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HPV Infection in Male Partners of Women with Squamous Intraepithelial Neoplasia and/or High-risk HPV

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In order to estimate the prevalence of HPV infection in male partners of women with squamous intraepithelial lesions and/or high risk HPV, we examined 25 men.

In situ hybridisation or polymerase chain reaction was used to detect HPV DNA in biopsied tissue or cell samples from the genital epithelium.

Twenty (80%) of the male consorts had clinical features suggestive of HPV infection. Of these, 18 (90%) had detectable HPV DNA, 11 (65%) of the high risk type. In 9 cases HPV DNA was detected by in situ hybridisation and in 9 by polymerase chain reaction. Concordance between female and male HPV type was found in 8 cases (32%), but regarding high-risk HPV carriage as such, 10 (40%) couples corresponded.

A search for HPV infection in male partners of women known to be infected with high-risk HPV seems worthwhile. ***Key words: genital human papillomavirus infection; contact tracing; partner notification; sexually transmitted diseases; in situ hybridisation; polymerase chain reaction.***

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The natural history of human papillomavirus (HPV) infection, probably the most common sexually transmitted disease (STD) in Western countries today, is still not fully understood. Much work is being focused on the infection in women, since HPV is known to be an important factor for the development of cervical cancer and other malignancies of the female genital tract. During recent years the identification of high-risk HPV, i.e. HPV 16/18 and to some extent 31/33/35 and 45 among the approximately 40 genotypes showing tropism for the genitoanal area, has partially narrowed the task (1). All sexually active people have the potential of becoming infected by HPV. Some results indicate that infections with the low-risk type of HPV are more likely than high-risk HPV to be eradicated by the immune system (2). If so, the high-risk infection might be of more interest regarding the natural course of the disease in view of its greater potential to be transmitted.

The detection of HPV in the male partners of women with HPV infection varies from 50% to 90% (3–5). In a large survey recently published (6), it was found that about 35% of the male partners of women with abnormal PAP smears harboured HPV DNA.

The aim of this study was to optimise the effort in looking for HPV infection in partners of women with this infection. Since high-risk HPV is likely to be shed for long periods and also contributes to malignant transformation of the squamous epithelium of the lower genital tract, it was considered important to identify at young age patients carrying these HPV types in order to prevent further transmission of the infection as far as possible.

MATERIAL AND METHODS

Patients

Twenty-five women were selected for the study by one of the investigators (ER) from patients referred to the colposcopy clinic because of recently detected HPV infection or an atypical PAP smear. Contributory reasons for examining the female patients were complaints of dyspareunia, a symptom sometimes associated with subclinical HPV infection both in women (7) and men (8). Some relevant characteristics of the patients are shown in Table I.

Twenty-three women with pronounced cervical lesions and 2 women with distinctly outlined aceto-white patches localised in the posterior part of the introitus vaginae in whom intraepithelial neoplasia and/or high-risk HPV was present, were asked to participate in the study. Because of these findings, they were also asked to have their partner referred to the STD clinic for examination.

Cervical lesions were chosen because of the predominance of high-risk HPV types found there, and vulvar lesions with dysplastic changes because of the relative rareness of this condition in the studied age group. Young patients were selected for the study, in the hope of finding recently infected couples in whom further transmission would supposedly be more likely to occur and the virus would also be easier to detect. A steady sexual relationship for at least 3 months was also required, as the minimum incubation period for HPV infection is estimated to be 6 to 8 weeks.

Clinical examination and sampling

The male partners were examined at the STD clinic by one of the authors (AS). The first sample was taken from the distal part of the urethra (2–3 cm) with a plastic probe of the type generally used for collecting samples for light microscopy for diagnosis of urethritis. From the glans penis, sulcus, preputium and penile shaft, a sample of cells was collected with a Cytobrush® (Medscand AB, Sweden). These two

Table I. *Patient characteristics*

Characteristics		Women (*n*=25)	Men (*n*=25)
Age – range (years)		16–31	18–39
– mean		23	25
– median		22	24
Number of partners (in lifetime)	– range	unknown*	1–>40
	– median	–	7
Duration of partnership	– range (months)	3–96	
	– median	12	
Previous STD			
– condyloma acuminata		2 (9.1%)	4 (18.2%)
– chlamydial infection		4 (18.2%)	3 (13.6%)
Symptoms			
– dyspareunia		6 (27.3%)	1 (4.6%)

* information on number of partners available only for the male partners attending the STD clinic.

