A colposcopic case-control study of cervical squamous intraepithelial lesions in women with anogenital warts

B A Evans, R A Bond, K D MacRae

Abstract

Objective—To assess whether anogenital warts, present or past, are an indication for women to be referred for colposcopy.

Design—A case control study comparing patients with and without a history of anogenital warts.

Setting—A department of genitourinary medicine in West London.

Patients—468 patients examined by colposcopy between January 1985 and December 1987 of whom 147 (31%) had abnormal cytology, 163 (35%) had anogenital warts and 158 (34%) had both.

Main outcome measures—Colposcopic findings and histology of cervical biopsies compared with behavioural and disease variables.

Results—Human papillomavirus infection (HPVI) of the cervix showed no relationship with a life time history of vulval warts, or with the presence of anogenital warts on clinical examination, or with any parameter of sexual behaviour included in the study. Cervical intraepithelial neoplasia (CIN) was strongly associated with current IUCD usage ($RR = 7.75$) and coitarche under 16 years of age ($RR = 3.72$), but a history of vulval warts yielded a negative association ($RR = 0.34$), suggesting a protective effect. This relationship held true when cytological dyskaryosis was made the dependent variable ($RR = 0.24$).

Conclusions—Anogenital warts are not a risk for subclinical cervical HPVI or for CIN and therefore not an indication for colposcopy.

Introduction

Numerous studies have defined risk factors for cervical intraepithelial neoplasia (CIN) and invasive cervical carcinoma by comparing patients with control groups drawn from women who presented with problems unrelated to sexual behaviour. If these lesions are sexually transmitted, controls from the general population might be expected to lead to the defining of characteristics associated with sexually transmitted diseases (STD) in general. Not surprisingly, therefore, all these studies have found strong relationships with sexual behaviour of patients or their sexual partners.¹⁻⁷ There is increasing evidence that certain types of human papillomavirus (HPV) are the agents responsible for promoting intraepithelial neoplasia.⁸⁻¹¹ More than 60 HPV

types have been defined by DNA/DNA hybridisation, only a few of which (HPV 16, 18, 31, 33, 35) have been detected in cervical neoplasia.¹⁰ Subclinical HPV infection (HPVI) can be detected by colposcopy.⁸ Screening for pre-cancerous changes by cytological examination has been criticised for lack of sensitivity and specificity.¹²⁻¹⁵ A new classification based on the concept of low and high grade squamous intraepithelial lesions (SIL) has been introduced to improve the management of abnormal cervical smears.¹⁶

This study set out to determine whether anogenital warts are a marker for subclinical HPVI or for CIN and are thus an indication for colposcopy, and also to look at other possible risk factors for HPVI or CIN in women attending a genitourinary medicine clinic. Patients with histologically defined lesions were compared with patients in whom these lesions were not found by the same detection procedures in a sample drawn from the same population by the same criteria.

Patients and methods

Patients attending the Department of Genitourinary Medicine at the West London Hospital between 1985 and 1987 were referred for colposcopy on the basis of two main indications. The first was if their cervical cytology was reported as showing either dyskaryosis or changes consistent with HPVI, principally koilocytosis. The other main indication was the presence of anogenital warts either on clinical examination or on past history. Patients with anogenital warts were not colposcoped until their lesions had resolved with treatment.

Prior to colposcopy all patients were given a self-administered questionnaire in which they were asked if they had ever had anogenital warts or whether they had a sexual partner with penile warts. The questionnaire also asked the age of coitarche and the total numbers of sexual partners in the patient's life. Additional questions were on current contraception, the date of the last period, previous pregnancies, cervical smears and average cigarette consumption.

Immediately prior to colposcopy, cultures were taken for *Candida albicans*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Cervical cytology was not performed in order to avoid distortion of cervical morphology and bleeding. After cervical cleaning with normal saline, the cervix was bathed in 5% acetic acid for at least one minute before examination. Biopsies were taken from

Department of
Genitourinary
Medicine, Charing
Cross Hospital,
London, W6 8RF
B A Evans

Charing Cross and
Westminster Medical
School, Charing Cross
Hospital, London, W6
8RF
R A Bond
K D MacRae

Address correspondence to:
Dr B A Evans, Director,
Department of GU
Medicine, Charing Cross
Hospital, London W6 8RF,
UK

Accepted for publication
24 April 1992

acetowhite areas according to the surface morphology. Thus, if the acetowhite epithelium (AWE) was of uniform appearance only one biopsy was taken. Specimens were processed by the routine histopathology service. This entailed examination by two observers, one of whom was always a consultant histopathologist, who worked on a monthly rotational basis in four separate teams.

