Extensive Human Papillomavirus Type 7-Associated Orofacial Warts in an Immunocompetent Patient

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Human papillomavirus (HPV) type 7 is frequently found in butchers’ warts and has been demonstrated in oral and facial warts of HIV-infected patients. The reservoirs of HPV7 and the route of transmission are still unclear. Here we describe an HIV-negative, otherwise healthy patient with extensive, recurrent orofacial papillomatosis whose immune status proved to be normal and who had no history of meat handling. HPV7 L1 gene DNA that differed in 3 point mutations from the HPV7 prototype could be detected in 2 morphologically distinct, perioral lesions by different PCR protocols. In situ hybridization confirmed the presence of HPV7 DNA in the nuclei of vacuolated cells of the granular layer. Our data show that HPV7 can lead to perioral, spiky warts and brownish plaques in immunocompetent patients who had never been working as a meat or fish handler. Key words: HPV7; butchers’ warts; HPV transmission.

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HPVs (A1–10 PCR). For A1–A10 PCR primers A1 (5’ CCYSCWYWGKGA CURCAYGG 3’), HPV16 L1 nt 487–507 and A10 (18) were employed in first-step PCR, and A2 (5’ SYATTSARGATGTTGAYATG 3’), HPV16 L1 nt 580–600) and A9 (5’ CCTTTRATAYWACMTCCAAA 3’), HPV16 L1 nt 1357–1366 in second-step PCR. PCR reactions were performed under the same conditions as described previously for A5–10 PCR (18). HPV typing was performed by direct sequencing of PCR products and comparison of the obtained sequences with a HPV database (13), as previously described (18). A2/A9 PCR products of biopsy 1 were cloned into the vector PCR-Blunt using the Zero Blunt PCR Cloning kit according to the manufacturer’s instructions (Invitrogen, Leek, Netherlands). Both strands of clones that carried an EcoRI insert were sequenced with M13 forward and M13 reverse primers.

In situ hybridization

Six-μm sections were cut from fresh frozen tissue, mounted on slides coated with 3-aminopropyltriethoxysilane and fixed in 4% PBS-buffered paraformaldehyde. Proteinase K digestion, acetylation and dehydration of sections were performed as described by Odenthal et al. (19). Sections were then incubated in prehybridization buffer (50% deionized formamide, 2 x SSC, 50 mM NaH2PO4, Na2HPO4, (pH 7.0), 1 mM EDTA, 1 mg/ml salmon sperm DNA) for 2 h at 42°C and denatured at 90°C for 2 min. Purified, denatured digoxigenin-labelled HPV7-specific A6/A8 PCR-product (140 ng/ml in prehybridization buffer) was used as hybridization probe (42°C, overnight; PCR DIG Probe Synthesis Kit, Boehringer Mannheim, Germany). After hybridization, sections were washed twice in 2 x SSC, and once in 1 x and 0.5 x SSC (42°C, 30 min). Blocking (30 min, 21°C, blocking reagent), incubation with alkaline-phosphatase labelled antidigoxigenin–Fab fragments (1:500; 1h, 37°C) and signal development with NBT/BCIP (dark blue indigo dye, 2–4h, 37°C) were performed according to the manufacturer’s instructions (Boehringer Mannheim). Sections were counterstained with methyl green and mounted with Kaiser’s glycerol gelatine (Merek, Darmstadt, Germany).

RESULTS

Detection of HPV DNA and HPV typing

Both biopsies were tested with 13 different PCR protocols for the detection of mucosal and cutaneous HPV types. Cutaneous (group B2, E) and cutaneous EV (group B1) HPV specific sequences could not be demonstrated in the biopsies, but 4 PCR protocols for the detection of group A HPVs yielded positive results in both samples (GP5+/6+, A5–10, A1–10, and CN2F/2R PCRs, Table 1). For HPV typing, GP5+/6+ (144 bp), internal A6/A8 (272 bp), and CN2F/2R (316 bp) PCR products were directly sequenced and the obtained L1 (major capsid protein) gene sequences were compared to an HPV database. In both biopsies only HPV7-specific sequences were detected. Additionally, internal A2/A9 PCR products of biopsy 1 were cloned and sequenced. Five different clones showed identical HPV7 sequences that differed slightly (3/738 nt) from the HPV7 reference sequence (20). The 3 nucleotide exchanges (HPV7 nt 6562 T→C, HPV7 nt 6599 and 6600 AG→GC) were also found by direct sequence analyses of both strands of CN2F/2R PCR products. This shows that the mutations were not artificially introduced during PCR amplification. The mutations at HPV7 nucleotides 6599–6600 led to an S368→A exchange in the deduced HPV7 L1 amino acid sequence.

Histology and in situ hybridization

In both biopsies, papillomatosis and hyperkeratosis with focal parakeratosis were found (Fig. 2A). Large clear cells were arranged in clusters in the upper epidermis, some with central nuclei, most of them containing no keratohyalin granules. These cells were surrounded by heavily stained granular cells containing small keratohyalin granules (Fig. 2B). In situ hybridization with a digoxigenin-labelled PCR-generated HPV7-specific probe revealed strong purple-blue signals in the nuclei of clusters of vacuolated cells in the granular layer (Fig. 3).

