

Subclinical penile human papillomavirus infection and dysplasia in consorts of women with cervical neoplasia

M J CAMPION,* D J McCANCE,† H S MITCHELL,* D JENKINS,‡ A SINGER,* J D ORIEL§

From the *Department of Gynaecology, Royal Northern Hospital; the †Department of Microbiology, United Medical and Dental Schools of Guy's and St Thomas's Hospitals; the ‡Department of Pathology, Whittington Hospital; and the §Department of Genitourinary Medicine, University College Hospital, London

SUMMARY Fifty men whose sexual partners were 50 women with histologically proved cervical intraepithelial neoplasia (CIN) grade III (severe dysplasia or carcinoma in situ) were studied. A further 25 men whose current regular sexual partners were 25 women with chlamydial cervicitis were recruited as controls. If either of the partners in either group had genital condylomata acuminata or a known history of similar lesions, the couple was excluded from the study.

Abnormal penile epithelium, which was detected by colposcopy after application of 5% acetic acid to the penile skin, was reported in 25 men in the study group compared with three in the control group. Histologically proved subclinical penile infection with human papillomavirus (HPV) was present in 23 men in the study group compared with three in the control group ($p < 0.01$). Of the 50 men in the study group, four had histologically proved severe penile dysplasia or carcinoma in situ with evidence of HPV infection, the disease being subclinical in each case and diagnosed on histology of a specimen obtained by colposcopically directed biopsy.

HPV DNA was detected on filter hybridisation of penile scrapes from 15 of the 23 men in the study group with histologically proved penile HPV infection, HPV16 DNA being detected in 10 of them. HPV DNA was detected on DNA-DNA hybridisation of biopsy material in seven of 18 men with histologically proved penile HPV infection. Five of these biopsy specimens were positive for HPV16 DNA. Only one man in the control group had HPV DNA detected in a penile scrape. This patient had histologically proved subclinical penile HPV infection. Such lesions may represent an important male reservoir of HPV types implicated in genital squamous carcinogenesis in both sexes.

The sexually transmitted nature of cervical squamous neoplasia is strongly suggested by epidemiological and demographic studies. This has led to the concept of a man at high risk who places his female sexual partner at increased risk of cervical neoplasia.¹ Specific features of such men have been described. Appreciably more women who are the wives of men with penile cancer die of cancer of the cervix.² Kessler showed a fourfold increased risk of cervical neoplasia in women whose husbands had, at some time, been married to another woman who developed cervical neoplasia.³ Neighbourhood clusters of cervical neoplasia have been reported in association with cohabitation relationships between affected women and specific

men.⁴ Buckley *et al* reported that husbands of monogamous women with cervical neoplasia had an above average number of sexual partners and had experienced first coitus at an early age.⁵ The importance of sexual behavioural characteristics of men in relation to the risk of cervical disease was underlined by Zunzunegui *et al*, who showed that women with cervical neoplasia were five times more likely to be married to a man who had had more than 20 sexual partners than were women with a normal cervix.⁶

Traditional demographic studies have correlated cervical neoplasia with the occupation of the husband. Highest standardised mortality rates for cervical cancer are found in wives of men whose occupations entail travel and absence from home for extended periods,⁷ a factor long known to be associated with a high risk of sexually transmitted disease (STD).⁸ Mortality from cervical cancer follows time trends in

Address for reprints: Dr J D Oriel, Department of Genitourinary Medicine, University College Hospital, Gower Street, London WC1E 6AU

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incidence of STD in men.⁷ Skegg postulated that men who have associated with prostitutes, a known reservoir of STD, place their wives at an increased risk of cervical cancer.⁹ He suggested that this explained the high incidence of cervical cancer in certain South American countries, such as Colombia, where women were strongly discouraged from having extramarital sexual relationships whereas men were expected to have many.

This evidence suggests that certain men may harbour some sexually transmitted agent associated with cervical neoplastic transformation. Recent research strongly implicates specific sexually transmitted types of human papillomavirus (HPV) in causing cervical cancer.^{10,11} HPV types 6 and 11 are considered to be the causative agents of the benign genital proliferations, condylomata acuminata, in men and women.¹² Malignant conversion of such lesions is rare, although notable exceptions have been reported.¹³⁻¹⁶ Women who are the sexual partners of men with penile condylomata acuminata have, however, been shown to be at increased risk of cervical neoplasia.¹⁷ Specific types of the heterogeneous group of HPVs are considered to confer high risk of neoplastic transformation. HPV types 16 and 18 were originally isolated direct from cervical cancer biopsy specimens,^{18,19} and HVP16 is detected in most biopsy specimens from patients with cervical neoplasia.^{18,20}

Most infection of the female genital tract with high risk HPV types is now thought to be subclinical, detected only by colposcopy after application of acetic acid. Despite the evidence implicating these specific HPV types in cervical carcinogenesis, and the apparent role of men in causing this disease, genital HPV infection in men has not been thoroughly investigated. In particular, the male reservoir of HPV types detected in cervical neoplasia is poorly understood. We now report the prevalence of subclinical penile HPV infection and associated HPV types in men who are the regular sexual partners of women with histologically proved cervical intraepithelial neoplasia grade III (CIN III) (severe dysplasia or carcinoma in situ). Subclinical penile HPV infection may represent an appreciable reservoir of HPV types implicated in cervical neoplasia.

