Presence of Human Papillomavirus Type 6f in Tonsillar Condyloma Acuminatum and Clinically Normal Tonsillar Mucosa

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An attempt was made to detect human papillomavirus (HPV) in 8 cases of oral papilloma by Southern blot hybridization. HPV type 6 was identified in a case of tonsillar condyloma acuminatum, but neither HPV type 6 nor 11 was found in the 7 other cases of squamous papilloma. The hybridization pattern of the HPV type 6 DNA after digestion with restriction enzymes revealed that the HPV was of the 6f subtype. DNA samples isolated from the condyloma acuminatum, 3 peripheral specimens of clinically normal tonsillar mucosa, a swab sample and saliva from the male patient with this tonsillar condyloma acuminatum were examined further by polymerase chain reaction (PCR). HPV type 6 DNA was detected in the tonsillar condyloma acuminatum and the 3 specimens of clinically normal tonsillar mucosa, but no HPV type 6 DNA was detectable in the normal tonsillar mucosa by Southern blot hybridization using a whole HPV DNA probe. The presence of a small amount of HPV in clinically normal tissue detectable only by PCR suggests latent infection of peripheral tissues with HPV produced from the condyloma acuminatum. Although HPV type 6 DNA was not detectable in the swab and saliva samples of this single case, further study is necessary for the clinical application of preoperative virological diagnosis by PCR using these samples.

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Key words: HPV—Oral papilloma—Condyloma acuminatum—Polymerase chain reaction—Southern blot hybridization

Introduction

Papillomaviruses induce hyperplastic, papillomatous, and verruaceous squamous cell lesions in the skin and at various mucosal sites in a wide range of host animals. Verruca vulgaris, focal epithelial hyperplasia, squamous papilloma and condyloma acuminatum are recognized as human oral papillomatous lesions.¹⁻³ Condyloma acuminatum in the anogenital region is a well-known papilloma induced by human papillomavirus (HPV) type 6 and/or 11 infection.¹⁻³ Recently, oral condyloma acuminatum associated with HPV has been reported.⁴⁻⁶ The main route of oral infection with HPV seems to be genito-oral sex.⁵⁻⁷ However, the presence of HPV in normal mucosa of the oral cavity has also been reported,⁸⁻¹¹ and caution has been urged when interpreting the presence of HPV in a tumor.¹² The aim of our present study was to clarify the type of HPV, if present, in oral papilloma, especially condyloma acuminatum, and normal oral mucosa. Furthermore, we attempted to detect HPV in swab samples and saliva by polymerase chain reaction (PCR) for virological diagnosis.

Materials and Methods

Specimens for Histological Examination and DNA Extraction

Tissue specimens of oral papilloma were obtained from patients aged 1-65 years (7 males and 1 female) treated at the Department of Otolaryngology, Okayama University Medical School (Table I). Tonsillar condyloma acuminatum and 3 specimens of
clinically normal different parts of the tonsillar mucosa were obtained from a surgically removed whole right tonsil with a papillomatous lesion in a 21-year-old male patient. Part of the papillomatous lesion, the swab sample of tonsillar papilloma and saliva from the same patient were kept frozen at -70°C until DNA extraction. The remaining portions of the papillomatous lesion were fixed with 5% formaldehyde buffer and processed for histological examination.

**Extraction of DNA**

DNA was isolated from tissue homogenates, the swab sample and 200 μl of saliva by proteinase K digestion, deproteinization with phenol, phenol-chloroform (1:1) and chloroform, precipitation with ethanol, resuspension and treatment with ribonuclease A, re-extraction with chloroform, and reprecipitation with ethanol as described previously.14

**DNA Labeling and Southern Blot Hybridization**

HPV type 6b DNA was a generous gift from Dr. H. zur Hausen (Heidelberg, Germany), and HPV type 11 DNA was cloned in our laboratory. The HPV DNA separated from the pBR322 vector was labeled with digoxigenin-deoxyuridine triphosphate using a kit obtained from Boehringer Mannheim, Germany. Restriction endonucleases were purchased from Takara Shuzo Co., Kyoto. Digestion with these enzymes was performed according to the methods indicated by the manufacturer. Electrophoresis of DNA (2–3.5 μg) was done after digestion with restriction endonucleases in 0.7% agarose gel in TEA-NaCl buffer (50 mM Tris-HCl, pH 8.0, containing 20 mM sodium acetate, 2 mM Na2EDTA and 18 mM NaCl). After gel electrophoresis, the DNA was transferred to Nytran Membrane (Schleicher and Schuell, Dassel, Germany) by Southern blotting. After hybridization under stringent conditions in 50% formamide at 42°C overnight, hybrids were detected by enzyme-linked immunoassay as described previously.15

**Detection of HPV by Polymerase Chain Reaction**

The sequences of polymerase chain reaction (PCR) primers were the same as those reported by W. Melchers et al.16 The primers for the E5 of HPV type 6 were 5'-TAGTGGGCGCTATGGCTCGTC-3' (base positions 4671–4691 as the sense primer), and 5'-TCCATTAGCCTCCACGGGTG-3' (base positions 4931–4951 as the antisense primer). The amplifier length was 280 base pairs (bp). These synthetic oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer (Foster City, CA). Amplification of DNA by PCR was done in 50-μl reaction volumes containing 0.5 μg template DNA in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.2 μM each primer, 200 μM each deoxyribonucleotide triphosphate, 0.01% gelatin and 1.25 unit of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT). The reaction was carried out on a DNA Thermal cycler (Perkin Elmer Cetus) for 30 cycles of 1 min at 94°C, 2 min at 55°C and 3 min at 72°C. Ten microliters of the PCR products was electrophoresed through a 3% agarose gel and visualized by ethidium bromide staining as described previously.17

