Acta Derm Venereol 2001; 81: 130–133

CLINICAL REPORT

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.*

(Accepted January 17, 2001.)

Acta Derm Venereol 2001; 81: 130–133

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Human papillomavirus (HPV) type 7 is the cause of so-called butchers' warts, which are benign hand warts of meat, fish, or poultry handlers (1–4); for review see Maitland *et al.* (5). HPV7 has also been found in oral or facial filiform or cauliflower-like, benign warts of HIV-1 infected patients (6–10). In 2 studies, HPV7 was detected in facial or oral papillomas of 4 persons with uncharacterized immune status (11, 12). Here, we describe a patient with recurrent, extensive, therapy-resistant HPV7-associated, orofacial papillomatosis, whose immune status was fully characterized and proved to be normal. Today, over 80 HPV types and at least as many new partial HPV sequences have been identified. Phylogenetically, papillomaviruses are divided into groups A–E. HPVs can be found in groups A (mainly mucosal/genital HPVs), B (cutaneous/epidermodysplasia verrruciformis (EV)-associated HPVs), and E (cutaneous HPVs). HPV7 belongs to group A8 and can infect both oral and cutaneous tissue (13).

CASE REPORT

A 26-year-old man presented in June 1998 with a one-year history of recurrent orofacial warts. The warts had been removed 5 times by carbon dioxide laser surgery and electrocautery, but always recurred rapidly. The patient was employed as a shop assistant in a fashion store and had never worked as a meat, fish, or poultry handler. He reported different sexual partners including some homosexual contacts in the past. The patient presented with spiky warts (diameter about 5 mm) in both angles of the mouth, which began to progress to the buccal mucosa. Additionally, we found some perioral verrucous and hyperkeratotic nodes and brownish plaque-like lesions (Fig. 1). The further examination revealed no other warts or dermatological abnormal findings at other sites of the body. The patient's past history was unremarkable. He reported a negative HIV-test performed 6 months before his presentation. Biopsies were taken from a spiky wart in the left angle of the mouth (biopsy 1) and from a perioral brownish plaque (biopsy 2). The full blood count revealed slight leucocytosis (12 600/μl), which was most likely caused by a known dental focus. The patient had normal absolute levels of IgG, IgA and IgM. IgE was elevated to 327 kU/l which is consistent with the patient's atopic predisposition (hay fever). All other routine laboratory parameters yielded normal values. CD4– (740/μl, 39%) and CD8– (490/μl, 26%) positive cells as well as the CD4/CD8 ratio (1.5) were within normal range. Absolute counts and relative numbers of B cells (CD19), natural killer cells (CD16–56) and IL-2-receptor-positive cells were also normal. A negative HIV screening test (HIV-1/HIV-2 Axsym-Test, Abbott, Wiesbaden, Germany) was obtained 5 weeks after the biopsies were taken. The same was true for Hepatitis B and C virus serology. Recall-antigen testing was performed with streptokinase/streptodornase, Candida albicans, Trichophython, mumps virus antigens and tuberculin. Normoergic skin reactions were detected after 24, 48 and 72 h for all antigens, with the exception of tuberculin and a NaCl-control which were negative. After histological and virological diagnosis, the patient was again treated with carbon dioxide laser and was free of warts at his last visit 8 weeks after surgery.

PCR and HPV typing

Tissue biopsies were processed with the QIAamp Tissue Kit (Qiagen, Hilden, Germany) and 10 μl of purified total cellular DNA were employed in each PCR reaction (25 ng/μl). Twelve different PCR protocols for the detection of mucosal and cutaneous HPVs were performed as previously described: GP5+/6+ and A5–10 general primer PCRs, TS6, TS11, TS16, TS18, TS31 type-specific PCRs (group A HPVs), CN1F/CN1R, CN2F/CN2R, CN3F/CN3R, C4F/C4R PCRs (group E, A4, A2, B2 HPVs, respectively) and CP65–70 PCR (group B1 HPVs) (14–18). The samples were additionally analysed with a newly established nested PCR for the detection of group A

Fig. 1. An immunocompetent patient with perioral HPV7-positive warts. Spiky warts in the left angle of the mouth and hyperkeratotic nodes and brownish plaque-like lesions on the perioral skin.

HPVs (A1–10 PCR). For A1–A10 PCR primers A1 (5′ CCYSCYWTWGGKGARCAYTGG 3′, HPV16 L1 nt 487–507) and A10 (18) were employed in first-step PCR, and A2 (5′ SYTATTSARGATGGTGAYATG 3′, HPV16 L1 nt 580–600) and A9 (5′ CCTTTARATYWACMTCCCAAAA 3′, HPV16 L1 nt 1357–1336) 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-aminopropyltrietoxysilane 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 × SSC, 50 mM NaH_2PO_4/Na_2HPO_4 (pH 7.0), 1 mM EDTA, 1 mg/ml salmon sperm DNA) for 2 h at 42°C and denaturated at 90°C for 2 min. Purified, denaturated 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 × SSC, and once in 1 × and 0.5 × SSC (42°C, 30 min). Blocking (30 min, 21°C, blocking reagent), incubation with alkaline-phosphatase labelled antidigoxigenin–Fab fragments (1:500; 1 h, 37°C) and signal development with NBT/BCIP (dark blue indigo dye, 2–4 h, 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 (Merck, 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 I). 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 S_{268}→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 I). 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 single, or in one study 2 different, HPV types have been found (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 extrafacial 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.

