IDENTIFICATION OF MULTIPLE HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES IN A PATIENT WITH ORAL CONDYLOMATA ACUMINATA

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ABSTRACT
Oral condyloma acuminatum is a papillomatous lesion that is transmitted most often sexually and associated with the human papilloma virus (HPV). This report describes a case of a 30-year-old woman with multiple oral condylomata acuminata, located on the lateral edges and on the dorsum of the tongue. The incisional biopsy showed histological features compatible with those of condyloma acuminatum. E6 viral oncogene of HPV types 6, 11, 16, and 33 was identified by means of polymerase chain reaction (PCR). The lesions were removed by radiodissecation and treated topically with trichloroacetic acid (80% and 40%) and interferon-alpha-2a. No recurrence was evident 8 months after the treatment. We report this case not only for the simultaneous presence of low- and high-risk HPV types in one patient and the rarity of the condition, but also for the good response to the combined surgical and medical treatment that we observed.

Introduction
Condylomata acuminata or venereal warts are hyperplastic lesions of the skin or mucous membranes and are caused by human papillomaviruses (HPVs) (1). Condylomata acuminata usually involve the anogenital region and occur rarely in the oral cavity (1). However, reports of oral condyloma are appearing more frequently due to the increase of the oral-sexual activity (2). The oral lesions generally appear one to three months after exposure to an infected individual and are usually found on the labial and buccal mucosae, tongue, gingiva, and palate (3).

Laboratory diagnostics of HPV infections is based primarily on molecular virology techniques, because it is not possible to propagate HPV in vitro (4). The presence of HPV DNA is usually established by nucleic acid hybridization assays with or without gene amplification. The most sensitive and widely used method for HPV detection and identification in clinical samples is polymerase chain reaction (PCR) (5). Through the use of PCR, this study investigates the presence of different types of HPV in a female patient with oral condylomata acuminata.

Case report
Our patient is a 30-year-old white woman who presented multiple, wart-like lesions on the lateral edges and on the dorsum of the tongue (Fig. 1). The lesions appeared nearly 2 years previously. The lesions were painless, but caused the feeling of discomfort and changes to the sense of taste. The
patient was otherwise in good general health. She was not on any regular medications.

Examination revealed a number of elevated, sessile masses with pinkish white color involving the lateral edges and the dorsum of the tongue. The lesions were soft to firm on palpation, and were covered by normal-appearing mucosa. They ranged in size from 3 to 8 mm. Genitalia, rectal mucosa and perianal skin showed no warty growth.

All the routine investigations were done. All values were within normal limits. The flocculation test for syphilis (VDRL) and enzyme linked immunosorbent assay (ELISA) for human immunodeficiency virus (HIV) types 1 and 2 on blood of the patient gave negative results. Incisional biopsy showed marked acanthosis, sharp deep margin and hyperkeratotic surface with vacuolated parakeratotic cells. These histological features were consistent with those of condylomata acuminata. All lesions were removed by radiodissecation with radiosurgical device (Ellman International, Inc. Hewlett, NY) using wireloop electrode. Three weeks later a few recurrent lesions were detected and trichloroacetic acid 80% and 40% was applied topically and interferon-alpha-2a was administered as adjuvant immunotherapy. The check-ups were made 2 and 8 months later and no recurrence was detected (Fig. 2).

Viral detection and typing

Biopsy material for HPV DNA analysis from the warts was collected in sterile jars containing transport medium and delivered for a study. Total cellular DNA was extracted by DNAzol (Invitrogen, USA) and portions of 1-2 μl (0.5-1.0 μg) were analyzed by PCR, using the protocols described previously (6, 7). In order to detect the presence of HPV DNA, the samples were initially tested by a set of consensus primers MY 09/11 (8). To internally control the quality of the target DNA, beta-globin-specific primers were used in the multiplex reaction with MY09/11 primers. Genotyping was carried out using the multiplex PCR kit (Maxim Biotech Inc., USA), which is designed to direct the simultaneous amplification of specific E6 gene of the HPV types 6, 11, 16, 18, and 33. All amplification products were electrophoresed in 2% (w/v) agarose gels containing 0.5 g/l ethidium bromide and amplicons visualized under ultraviolet light. All standard precautions were used to prevent contamination and false positives in PCR reactions. In the biopsy sample was established the presence of E6 gene of the HPV types 6, 11, 16, and 33 (Fig. 3).
Fig. 3. The electrophoretic pattern of the HPV PCR products. Lane 1 - clinical sample; lane 2 - negative control; lane 3 - positive control; M – molecular weight marker.

Results and Discussion

Oral condyloma acuminatum is a papillomatous lesion that is caused by human papillomaviruses. Most often a history of oral-genital sex can be elicited from the patient (9). Some patients with condyloma acuminatum have concurrent genital or anal warts. Oral condyloma may be also a manifestation of HIV infection (10). Our patient has no anogenital lesions and is HIV negative. Because she admits oro-genital contacts, this way of acquisition of oral condyloma is highly possible.

HPVs are highly epitheliotropic viruses, of which more than 180 genotypes have been identified so far. The genotypes are divided into high-risk (e.g. 16, 18, 31, 33) and low-risk (e.g. 6, 11), depending on the association with malignant changes (11). The high-risk types have been implicated in the majority of cervical cancers and their high-grade precursor lesions. Moreover, these HPV types have emerged as one of the most important identified risk factors for human cancers (12).

The viral oncoproteins E6 and E7 are required for the initiation and maintenance of the malignant phenotype in HPV-positive cancers. Both proteins are multifunctional and interfere with important cell cycle regulatory proteins. E6 and E7 could immortalize various human cell types, inactivate host proteins (such as p53 or pRb), and induce mutations in the host cell DNA (12). In this study, the HPV genotyping analysis was performed by targeting the E6 viral oncogene of multiple HPV types.

Oral condyloma is associated most often with the low-risk HPV types 6 and 11, and less commonly with the high-risk types 16, 18, 31, 33, and 35 (13). In our patient was revealed the coexistence of E6 sequences of the HPV types 6, 11, 16, and 33. Because several studies have implied the role of high-risk HPVs in the development of oral malignancy (14; 15), their presence in the biopsy of our patient is of great concern. It has been suggested that high-risk HPV DNA may be latent for a long time in the oral mucosa, and it may be responsible for the initiation and development of a tumoral growth as a result of a multicarcinogenic interaction together with some other carcinogens and co-carcinogens (16). Therefore, the cases with high-risk HPV DNA positivity suggest that these lesions should be more carefully followed up.

No treatment is completely satisfactory; relapse is frequent and requires re-treatment (13). It has been suggested that a combination of surgical and medical treatment is most effective in clearing lesions completely and in reducing the relapse rate (17). Oral warts can be removed by electro- or radiosurgery, laser surgery, cryotherapy, or surgical excision. Local medical treatments include topical application of trichloroacetic acid, podophyllum, cidofovir, imiquimod, and intralesional injection of antiviral agents (10). Interferon appears to be a promising area of immunotherapy for condyloma acuminatum and
may play a role in controlling the recurrence (13). The lesions of our patient were partially removed by radiodissecation and then treated topically with 80% and 40% trichloroacetic acid and adjuvant interferon-alpha-2a. The lesions showed complete resolution and no recurrence was evident eight months after the treatment.

In conclusion, we reported this case not only for the rarity of the condition and the simultaneous presence of low- and high-risk HPV types in the lesions of one patient, but also for the good response to the combined surgical and medical treatment that we observed.

REFERENCES