Patients and methods

STUDY GROUP

We recruited 50 men, each of whom was the current regular sexual partner of a woman attending the colposcopy clinic of the Royal Northern Hospital, London, for management of histologically proved CIN III, which had been diagnosed according to the criteria of Buckley *et al.*²¹ Histological evidence of HPV infection according to criteria defined by Dyson

*et al.*²² was present in 34 of the 50 specimens obtained by colposcopically directed cervical biopsy.

The median age of the women was 25 (range 17-34) years, and the median age of the male partners was 27.5 (range 21-38) years. The median duration of the sexual relationship was 2.5 years. If either partner had, or had a history of, genital condylomata acuminata the couple was excluded from the study.

CONTROL GROUP

We recruited a control group of 25 men, each the current regular sexual partner of a woman with chlamydial cervicitis that had been diagnosed by enzyme immune assay of an endocervical swab by the genitourinary medicine department of University College Hospital, London. Women who had, or had a history of, an abnormal cervical smear or colposcopy were excluded. If either partner had, or had a history of, genital warts the couple was excluded from the study.

The median age of the women was 23 (range 18-29) years, and the median age of the male partners was 26 (range 19-32) years. The median duration of the sexual relationship was 3.0 years.

INVESTIGATIONS

A medical, social, and sexual history was obtained from each man in both the study and the control groups. Each man was questioned regarding a history of STD, particularly genital condylomata acuminata. Urethral specimens were collected with a plastic loop for Gram staining and culture for *Neisseria gonorrhoeae*, and an endourethral specimen was obtained from each man to detect *Chlamydia trachomatis* by enzyme immune assay (Chlamydiazyme, Abbott). A specimen of urine was examined for albumin and sugar. Blood was taken for serological tests for syphilis.

PENILE COLPOSCOPY

Using the Zeiss OPMI 1 colposcope at $\times 10$ and $\times 16$ magnifications, we examined the penile epithelium, including that of the distal urethra, of each man in the study and control groups. The penile skin was first examined for the presence of abnormal epithelial vascular patterns, particularly punctation, as indicators of possible subclinical HPV infection. It was then swabbed with 5% acetic acid with a large soaked cotton wool swab and carefully examined again with the colposcope. Areas of penile skin that became white after application of acetic acid (acetowhite epithelium) were mapped, photographed, and biopsied.

PENILE SKIN BIOPSY

We performed biopsies of the penile skin, under local anaesthesia, from each man in whom subclinical penile

Table 1 Incidence of colposcopic penile atypia and histology results of specimens obtained by colposcopically directed biopsies

	Study group (n = 50)	Control group (n = 25)
Acetowhite epithelium	25	3
Histology results:		
Normal	2	0
HPV infection alone	13	2
HPV infection with atypia	6	1
Severe dysplasia or carcinoma in situ with evidence of HPV infection	4	0

Study group = sexual partners of 50 women with cervical intra-epithelial neoplasia grade III (CIN III) (severe dysplasia or carcinoma in situ).

Control group = sexual partners of 25 women with chlamydial cervicitis.

lesions were detected. We injected 1% xylocaine at the site of specific lesions using a dental syringe with a 26 gauge needle. We took a small specimen of the atypical penile skin with either a 2 mm Keyes dermatological punch or Eppendorf biopsy forceps, and applied ferric subsulphate (Monsel's solutions) to the biopsy site to secure haemostasis. The biopsy specimens were fixed in formol sublimate for histological examination. From 18 men in the study group who had more

Campion, McCance, Mitchell, Jenkins, Singer, Oriol extensive subclinical acetowhite epithelium, we took a further specimen from an adjacent area and snap froze it in liquid nitrogen for DNA-DNA hybridisation.

DNA-DNA HYBRIDISATION OF PENILE SCRAPES

A sample of superficial penile epithelial cells was obtained from each man in the study and control groups before colposcopic examination. The entire penile skin, including the foreskin and the glans, was scraped vigorously with a plastic spatula. The cells were washed in phosphate buffered saline (PBS). Epithelial cells from the distal urethra were sampled using the Mediscan cytobrush (Colgate) and also washed in PBS. Filter DNA-DNA hybridisation using the methods described by McCance *et al.*³ was performed on the penile scrapes to identify associated HPV types. The filters were hybridised against linear HPV6 and 16 DNA labelled with radiophosphorus (³²P) under moderately stringent conditions (melting temperature (T_m) - 25°C), but washed at high stringency (T_m - 12°C).