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Table II. *Results of clinical evaluation, histopathological examination and in situ hybridisation (ISH) of directed biopsies, and polymerase chain reaction (PCR) on biopsy specimens or cell samples in the 25 men*

Patient No.	Age yrs	No. of partners during lifetime	Previous genital HPV infection	Type of lesion			Histology		HPV type		
				acuminate	papular	subclinical	koilocytosis	PIN[2]	ISH	PCR/hybridisation biopsy	cell sample
1	21	3				x	x		**31/33/35**	nd	nd
2	28	>20				x	x	II	neg	**16**	nd
3	23	9			x	x	x		**31/33/35**	nd	nd
4	29	10					–		nd	nd	neg
5	18	unknown		x			x		**6/11**	nd	nd
6	22	unknown	x			x	x	II	**6/11**	nd	nd
7	21	9				x	x	I	**16**	nd	nd
8	20	>40	x		x		x	II	neg	**33**	nd
9	23	7		x		x	x		neg	**16**	nd
10	20	21				x	x		neg	neg	**18**
11	23	13				x	–		**16**	nd	nd
12	33	15					nd		nd	nd	neg
13	31	4				x	–		neg	**18**	nd
14	25	17					nd		nd	nd	neg
15	19	4			x		x		**6/11**	nd	nd
16	19	unknown					nd		nd	nd	**6**
17	25	3				[1]	–		neg	neg	neg
18	25	7				x	x		**6/11**	nd	nd
19	28	3				x	nd		nd	nd	neg
20	27	15	x			x	x		neg	neg	**18**
21	28	>10			x		x		neg	**pos**	nd
22	23	12				x	x		neg	neg	neg
23	39	15	x				nd		nd	nd	neg
24	31	12				x	x		**pos**	**16**	nd
25	32	>30			x		–		neg	neg	neg
Total			4	2	5	14 (15)	15	4	9	6	3

[1] = subclincal but not typical for HPV
[2] PIN = penile intraepithelial lesion

samples were placed together in one plastic tube containing Tris-HCl, EDTA-buffered saline.

A careful examination of the skin and mucous membranes was then performed, including application of 5% acetic acid for 2 min and peniscopy with a Zeiss colposcope. Lesions were classified as acuminate, papular or macular (subclinical). The subclinical lesions were considered typical for HPV infection if elevated with a well demarcated border, and with punctuated vessels, sometimes also with epithelial fissures, and as non-typical when they had no elevated border and only showed a scattered, dot-like aceto-whitening.

A biopsy specimen was obtained if any clinical abnormality was disclosed. This was placed in a 4% formalin saline buffered to pH 7.2, processed routinely and stained with haematoxylin and eosin for histological examination.

Histological examination

The histological criteria for HPV infection, i.e. koilocytosis, hyperplasia, parakeratosis, hyperkeratosis and papillomatosis and also for the two conditions claimed by Rock et al. (10) to be significantly related to the histology of HPV infection, namely inflammation and dysplasia, were all present to variable degrees. Only data for koilocytosis and dysplasia, i.e. dyskaryotic cells and, usually, atypical mitotic figures, indicating cervical (CIN) and penile intraepithelial neoplasia (PIN), are shown in Table II.

Detection of HPV DNA

In situ hybridisation (ISH)

All biopsies were first tested with ISH using a biotinylated *pan* probe (Omniprobe, Digene, Silversprings) that detects a broad spectrum of HPVs: 6, 11, 16, 18, 31, 33, 35, 42–44, 51, 52, 56 and 58 under conditions of low stringency. Sample handling, protease treatment, denaturation, hybridisation, posthybridisation and enzymatic detection were carried out as previously described (11, 12). The positive biopsies were typed with Digene's genomic, type-specific and biotinylated HPV probes 6/11, 16/18 and 31/33/35. All biopsies that did not show positive results with ISH were subsequently tested utilising the polymerase chain reaction, as were the cell samples in cases without clinical abnormatilies, i.e. when no biopsy was taken.

Polymerase chain reaction (PCR)

DNA preparation. Three 10-μm sections were placed in an Eppendorf tube and digested in 100–250 μl digestion buffer (200 μg proteinase K (Boehringer Mannheim, Mannheim, Germany) per ml lysis buffer: 50 mM Tris-HCL, pH 8.5, 1 mM EDTA, pH 8.0 and 10% Tween 20 non-ionic detergent) for 2 h at 65°C. The proteinase K was then destroyed by incubation at 95°C for 5 min and the tubes were centrifuged at 13,000 *g* for 5 min. For amplification 10 μl and 20 μl were used (13).

