Subclinical Human Papilloma Virus Infection in Condylomata Acuminata Patients Attending a VD Clinic

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In 37 (77%) of 48 patients with external genital warts, application of 5% acetic acid revealed areas of acetowhite epithelium. The lesions were not clinically apparent before acetic acid was applied but were easily detected without the use of a colposcope. In a control group of 20 patients with chlamydial urethritis and no history of genital warts, none had acetowhite genital lesions. Histological examination of biopsy specimens from the flat acetowhite lesions showed HPV infection with koilocytosis in 29 (78%) and in 3 (8%) intra-epithelial neoplasia grade II-III. Using *in situ* hybridization with commercially available biotinylated DNA probes, HPV types 16/18 could be detected in 7 (24%) patients with koilocytosis and in 3 (100%) patients with dysplasia. Simultaneous infection with HPV types 6/11, 16/18, and 31/33/35 was found in 8 of the 13 HPV DNA-positive patients. It is concluded that subclinical HPV-induced acetowhite lesions are common among patients with genital warts and that these flat lesions may be associated with a high grade of dysplasia. Consequently, routine use of the acetic acid test on the genital epithelium is recommended in patients with condylomata acuminata in order to diagnose and treat all HPV-infected areas.

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Application of 5% acetic acid to the penile, vulvar or anal epithelium causes a characteristic whitening of HPV-infected and dysplastic areas of epithelium (1–3). Most infections of the female genital tract with the neoplasia associated HPV types 16, 18, 31, 33, and 35 are now thought to be subclinical. Subclinical penile HPV-induced infection in acetowhite elements has been described in 46% of male partners of women with grade III cervical intra-epithelial neoplasia (4). HPV DNA was detected in 39% of the biopsy specimens from these lesions (4). In women with symptomatic HPV vulvitis, HPV of known genotype could be demonstrated in 32% of the flat acetowhite lesions (5).

To our knowledge, unselected patients with external condylomata acuminata have not been examined for genital foci of subclinical HPV infection. We now report on the prevalence of subclinical HPV infection and associated HPV types in patients with genital warts who were attending a VD clinic in Copenhagen.

PATIENTS AND METHODS

Forty-eight heterosexual patients presenting with acuminate or papular external genital warts from November 1989 to February 1990 were enrolled in the study. The genital warts were located on the penile skin in 23 male patients and on the vulvar or perianal skin in 25 female patients. The mean duration of warts was 16 weeks (range 2 weeks to 5 years). Thirty of the patients had previously been treated with podophyllin. In all patients, a gauze moistened with a 5% acetic acid solution was applied to the genital epithelium for 2–3 min and a biopsy specimen was taken if acetowhite flat areas appeared, for histological examination and HPV typing. The acetic acid test was also applied to a control group of 20 patients (10 males, 10 females) with chlamydial urethritis and with no sign or history of genital warts.

In situ hybridization was performed on routinely formalin-fixed and paraffin-embedded tissue using commercially available DNA-probes against HPV types 6/11, 16/18, 31/33/35 (Vira Type *in situ* HPV Tissue Hybridazation Life Technologies, Inc., Gaithersbury, Maryland, USA). Four-μm-thick sections were deparaffinized in petroleum and rehydrated through graded ethanols. Sections were pretreated with a digestive solution and a biotinylated probe was added. A positive and a negative control were included for each biopsy. After DNA denaturation by heating (90°C for 10 min) the slides were transferred to a moist chamber for hybridization (42°C overnight). For visualization we used the alkaline phosphatase/Nitroblue tetrazolium method from the kit. Light-microscopic examination was performed by only one person.

RESULTS

In 37 (77%) (19 females, 18 males) of the 48 patients with acuminate genital warts, distinct flat areas of white epithelium appeared after acetic acid applica-

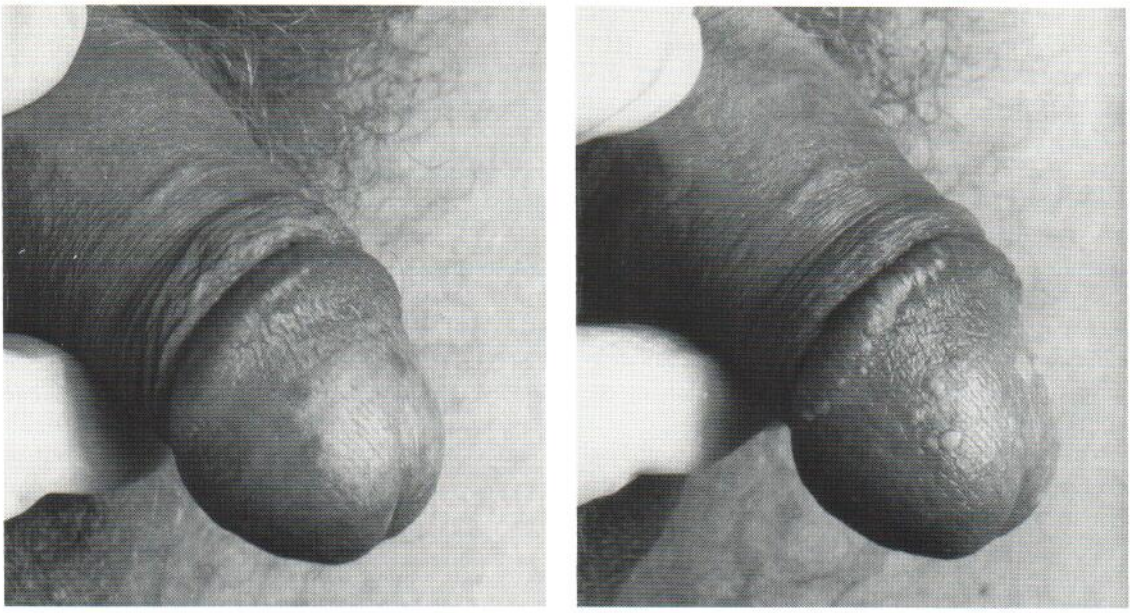

Fig. 1. Human papillomavirus infection on penile skin before (A) and after (B) application of a 5% acetic acid solution. Macular lesions with acetowhite epithelium are seen in (B).