DNA-DNA HYBRIDISATION OF PENILE BIOPSY SPECIMENS

Total DNA was extracted from the biopsy specimens by the method described by Heritage *et al.*²⁴ The DNA

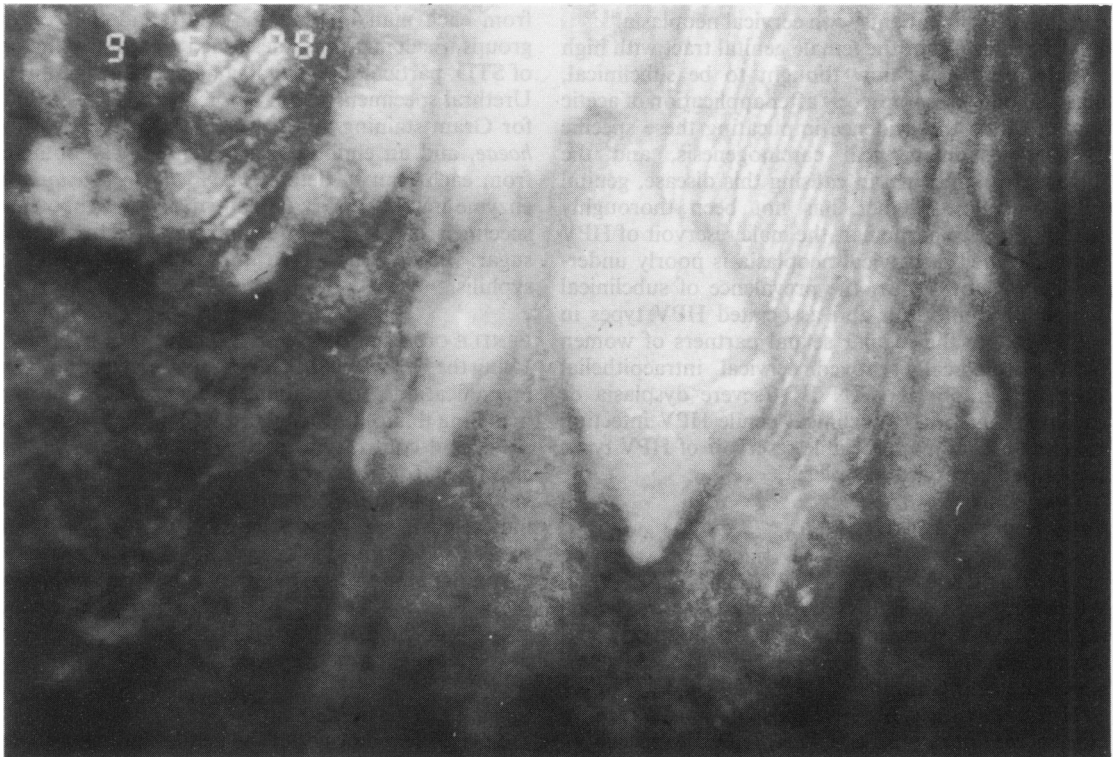


Fig 1 Colpophotograph showing subclinical penile acetowhite epithelium on the shaft of the penis.