DISCUSSION

HPV7 was first detected in butchers’ warts which are warts with a particular histology found on the hands of persons that work with meat or fish (1–3, 21). Apart from the hands of meat handlers, HPV7 has also been found in facial or oral warts of HIV-infected persons (6–10, 22). HPV7-induced warts are extremely rare in the general population (23). It is not clear how HPV7 is transmitted, and it has been speculated that HPV7 is widespread, but only causes clinical disease under specific conditions such as direct contact with meat or immunosuppression (5, 22). In two screening studies, de Villiers et al. have detected HPV7 in 4 persons with uncharacterized immune status: 2 non-meat handlers suffered from recurrent filiform warts on the face and at other body sites and 2 further patients had HPV7-positive papillomas of the oral mucosa (11, 12). In another study the same authors state that HPV7 could not be demonstrated in any oral warts of immunologically normal patients (6). The patient investigated in our study was fully immunocompetent and had no health problems apart from recurrent orofacial papillomatosis. (Fig. 1). An HIV-test performed 5 weeks after the biopsies were taken (and over a year after the first appearance of the orofacial warts) excluded an early HIV infection. HPV7 DNA could be detected in 2 morphologically different warts by 4 PCR-protocols suitable for the detection of HPV group A DNA (Table 1). Direct sequencing of the different PCR-products yielded unambiguous results and 5 cloned PCR products had identical sequences, making an infection with more than one HPV type unlikely. This is in accordance with clinically similar warts of HIV-infected patients in which only PCR protocols for the detection of group AHPV yielded single, or in one study 2 different HPV types (7–10). HPV7 could also be demonstrated by in situ hybridization, pointing to relatively high copy numbers of the infecting virus (Fig. 3). Histologically, the biopsies investigated here shared several aspects typical of HPV7-positive butchers’ warts as clusters of large clear cells without keratohyalin granules surrounded by heavily stained cells with keratohyalin granules (Fig. 2B) (2). Some authors have emphasized the presence of vacuolated cells, especially in the rete ridges (24). We did not notice this in our biopsies.

The route of infection in our patient is unclear. He has never worked as a butcher, meat or fish handler. His hands, as well as other extracutaneous body sites, were free of warts at the time of investigation and reportedly also in the past, making autoinoculation unlikely. One might speculate that the patient had acquired the warts by sexual contacts with men, who might have been HIV-infected and might have had HPV7 associated oral warts. We cannot exclude that the patient suffered from any preceding predisposing conditions associated with microabrasions of the oral/perioral area, such as cheilitis or herpes. Perhaps his atopic predisposition (hay fever) facilitated the infection. Keefe et al. (23), however, have not found an association between butchers’ warts and atopy.

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Table I. **HPV PCR and typing results**

<table>
<thead>
<tr>
<th>Sample</th>
<th>PCR</th>
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<tbody>
<tr>
<td></td>
<td>GP5+ /6+ group A</td>
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<tr>
<td>Biopsy 1</td>
<td>Nested A5–A10 group A</td>
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<tr>
<td></td>
<td>Nested A1–A10 group A</td>
</tr>
<tr>
<td>Direct sequencing</td>
<td>+ HPV7</td>
</tr>
<tr>
<td>Sequencing of clones</td>
<td>HPV7 (5 clones, 3 nt Δb)</td>
</tr>
<tr>
<td>Biopsy 2</td>
<td>Nested CN3F/3R group A2</td>
</tr>
<tr>
<td>Direct sequencing</td>
<td>+ HPV7</td>
</tr>
</tbody>
</table>

Results of HPV group B1, B2 and E PCR s are not shown, since they yielded negative results for both biopsies. For details of PCR see Materials and methods and Refs 15–18.

* HPV7 is a member of the HPV group A8. Due to sequence similarities with group A4 HPVs, such as HPV 2 or 27, HPV7 DNA could also be amplified with CN2F/2R primers.

* 3 nt Δ: Sequence analyses revealed 3 mutations compared to the HPV7 reference sequence; for details see text.

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**Fig. 3. In situ** hybridization of a HPV7-containing perioral biopsy (biopsy 2). HPV-7 DNA is detectable in the nuclei of vacuolated granular cells with a digoxigenin-labelled HPV7-specific probe (for experimental details see Material and methods, original magnification ×100).

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To our knowledge, this is the first description of extensive, recurrent HPV7-induced perioral papillomatosis in an immunocompetent HIV-negative patient whose immune status was fully characterized. Further typing of perioral warts should be done and may possibly help to understand the epidemiology of HPV7. As in butchers’ warts and in orofacial warts of HIV-infected patients, the lesions investigated here were difficult to treat and had a strong tendency to recur (11, 25), which might be attributed to the HPV type 7.

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**REFERENCES**