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### Table I. HPV DNA in Oral Papilloma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Derived from</th>
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<th>PHV DNA</th>
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<tbody>
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<td>M</td>
<td>21</td>
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<td>Tonsil</td>
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<tr>
<td>2</td>
<td>M</td>
<td>1</td>
<td></td>
<td>Tonsil</td>
<td>-</td>
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</tr>
<tr>
<td>3</td>
<td>M</td>
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<td>Tonsil</td>
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<tr>
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<td>Tongue</td>
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<tr>
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<td>Tongue</td>
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<td>M</td>
<td>58</td>
<td></td>
<td>Oral floor</td>
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</tbody>
</table>

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**Results**

**Histopathological Findings of Tonsillar Papillary Tumor**

As shown in Table I, all of the oral papillomatous tumors were benign papillomas. Except for case 1, no koilocytosis, as defined by pyknotic nuclei with perinuclear halos specific for HPV infection in the upper layer, was observed. Figure 1 shows the papillomatous lesion from the right tonsil.
characterized by squamous metaplasia and epithelial hyperplasia with koilocytosis. This lesion was diagnosed as condyloma acuminatum.

**Detection of HPV Type 6 by Southern Blot Hybridization**

DNA samples extracted from oral papillomas after EcoRI digestion were examined by Southern blot hybridization with digoxigenin-labeled DNA probes for cloned HPV type 6b and 11. Only the DNA from tonsillar condyloma acuminatum showed a single band at a site corresponding to approximately 7.9 kilobase pairs (kb) (Fig. 2, lane 1). The remaining 7 samples of squamous papilloma showed no detectable bands (Fig. 2, lanes 2–8). DNA samples extracted from the tonsillar condyloma acuminatum and from 3 samples of clinically normal tonsillar mucosa were further examined using a digoxigenin-labeled DNA probe of cloned HPV type 6b. Except for the positive control of cloned HPV type 6b DNA (100 pg), only the DNA from tonsillar condyloma acuminatum showed a single hybrid band at a site corresponding to approximately 7.9 kb. (Fig. 3a lane 1). None of the 3 samples of clinically normal tonsil showed detectable bands (Fig. 3a, lanes 2–4 and Table I). To clarify the subtype of the HPV type 6 detected in the tonsillar condyloma acuminatum, the DNA was further digested with BamHI, HindII, PstI, HpaII and EcoRI, and examined by Southern blot hybridization with the HPV type 6b DNA probe (Fig. 3a, lanes 6–8). A band of approximately 7.9 kb was detected after BamHI, HpaII and EcoRI digestion (Fig. 3a, lanes 6, 10 and 11), and a band of approximately 7.6 kb was found after HindII digestion (Fig. 3a, lanes 6). Four bands of approximately 3.5, 1.6, 1.4 and 1.0 kb were detected after PstI digestion (Fig. 3a, lane 8). Two bands were detected in the non-digested DNA (Fig. 3a, lane 9), suggesting the presence of episomal forms I and II HPV DNA. These hybridization patterns after digestion with restriction enzymes were specific for HPV type 6/17, 18

**Detection of HPV Type 6 by PCR**

PCR products obtained using HPV type 6 specific primers of the DNA from tonsillar condyloma acuminatum (Fig. 3b, lane 1) and 3 samples of clinically normal tonsillar mucosa (Fig. 3b, lanes 2–4) showed amplified DNA bands of the expect-
Condyloma acuminatum is a well known genital disease thought to be caused by HPV type 6/11 infection and transmitted by sexual contact. Oral condyloma acuminatum is a rare disease and thought to be transmitted by genito-oral sex. In this study, HPV type 6 was detected in a tonsillar condyloma acuminatum but not in other oral squamous papillomas derived from the tonsil, tongue, lips and oral floor. HPV type 6 DNA was further detected not only in the tonsillar condyloma acuminatum but also in 3 specimens of clinically normal tonsillar mucosa from the same patient by the most sensitive detection method available, PCR. However, HPV type 6 was not detected in the 3 clinically normal tonsillar mucosa specimens by Southern blot hybridization using the whole HPV DNA probe. In our system, the end-point dilution of HPV DNA for PCR was 0.01 pg, while that for Southern blot hybridization was 10 pg. Detection of HPV DNA by PCR but not by Southern blot hybridization in clinically normal tonsillar mucosa seems to be due to the difference in sensitivity for HPV DNA detection between the two methods. The upper layer of the condyloma acuminatum contained many koilocytes, which are believed to produce HPV. Accordingly, it is suggested that the small amount of HPV type 6 detectable only by PCR in clinically normal specimens of tonsillar mucosa were due to latent infection with progeny virus from the condyloma acuminatum. Radical tonsillectomy seems to have been a reasonable treatment in view of the fact that the peripheral clinically normal tonsillar mucosa was also infected with HPV.

A method for HPV detection using swab samples from female genitalia has been established, and the detection of herpes simplex virus in saliva sample using PCR has been reported. However, HPV was not detectable in either the swab sample or saliva. HPV type 6/11 was not detected in 7 cases of oral papilloma in the present study. It is still possible that types of HPV other than 6 or 11 may be present in these oral papillomas, although none had koilocytosis (Table I). The etiological agents or factors involved in oral papilloma without HPV are still unknown.

**References**