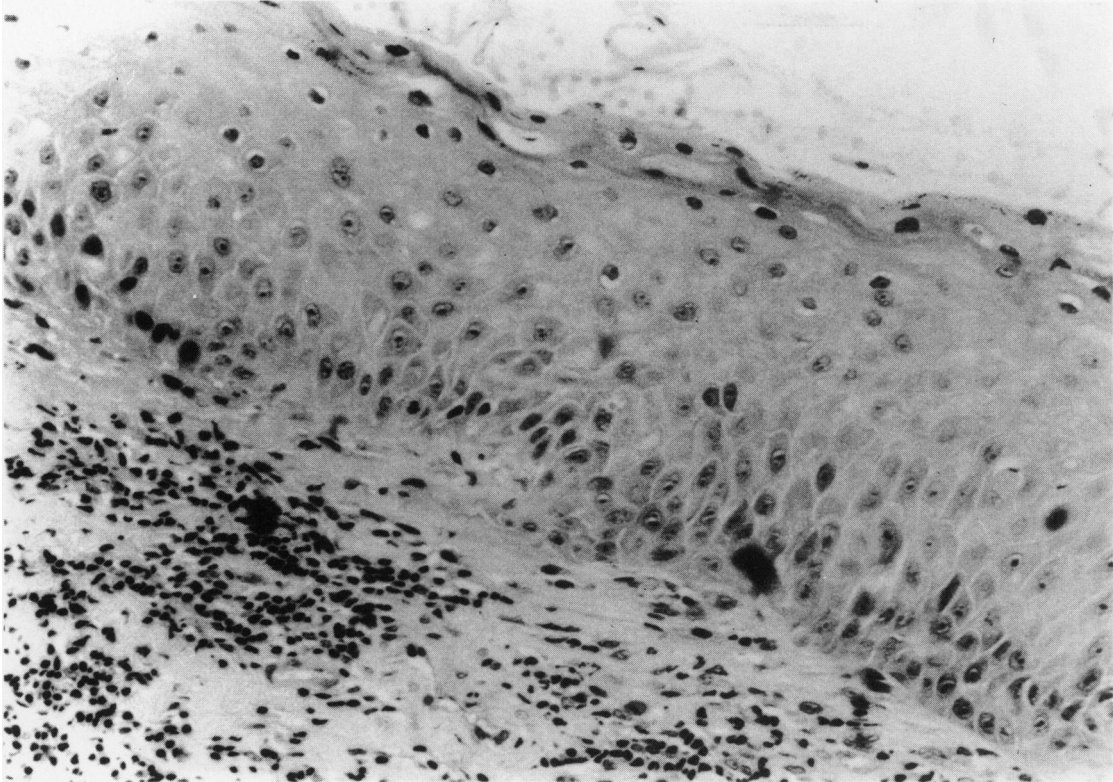


Fig 2 Histology of biopsy specimen from subclinical penile lesion reported as showing evidence of HPV infection alone.

was then transferred to nitrocellulose filters by the Southern blot method.²⁵ Hybridisation of the nitrocellulose filters was performed at low stringency ($T_m - 40^\circ\text{C}$) with either HPV6, HPV16, or HPV31 linear probes labelled with ^{32}P and then at high stringency ($T_m - 10^\circ\text{C}$) and re-exposed to Fuji x ray film. This gave a means of detecting HPV6, 16, and 31 related species as well as prototype viral DNA. The HPV6 probe was a gift from Drs L Gissmann and H zur Hausen. HPV16 had been cloned by one of us (DJMcC) from a CIN III lesion. The HPV31 probe was a gift from Dr A Lorincz.

Results

MICROBIOLOGY

One of the 50 men in the study group was diagnosed as having *N gonorrhoeae* infection. *C trachomatis* was not detected in endourethral swabs from the men in the study group, but 16 of the 25 men in the control group, whose regular sexual partners had chlamydial cervicitis, were diagnosed as having chlamydial urethritis.

PENILE COLPOSCOPY

Table 1 shows the prevalence, in the study and control groups, of subclinical penile lesions diagnosed as acetowhite epithelium after application of 5% acetic acid. Of the 50 men in the study group, 25 had flat penile lesions that became white after application of acetic acid and that were not clinically apparent before colposcopy. Only three of the 25 men in the control group had similar lesions. Abnormal capillary patterns could be detected on the penile skin in 11 men in the study group before acetic acid was applied. The addition of acetic acid, however, produced prominent whitening that permitted diagnosis. None of the control group men had visible penile lesions before the application of acetic acid. Figure 1 shows an area of acetowhite epithelium on the shaft of the penis. This lesion was not detected before acetic acid was applied to the penile skin.

HISTOLOGY

Table 1 also shows the histology results of biopsy specimens from the acetowhite penile epithelium, which were classified as normal; showing HPV infection alone, characterised by koilocytotic atypia and