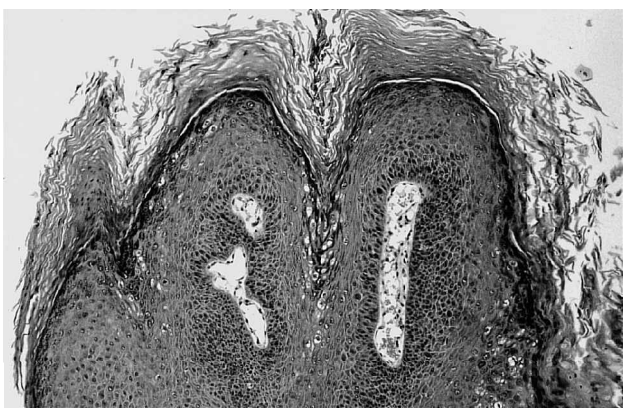
Table I. *HPV PCR and typing results*

Sample	PCR					
	GP5+/6+ group A	Nested A5–A10 group A	Nested A1–A10 group A	Type specific PCRs HPV6, 11,16,18,31	Nested CN3F/3R group A2	Nested CN2F/2R group A4[a]
Biopsy 1	+	+	+	–	–	+
Direct sequencing	HPV7					HPV7[a] (3 nt Δ[b])
Sequencing of clones			HPV7 (5 clones, 3 nt Δ[b])			
Biopsy 2	+	+	+	–	–	+
Direct sequencing		HPV7				HPV7[a]

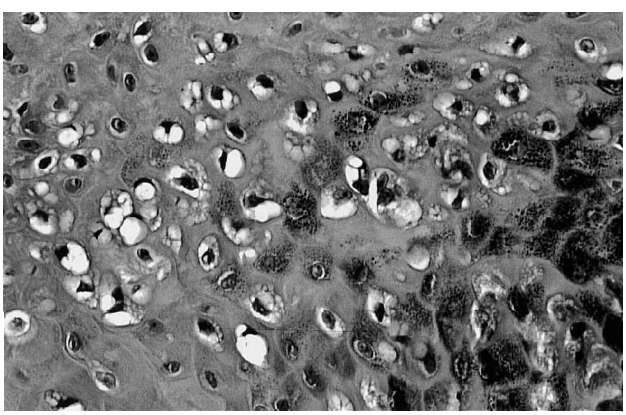
Results of HPV group B1, B2 and E PCRs are not shown, since they yielded negative results for both biopsies. For details of PCR see Materials and methods and Refs 15–18.

[a] 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.

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



(*a*)



(*b*)

Fig. 2. HPV7-infected spiky perioral wart (biopsy 1). (a). Papillomatosis, hyperkeratosis and focal parakeratosis. Large, clear cells are visible in the upper epidermis (haematoxylin–eosin, original magnification ×40). (b). Vacuolated clear cells with centrally located nuclei lacking cytoplasmatic keratohyalin granules were surrounded by heavily stained granular cells with small keratohyalin granules (haematoxylin–eosin, original magnification ×400).

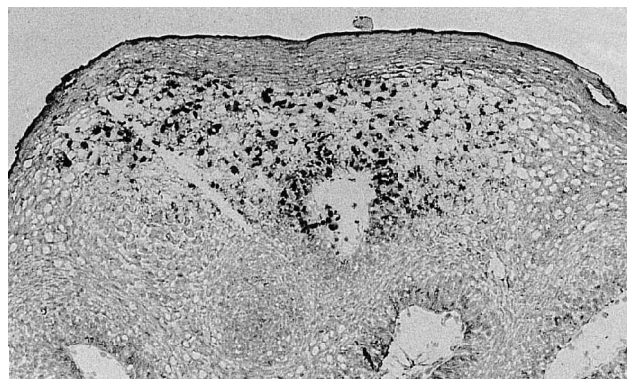


Fig. 3. In situ hybridization of a HPV7-containing perioral biopsy (biopsy 2). HPV-7 DNA is detectable in the nuclei of vacuolated granular cells with a digoxigenin-labelled HPV7-specific probe (for experimental details see Material and methods, original magnification ×100).

To our knowledge, this is the first description of extensive, recurrent HPV7-induced periorofacial 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.

ACKNOWLEDGEMENTS

We would like to thank M. Odenthal, Institute of Pathology, University of Koeln for valuable technical advice concerning *in situ* hybridization. This work was supported by the Köln Fortune Program/Faculty of Medicine, University of Cologne, project number 84/1998.

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