Amplification. This was performed in two ways (14). First, the consensus primer pair GP5 and GP6 (15), which spans nucleotides 6764 to 6883 within the L1 open reading frame (ORF) in HPV type 6b and the corresponding regions of the other genital HPVs, was used in a one-step PCR with the DNA extracted from the biopsies. Second, all negative cases were then subjected to a two-step PCR using the consensus primers MY09 and MY11 (16) as outer primers and GP5 and GP6 as inner primers. The former primer pair spans nucleotides 6722 to 7170 in HPV 6b and the corresponding regions in other HPVs. All biopsies

Table III. *Location of lesion and histopatholgical findings in the 25 women*

CIN = cervical intraepithelial neoplasia; VIN = vulvar intraepithelial neoplasia

Location of lesion	*n*	HPV neg	HPV type		
			6/11	31/33/35	16/18
Cervical lesions					
HPV infection	5		–	1	4
CIN I+II	11	1	–	2	8
CIN III	7	1	1	–	5
Vaginal lesions					
HPV infection	–		–	–	–
VIN I+II	1		–	–	1
VIN III	1		–	–	1
Total	25	2	1	3	19

were also amplified with the β-globin primers PC03 and PC04 (17) to exclude false negative results.

For a one-step PCR the following was mixed in a 500-μl tube up to the final volume of 50 μl: 25 pmol of the GP5 and GP6 primers, 50 mM KCl, 10 mM Tris-HCL pH 8.3, 3.5 mM $MgCl_2$, 200 μM of each dNTP, 0.01% gelatin, 2 U *Taq*polymerase (Perkin Elmer Cetus at La Roche) and 10–20 μl DNA. The mixture was overlaid with 50 μl mineral oil (Sigma) and subjected to 40 amplification cycles in a Techne PHC-3 heating block according to the following profile: initial denaturation at 95°C for 3 min, thereafter 1 min; annealing at 40°C for 2 min and elongation at 72°C for 1.5 min. The final elongation step was prolonged to 5 min.

For a two-step PCR, 0.2 pmol of the MY09 and MY11 primers and 10–20 μl DNA were added to the amplification buffer as described above and a total of 20 cycles were run as follows: denaturation at 95°C for 30 s, annealing at 45°C for 30 s and extension at 72°C for 30 s. A total of 20 pmol of the GP5 and GP6 primers was then added together with 2 U *Taq*polymerase to the amplification mixture and 30 additional cycles were performed.

All PCRs were carried out that every tenth sample was a negative control. All such controls were negative. As positive controls, we used previously positive, clinical biopsy samples.

Detection and slot blot hybridisation. For detection, 10 μl of the PCR product were subjected to 1.5% agarose gel electrophoresis in Tris-borate buffer and ethidium bromide. For HPV-typing, 5 μl of the PCR product were blotted and hybridised as described earlier (14), testing for HPV 6, 11, 16, 18, 31, 33 and 35.

RESULTS

Of the 25 women with distinct cervical or vulvar lesions who served as index patients, 20 had neoplastic changes, 18 CIN and 2 vulvar intraepithelial neoplasia (VIN) (Table III). The 5 women without squamous intraepithelial neoplasia all harboured high-risk HPV.

The median age of the 25 males was 24 years (range 18–39 years). The number of sexual partners during the men's lifetime varied from 1 to >40. The duration of the present sexual relationship ranged from 3 to 96 months (median 12 months). Of the male partners 4 (18%) had a medical history of genital HPV infection and 3 (14%) had experienced at least one episode of chlamydial infection (Table I).

The complete results of the clinical and histopathological evaluations and the screening for HPV DNA in the partners are summarised in Table II. Peniscopy revealed lesions strongly suggestive of HPV infection in 80%; these were most frequently subclinical (15/25; 60%), followed by papular (5/25; 20%) and acuminate (2/25; 8%). Altogether 20 males thus showed clinical features suggestive of HPV infection, but only 3 men were aware of any penile lesions. Not having suspected genital warts or anything that could be referred to as an STD, they had not sought medical care until advised to do so by their partner. Histopathological examination revealed lesions conclusive for HPV infection, e.g. koilocytosis in 15 cases (79%), and PIN in 4 (21%). HPV DNA was detected in 18 of the 25 males (72%). Nine of these 18 cases were detected by the less sensitive ISH and another 9 with PCR, 6 in biopsy tissue samples and 3 in cell samples.