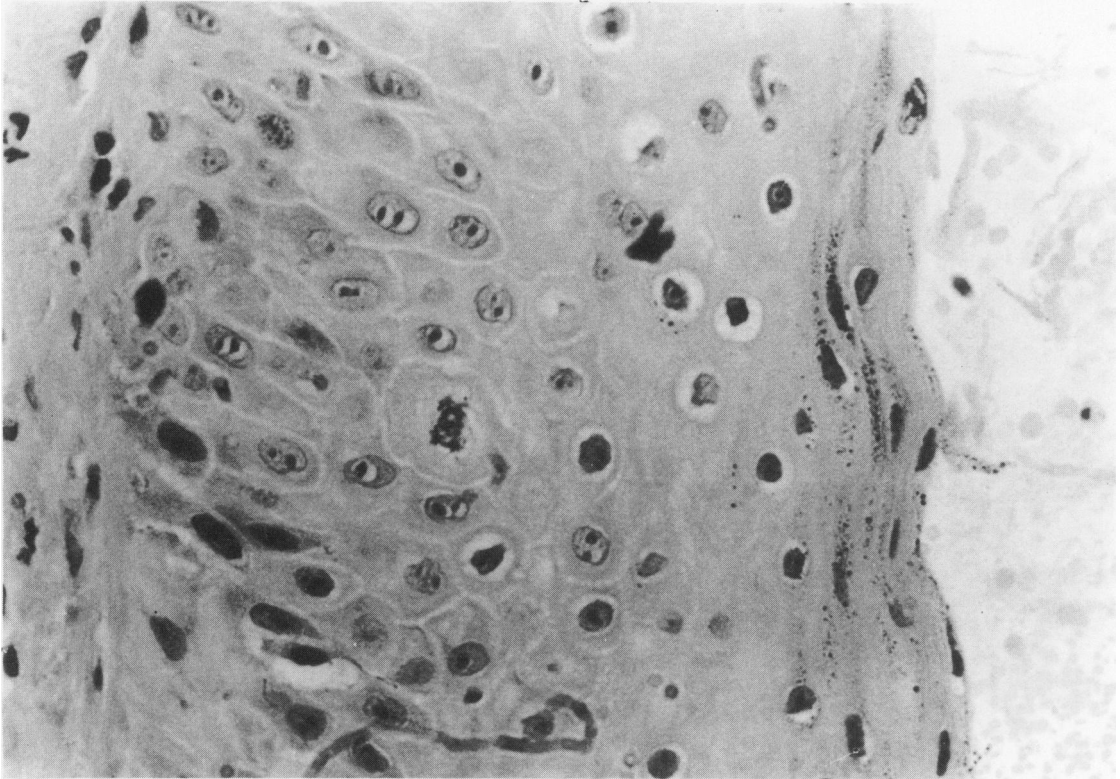


Fig 3 *Histology of biopsy specimen from subclinical penile lesion reported as showing evidence of HPV infection with epithelial atypia not amounting to severe dysplasia.*

dyskeratosis (fig 2); showing HPV infection with atypia, characterised by koilocytotic atypia with basal hyperplasia, basal nuclear abnormalities, and abnormal mitotic figures (fig 3); or showing severe dysplasia or carcinoma in situ of the penile epithelium (fig 4).

Of the 50 men in the study group, 23 had histologically proved penile HPV infection with or without more severe epithelial transformation. The lesions were in each case detected only by colposcopy after application of acetic acid, and HPV infection in each case was diagnosed by histology of a specimen obtained by colposcopically directed biopsy. Subclinical HPV infection was confirmed by histology in only three of the 25 men in the control group ($p < 0.01$).

Four of the 50 men in the study group had histologically confirmed severe dysplasia of the penile skin, which amounted to carcinoma in situ in three cases (figs 4 and 5). The area of penile atypia in each case could not be detected readily before acetic acid was applied.

DNA-DNA HYBRIDISATION OF PENILE SCRAPES

Table 2 shows the results of DNA-DNA hybridisation

of desquamated epithelium harvested by penile scraping. In the study group, 15 of the 23 men with histologically proved penile HPV infection were positive for HPV DNA, 10 of whom were positive for HPV16. Penile scrapes from the two men with colposcopically detected lesions that were reported as normal on histology (table 1) were both negative for HPV DNA. Three of the four men with penile severe dysplasia or carcinoma in situ (table 1) had penile scrapes positive for HPV16 DNA. Of the 25 men in the study group who had neither clinical nor colposcopic evidence of penile disease, four (16%) were positive for HPV DNA but only one was positive for HPV16.

In the control group of 25 partners of women with chlamydial cervicitis, only one was positive for HPV DNA. This man had histologically proved penile HPV infection with atypia, and HPV16 DNA was detected.

DNA-DNA HYBRIDISATION OF BIOPSY SPECIMENS

HPV DNA was detected by DNA-DNA hybridisation in seven biopsy specimens from 18 men in the study group who had more extensive areas of penile aceto-white epithelium, although HPV infection with or without epithelial atypia was confirmed on histology