tion. The lesions were clinically not apparent before the acetic acid was applied (Fig. 1A), but were easily recognized with the naked eye after using the acetic acid test (Fig. 1B). No flat acetowhite lesions were revealed in the control group of 20 patients with chlamydial infection.

The results of the histological examination of biopsies are shown in Table I. HPV-induced koilocytosis could be detected in 29 (78%) of the 37 specimens. Penile intra-epithelial neoplasia (PIN) were detected in 3 male patients previously not treated with podophyllin (Table I). PIN grade II (moderate dysplasia) was associated with koilocytosis in one patient and 2 patients had PIN grade III (carcinoma *in situ*). Thus, the vast majority of the acetowhite lesions, 32 (86%) of 37 biopsy specimens, evidenced changes associated with HPV infection.

Using *in situ* DNA hybridization, HPV DNA could be visualized in 13 (35%) of the 37 biopsy specimens. HPV types 6/11, 16/18, and 31/33/35

Table I. *HPV-types in relation to histologic findings in 37 biopsy specimens from acetowhite external genital lesions in patients with condylomata acuminata*

Histology	No.	HPV-type				
		Negative	6/11	16/18	31/33/35	6/11+16/18+ 31/33/35
Koilocytosis	29	21	1	1	–	6
Inflammation	2	1	–	–	1	–
Acanthosis	1	–	1	–	–	–
Other[a]	2	2	–	–	–	–
Penile intra-epithelial neoplasia						
Grade II[b]	1	–	–	–	–	1
Grade III	2	–	–	1	–	1
Total	37	24	2	2	1	8

[a] normal skin and hyperkeratosis.
[b] associated with koilocytosis.

were found simultaneously in 6 of the 29 koilocytotic lesions and in 2 of the 3 dysplastic lesions. HPV 16/18 was demonstrated in all 3 patients with PIN grade II-III.

DISCUSSION

The vast majority of our patients with condylomata acuminata also had subclinical acetowhite HPV-induced elements in the genital area. These flat lesions were easily seen without the use of a colposcope and clinicians who are not familiar with its use can diagnose the infection by careful examination of the epithelium after application of acetic acid.

This study once again points to the multifocal character of the genital HPV infection, since the flat lesions were located not only close to but also several centrimetres from the visible acuminate warts. The disseminated distribution of the HPV infection might explain the low rate of cure obtained in patients with genital warts in spite of radical treatment.

It must be emphasized that when dealing with the flat acetowhite lesions we do not know their natural course, contagiousness or the optimal form of therapy. It does seem, however, most logical not only to focus on the acuminate warts but also on the concomitant flat acetowhite elements. We have previously reported a cure rate of approximately 25% in patients with genital warts treated with CO_2-laser (6). A more radical therapeutical approach might be obtained if the acetic acid test were used routinely in wart patients attending VD clinics.

It is well known that inflammatory conditions such as fungal infections and eczematous reactions can be the cause of false positivity of the acetic acid test. The specificity of the simple acetic acid test seems to be fairly high, as 86% of the acetowhite elements had histological abnormalities associated with HPV infection or HPV-induced dysplasia.

Another argument for the routine use of the acetic acid test is the alarming finding of 2 patients with carcinoma *in situ* and one patient with PIN grade II in the biopsy specimens. Dysplasia has previously been found in penile, vulvar and anal acetowhite epithelium (1–3). Hence histological evaluation of the acetowhite lesions is advisable not only to confirm the HPV nature of the lesions but also to exclude associated dysplasia. We do realize that a single biopsy specimen may be insufficient to evaluate this aspect. It must be pointed out that our patients with dysplasia were previously untreated, and the lesions were therefore not podophyllin-induced.

HPV DNA was detected by *in situ* hybridization in 35% of the 37 biopsy specimens. In two previous reports, HPV DNA was found in 39% of penile and 32% of vulvar subclinical acetowhite lesions, using dot-blot and Southern blot assays (4, 5). In this respect, it is important to stress that HPV DNA of unknown genotype was seen in 12% of the acetowhite lesions from women with flat vulvar lesions (HPV vulvitis) (5). Some of our patients may have been infected with unknown HPV types, but the *in situ* hybridization assay does not allow us to evaluate this aspect. The sensitivity of the *in situ* hybridization method is, however, considerably lower than the recently published polymerase chain reaction (7).

The neoplasia-associated HPV types 16/18 and 31/33/35 were found in 11 of our patients. A similar number of patients had type 6/11 HPV in the biopsy specimens, and simultaneous infection with more than one HPV type was frequently encountered. Dual infection with HPV types 16 and 11 has recently been demonstrated in a high percentage of cervical smears from women with cytologic abnormalities (7).

Our study confirms the flat acetowhite lesions to be a major reservoir for the HPV types related to neoplasia. This fact may explain why 3 of the patients had associated dysplasia. Based on our experience, we recommend routine application of 5% acetic acid on the genital epithelium in patients with condylomata acuminata in order to diagnose and treat all HPV-infected lesions.

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