCR, the amplified DNAs were transferred onto a nylon filter and hybridized with a ^{32}P-labeled oligomer probe specific for E6 of HPV 16 (H4: 5′ATGGAACAACATTAGAACAGCAATACAACAAACCGTTGTG3′) or HPV 18 (H5: 5′ATGGAGACACATTGGAAAAACTAACTAACACTGGGTTATA 3′) by the Southern blot method. Caski cells were used as the positive control for HPV 16 and the negative control for HPV 18. Similarly, HeLa cells served as the negative control for HPV 16 and the positive control for HPV 18. The sensitivity of our PCR assay is such that 20 viral copies per sample can be detected.

Figure 1 shows the results of Southern blot hybridization for PCR products. The PCR products amplified with two primers (H1 and H2) specific for HPV 16 or two primers (H1 and H3) specific for HPV 18 were observed as a specific band of the expected size (109 bp) in the positive controls (lane 1 for HPV 16 and lane 4 for HPV 18). No band was detected in lanes 2 and 3, which were used as negative controls for HPV 18 and 16, respectively. In a couple, a specific band for HPV 16 was detected in both cervical cytology samples from the female (lane 5) and urine from the male consort (lane 7). HPV 18 was not detected in any of the samples (lanes 6 and 8).

The results of the present study are summarized in Tables I and II. Five (33%) of the 15 patients with carcinoma *in situ* and four (50%) of eight patients with invasive cancer were positive for HPV-DNA. Several

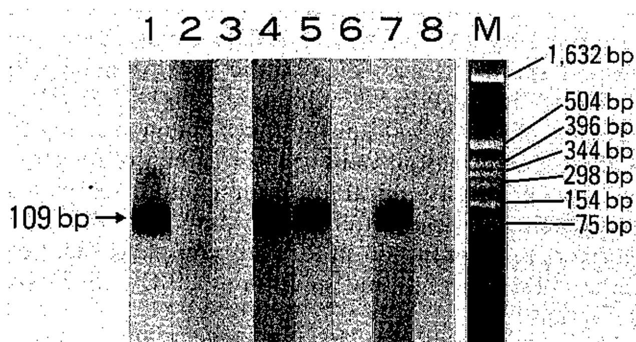

Fig. 1. Detection of HPV 16 and 18 in cervical scrape samples from females and urine samples from their male consorts. Lane 1. Caski cells, positive control for HPV 16. Lane 2. Caski cells, negative control for HPV 18. Lane 3. HeLa cells, negative control for HPV 16. Lane 4. HeLa cells, positive control for HPV 18. Lanes 5–8. In one couple, a specific band for HPV 16 was detected in both the cervical cytology sample from a female (lanes 5 and 6; HPV 16 and HPV 18 hybridization, respectively) and urine from the male consort (lanes 7 and 8; HPV 16 and HPV 18 hybridization, respectively). Lane M. DNA size marker, Hinf-1 digest of PBR-322.

Table I. PCR Detection of Human Papillomavirus Types 16 and 18 in Cytological Samples from Females with Cervical Neoplasms

Diagnosis	No. of cases	PCR positive for		
		HPV 16	HPV 18	HPV 16 or 18
Carcinoma *in situ*	15	5 (33%)	0	5 (33%)
Invasive squamous cell carcinoma	6	3 (50%)	0	3 (50%)
Adenocarcinoma	2	0	1 (50%)	1 (50%)
Total	23	8 (35%)	1 (4%)	9 (39%)

Table II. Correlation between PCR Detection of HPV in Female Patients and Their Male Consorts

Female patients	No. of cases	Male consorts		
		PCR-positive		PCR-negative
		HPV 16	HPV 18	
HPV positive	9	2 (22%)	0	7
HPV type 16	8	2 (25%)	0	6
HPV type 18	1	0	0	1
HPV negative	14	1 (7%)	0	13

reports with or without the PCR method from Western countries have shown a higher incidence of HPV-DNA in exfoliated cells of the uterine cervix.[11-14] However, the low incidence of HPV infection in the present series is not surprising because Southern blot analysis of tissue specimens has also shown a lower incidence in Japan[3, 4] than in the people of other countries.[2] A few investigators have tried to improve the sensitivity of detection of HPV in tissue samples from Japanese women.[15] In addition, a recent report using a filter *in situ* hybridization method[16] or PCR method[17] for cytological samples from Japanese women showed an incidence of HPV infection similar to our result.

The present PCR method showed that two (25%) out of eight male consorts of DNA-positive women harbored the same HPV type as the female, whereas only one (7%) out of 14 consorts of DNA-negative women harbored HPV. None of the male sexual partners showed any clinical evidence of HPV infection at the time of examination or during the follow-up period of 6 months. There was no difference in the extent of disease in the females as a function of whether the male consort was positive or negative for HPV. It should also be mentioned that a sufficient number of epithelial cells were collected even in PCR-negative samples. However, which cells, i.e., transitional cells, squamous cells, glandular cells or non-epithelial cells, harbored the HPV is not clear in the present study. Our conclusion that HPV is sexually transmissible between sexual partners is consistent with the findings of previous reports using Southern blot analysis for swab or biopsy materials from the cervix and penis.[5, 6] The present study also showed that the PCR method using urine samples works as a non-invasive, sensitive and convenient diagnostic tool for detecting HPV infection of the urogenital tract of males, as some investigators have demonstrated for swab materials from the uterine cervix of females.[13, 14, 18] It is, however, important to obtain sufficient sediment from a large volume of urine if few cells are present.

A definitive correlation between the presence of HPV 16 in sexual partners and the development of cervical cancer remains to be established since the number of samples assayed in the present study was not very large. However, virus detection in urine samples by the PCR method will facilitate large epidemiologic studies and investigations of the mechanisms of transmission in viral STDs.

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