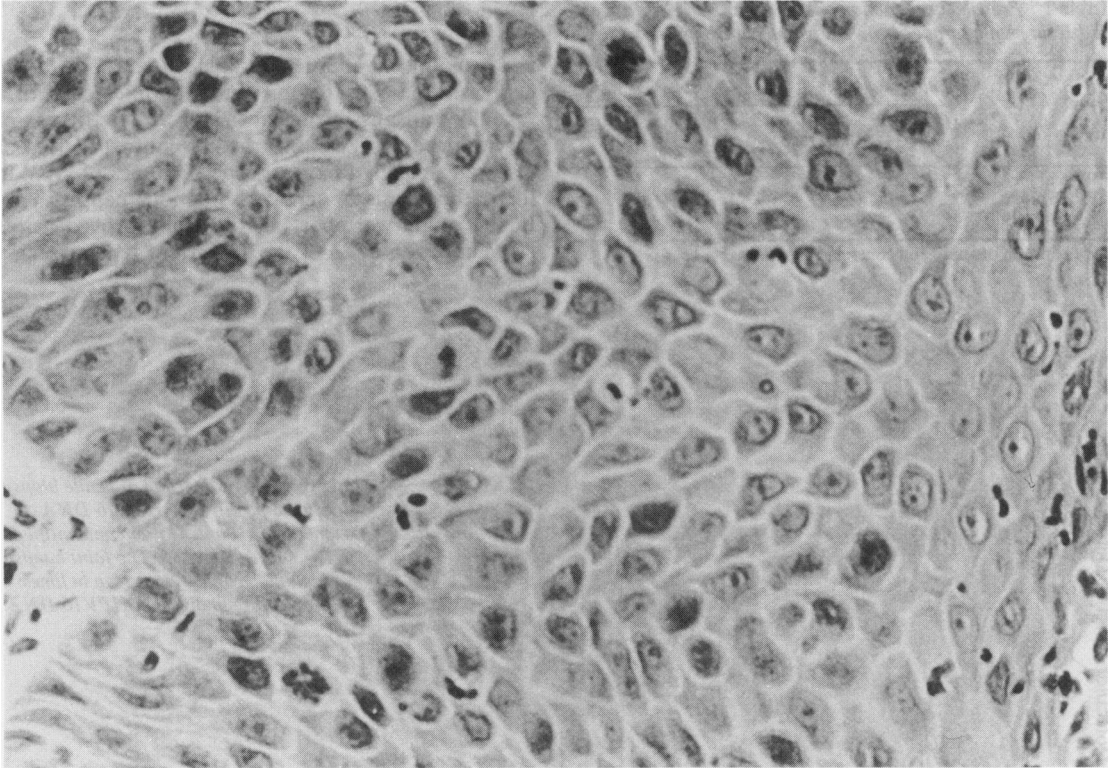


Fig 4 *Histology of biopsy specimen from subclinical penile lesion reported as showing appreciable epithelial dedifferentiation amounting to severe dysplasia.*

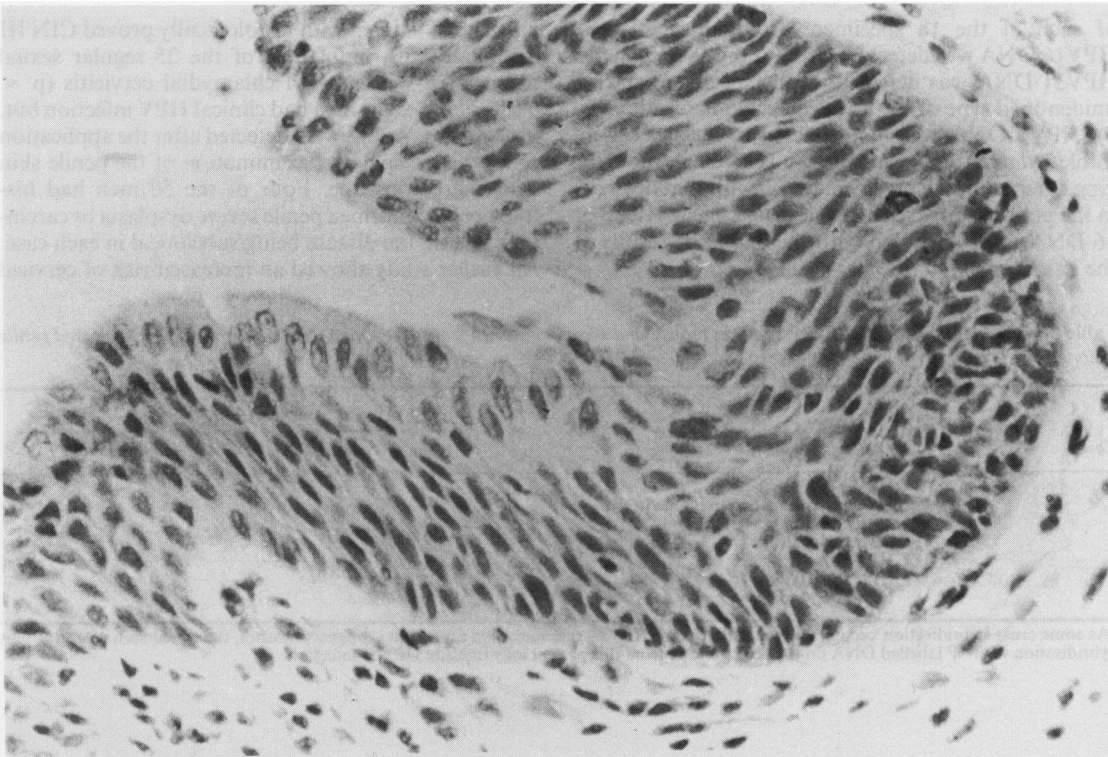


Fig 5 *Histology of a biopsy specimen from subclinical penile lesion showing full thickness dedifferentiation of epithelium diagnosed as penile carcinoma in situ (penile intraepithelial neoplasia).*

Table 2 Prevalence of human papillomavirus (HPV) types 6 and 16 in penile scrapes from study and control groups

	Study group with:		Control group with:	
	HPV (n = 23)	No HPV (n = 27)	HPV (n = 3)	No HPV (n = 22)
HPV 6 alone	5	3	0	0
HPV 16 alone	7	1	1	0
HPV 6 and 16	3	0	0	0

For definition of study and control groups see table 1.