The concordance of HPV types between the partners is shown in Table IV. Eight of the 25 partners (32%) harboured the same HPV type. When a comparison was made regarding high-risk HPV carriage as such, without consideration of HPV type, corresponding results were found in 10 out of 25 couples (40%).

DISCUSSION

Several investigators have studied the presence of HPV in male partners of women with the infection. Mostly subclinical lesions have been observed in the males (3–5, 18). The correlation between men and women, regarding HPV infection without consideration of HPV types, has varied between 50% and 90% in different studies. With respect to concordance of HPV types in infected couples, lower figures have been found ranging from 12.5% (19) to 23% (6). In the study reporting the lower rate of identical HPV types, variants of the HPV 16 DNA were observed on combining PCR with sequencing, a very promising method for detecting variability in the pathogen under study. With this approach the route of transmission can be traced for any organism, such as the human immunodeficiency virus in a

Table IV. *Concordance of HPV type in 25 women and their partners*

HPV detection in women	*n*	HPV detection in men				
		Negative	6/11	31/33/35	16/18	Unknown (HPV"X")
Negative	2	1	–	–	1	–
6/11	1	1	–	–	–	–
31/33/35	3	–	1	**1**	–	1
16/18	19	6	4	**2**	**7**	–
Total	25	8	5	3	8	1

dental clinic (20). This subtyping of the detected virus was not performed in the present study.

In the above cited studies the mean ages of the patients and their partners were higher, 33 years in the males of the study by Barasso et al. (3) and 30 years in the women and 37 years in the men in that by Schneiders et al. (18), compared with the mean ages of 23 years in women and 25 years in men in our material. STDs are more frequent at a younger age, for which reason we chose to investigate somewhat younger couples. At a younger age the HPV infection may be assumed to have been more recently acquired and not reactivated. A latent infection that is reactivated would rather point to discordant HPV types in the couples, reflecting acquisition of the virus in a previous sexual relationship.

In our study we focused attention on high-risk HPV carriers, considering this to be cost-effective, even though classical genital warts (condyloma acuminatum), which are usually caused by low-risk HPV in males, seem to be a contributory factor for malignancies of the lower genital tract in the female partner (21). That finding could be a marker for the HPV types with which the women had been infected in previous sexual encounters but which had not been detected at the time of the investigation.

Our results show 32% concordance with respect to HPV type, and 40% concordance when high-risk carriers were considered as one group. Despite the low power of our study due to the small number of patients, an effect of the strict exclusion criteria with respect to age and HPV type, we believe the results to be of interest. Our finding of a rather low rate of accordance in HPV type in young couples with a clinical infection supposedly quite recently acquired gives rise to some considerations.

It may be speculated whether, when transmitted, several types of virus can be shed and inoculated at the same time. The amount of different types of virus shed, the local immunoresponse and presumably other factors will all influence the outcome of this process and the question as to which virus will be detected at a given time of sampling. It has been shown that detected HPV types vary over time and that viral shedding is transient (2, 22). Another possibility is that the detected virus has not been transmitted within the ongoing sexual relationship but has only been reactivated as a predominantly subclinical infection.

In our study the number of male partners with detected HPV DNA (68%) was higher than expected in view of the prevalence of HPV infection found in this age group and in this setting, approximately 30% (23). Through our highly selected group of HPV-infected women we have identified a group of men carrying HPV infection of a high-risk type of which they were unaware and therefore also unintentionally at risk of transmitting to a new partner, something men are known to fear (24).

As in other studies undertaken on subclinical penile lesions, a relatively high proportion of neoplastic changes was revealed, 21%. This is in accordance with the report by Löwhagen et al. (25), who diagnosed dysplasia in 29% of male patients with mostly macular lesions and Barasso et al. (3), who found features of intraepithelial neoplasia in 24% of the biopsied male partners to women with CIN.

The epidemiological aspects of HPV infection have to be considered, at least regarding the high risk types. The question arises whether HPV should be screened for like chlamydial infection. There are objections to screening for HPV in general (26) but with a targeted screening like ours, aimed to find carriers of high-risk HPV, we believe this to be more effective than blind screening of special age groups or STD clinic attendants.

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