Discussion

Epidemiological and demographic studies of cervical neoplasia indicate that men may be infected with a sexually transmissible agent that increases the risk of cervical neoplasia in their sexual consort.²⁻⁹ Recent research strongly implicates specific types of HPV in the pathogenesis of cervical squamous neoplasia.^{10,11} In this study we report histologically proved penile HPV infection in 23 of the 50 current regular sexual

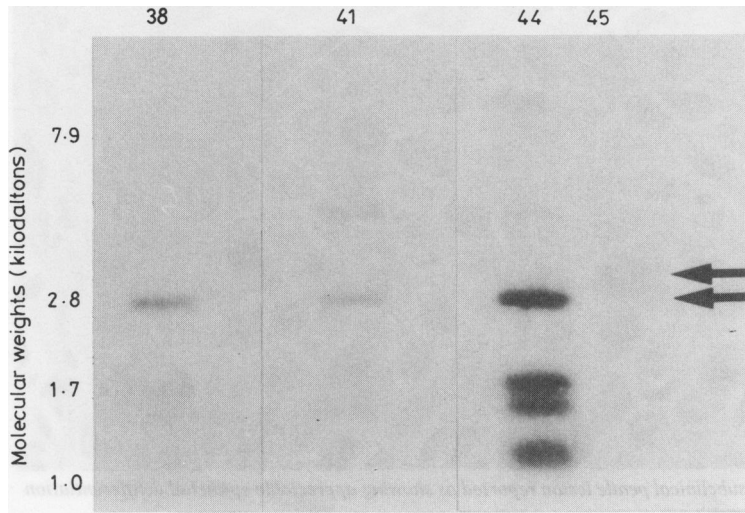


Fig 6 Autoradiograph of penile biopsy specimen positive for HPV16 DNA (lanes 38, 41, and 44) and unidentified HPV DNA (lane 45). The faint bands of high molecular weight seen in lanes 41 and 44 are undigested HPV16 DNA.

of each of the 18 specimens from adjacent sites. HPV16 DNA was detected in five of the 18 specimens. HPV31 DNA was detected in one specimen, and an unidentified type of HPV was detected in another (fig 6). HPV6 DNA was not detected in any specimen. Table 3 summarises these data and correlates the HPV type detected in the biopsy material with that detected in the penile scrape (using ³²P labelled linear HPV6 or 16 DNA as probes) and with the histology results of the lesion.

partners of women with histologically proved CIN III compared with only three of the 25 regular sexual partners of women with chlamydial cervicitis (p < 0.01). None of the men had clinical HPV infection but, in each case, lesions were detected after the application of 5% acetic acid and examination of the penile skin with the colposcope. Four of the 50 men had histologically confirmed penile severe dysplasia or carcinoma in situ, the disease being subclinical in each case. An earlier study showed an increased risk of cervical

Table 3 Histology results and HPV types in biopsy and scrape material from seven men with colposcopically diagnosed penile lesions

Case	Histology results	HPV types in:	
		Biopsy (Southern blot)	Scrape (Dot blot)
1	HPV alone	16	6 and 16
2	Severe dysplasia or carcinoma in situ	16	16
3	HPV and atypia	16	6
4	Severe dysplasia	16	6 and 16
5	HPV alone	16	16
6	HPV and atypia	31	16*
7	HPV and atypia	Unidentified	None

*As some cross hybridisation occurs between HPV16 and HPV31 genomes even after high stringency washes, the signal seen after dot blot hybridisation with ³²P labelled DNA on the penile scrape from this patient may indicate HPV31 infection.

HPV infection and CIN in women exposed to genital HPV infection by sexual intercourse with men with penile condylomata acuminata.¹⁷ Viral transmission between the sexes was also shown. The current study, however, shows the high incidence of subclinical penile HPV infection and penile epithelial atypia in the absence of clinical HPV infection in men recruited as partners of women with CIN. It shows for the first time the high prevalence of HPV16 in association with penile subclinical HPV infection.

Of the 23 men in the study group with histologically proved HPV associated penile epithelial atypia, HPV DNA was detected in filter hybridisation of a penile scrape in 15, HPV16 DNA being detected in 10 including three of the four men with histologically proved penile severe dysplasia or carcinoma in situ. Of the 27 men in the study group with no histological evidence of penile atypia, four were positive for HPV DNA and only one of them was positive for HPV16 DNA. Only one man in the control group of 25 men had HPV DNA detected in a penile scrape. This patient had histologically proved subclinical penile HPV infection, and HPV16 DNA was isolated from the penile scrape.

Of the 25 men in the study group with subclinical penile acetowhite epithelium, 18 had lesions sufficiently large to permit two adjacent biopsy specimens to be taken for histology and DNA-DNA hybridisation. HPV DNA was detected in seven, although histological evidence of penile HPV infection with or without epithelial atypia was reported in each of the 18 cases. HPV16 DNA was detected in five biopsy specimens including two from patients reported histologically as having severe dysplasia. HPV31, a type of virus detected rarely in cervical neoplasia biopsy specimens in the United Kingdom but in up to 20% of CIN specimens in North America, was detected in one patient. An unidentified HPV type, which cross hybridised with HPV16 probe at low stringency, was detected in another. The detection of unidentified HPV types in lesions associated with penile HPV has been reported previously,²⁶ and may partly explain the relatively low incidence of specific HPV DNA in the penile biopsies performed in this study.

The classic genital lesion associated with sexually transmitted HPV infection in both sexes is the condyloma acuminatum. The reported incidence of condylomata acuminata has increased appreciably in both sexes in the past decade.²⁷ Vulval condylomata acuminata are associated with an increased risk of preinvasive cervical disease,²⁸ and a man with penile condylomata acuminata appears to place his female sexual partner at increased risk of cervical neoplasia.¹⁷ Condylomata acuminata are, however, characteristically associated with HPV6 or 11 infection.¹² HPV16, the viral type detected most often in CIN and cervical

squamous carcinoma,^{18,20} is rarely detected in condylomata acuminata.²⁹

Concomitantly with the increased diagnosis of clinical genital HPV infection the diagnosis of cervical neoplasia has increased, particularly in young women. Histological or molecular biological evidence of HPV infection is present in over 80% of CIN biopsy specimens. This HPV infection is usually subclinical, and HPV16 DNA is often isolated from both biopsy and cytological specimens. The high incidence of cervical disease associated with HPV16 suggests great disparity between the sexes in the reservoir of high risk HPV types, as no similar reservoir has been reported previously in men.

The apparent difference between the sexes in the incidence of genital HPV infection, the association between genital HPV infection in men and cervical neoplasia, and the incidence of subclinical genital HPV infection in women led to the use of the colposcope to screen the sexual partners of women with CIN.³⁰ This permitted the more accurate detection of small condylomata acuminata and other clinical lesions associated with HPV. The addition of acetic acid to the penile epithelium and colposcopic examination, however, permits the detection of subclinical lesions that appear as white epithelium, often with abnormal capillary patterns, on previously normal non-elevated skin.

In this study, 23 of the 50 regular sexual partners of women with CIN III had histologically proved subclinical penile HPV infection; HPV16 DNA was detected in the penile scrapes of 10 of these men and in five out of 18 biopsy specimens from such subclinical lesions. The strong association of subclinical penile HPV infection with CIN in the men's sexual partners and the high prevalence of HPV16 in this group suggests that these lesions may represent an appreciable reservoir in men of the high risk types of HPV implicated in cervical neoplasia.

We do not know the malignant potential of the penile intraepithelial atypia reported in this study, which amounted to severe dysplasia or carcinoma in situ in 8% (4/50) of men in the study group. Penile carcinoma has been reported in association with recalcitrant penile condylomata acuminata, and both these conditions are associated with an increased risk of cervical neoplasia in female sexual partners.^{2,17,31} McCance *et al* reported detecting HPV16 DNA in 49% of penile carcinomas and HPV18 DNA in a further 9%.³² Though penile carcinoma remains uncommon in Western communities, the finding of penile severe dysplasia or carcinoma in situ in 8% of the sexual partners of women with CIN III may be a cause for concern. The oldest of the men with severe penile epithelial disease was 32. An association between genital squamous carcinoma in male and female

sexual partners is established. Studies from Puerto Rico² and New York State³³ of men with penile cancers have shown significantly more cases of cancer of the cervix in their marital partners. This was not the case for cancers at other sites. Smith *et al* reported the mortality of women married to men with penile cancer in England and Wales.³⁴ There was a slight excess of deaths from cancer among these women, but the only site for which there was a significant excess was the cervix. An increased risk of cervical neoplasia in the sexual partners of men with penile carcinoma in situ has been reported.²⁶

Despite dissimilarities in the epidemiology of penile and cervical squamous cancer, there is strong evidence that the two diseases have a common aetiology. The detection of histologically proved penile severe dysplasia or carcinoma in situ in 8% of sexual partners of women with similar cervical disease in this study provides further evidence. The association of pre-invasive and invasive genital squamous neoplasia in both sexes with specific sexually transmitted HPV types suggests that this virus may be important in genital carcinogenesis. So that these findings do not alarm the spouses of patients with genital neoplasia, however, it is important to note that cancer of the penis remains a rare disease, affecting about 250 men each year in England and Wales. A threefold increased risk of cancer of the penis in men whose wives have had cervical cancer represents a very small absolute increase.³⁴ Of the 4000 new cases of cancer of the cervix uteri diagnosed each year in England and Wales, only about 10 or 11 are likely to be associated with cancer of the penis in the husband.

In considering the aetiology of cervical neoplasia, the greatest enigma is the role of men in this process.³⁵ This study suggests that subclinical penile HPV infection provides an important reservoir for high risk types of HPV that are implicated in cervical and, indeed, penile carcinogenesis and may define the man who places his female sexual partner at high risk for genital neoplasia. Multidisciplinary co-operation is required to assess the prevalence and natural history of subclinical genital HPV infection, associated HPV types, risk of neoplasia, and appropriate management.

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