Data were analysed on the University of London's computer system using the SPSS-X package. Statistical analysis utilised the chi-square test and stepwise logistic regression was carried out on BMDP statistical software. The criteria for removal and entry in the model were $p < 0.05$. This analysis provided estimates of relative risk (RR) for associations between various possible behavioural and disease risk factors.

Results

Between January 1985 and December 1987, 468 patients underwent colposcopy. Their mean age was 25.2 years (range 17–54) with 97% less than 40 years of age. The incidence of sexually transmitted diseases among this group did not differ significantly from the clinic population as a whole apart from selection for anogenital warts. Likewise, age and parameters of sexual behaviour did not differ from those reported in a study of female sexual behaviour carried out during the same period.¹⁷

The criteria for colposcopy are shown in table 1. Approximately one-third had abnormal cytology, one-third anogenital warts and one-third had both.

The results of colposcopy are shown in table 2. Three hundred and sixty-six patients (78%) had AWE and underwent biopsy and 102 (22%) had no abnormality. Of the 468 patients, 179 (38%) had biopsies showing histological evidence of HPV and 99 (21%) had biopsies showing CIN.

Patients with anogenital warts

There were 286 patients who had clinically diagnosed anogenital warts. Their average age

was 24.3 years and 116 (40.5%) had cytological evidence of HPV. Nuclear dyskaryosis was reported in 98 (34.2%), in whom it was moderate in 6 (2.1%) and severe in 1 (0.3%). Colposcopy was normal in 71 (24.8%). 112 (39.2%) showed histological evidence of HPV and CIN was found in 46 (16.0%), of whom 26 (9.1%) had CIN2 and 5 (1.7%) had CIN3. Inclusion of patients with a past history of anogenital warts made negligible differences in these percentages.

Cytology and histology compared

The relationship between cytological and histological evidence of HPV is shown in table 3. Histological evidence of HPV was found in 77 of the 183 patients in whom there was no cytological evidence (negative predictive value: 58%). On the other hand, the cytological finding of wart virus infection was confirmed in only 102 of the 183 (positive predictive value: 56%). The relationship between cytology and histology for CIN is shown in table 4. Although the negative predictive value of 90% is very good, 10 of the 177 patients with negative cytology (5.6%) had high grade lesions (CIN2 or 3). The positive predictive value of 43% stems from the inclusion of patients whose smears showed occasional or borderline dyskaryosis. Nevertheless, 44 of the 72 (61%) patients with high grade lesions had smears that were reported as showing no more than mild dyskaryosis.

Univariate analysis

The relationship of age and behavioural variables with cervical HPV showed only two significant associations. First was a negative association with age above 25 years in which 55 of 179 patients with HPV were aged over 25 compared with 116 of 289 patients without HPV ($p < 0.05$). There was also a significant association with the intra-uterine contraceptive device (IUCD) which was used by 21 of 176 patients with cervical HPV compared with 15 of 287 patients without ($p = 0.01$). The behavioural variables examined were coitarche at less than 16 years and less than 17 years of age, more than five lifetime partners, use of no contraception, oral contraception and IUCD,

Table 1 Criteria for colposcopy

	Inclusive	Exclusive
Abnormal cytology	310 (66%)	144 (31%)
Dyskaryosis	208	
Koilocytosis	211	
Anogenital warts	324 (69%)	158 (34%)
Current	286	
Past > 1 year	50	
Both		166 (35%)
Total patients		468 (100%)

Table 2 Results of colposcopy

	102 (22%)	366 (78%)
Normal		
Abnormal		
0	267	126 (47%)
1	27	19 (70%)
2	51	27 (53%)
3	21	7 (33%)
Total	366	179 (49%)

Table 3 Cytology and histology: HPV

Cytology	Histology				
	–	106	77	183	NPV 58%
Koilocytosis	+	81	102	183	PPV 56%
		187	179	366	
		Spec 57%	Sens 57%		

Table 4 Cytology and histology: CIN

Cytology	Histology				
	0	1	2	3	
	0	159	8	2	177 NPV 90%
	1	101	15	29	15 160
Dyskaryosis	2	3	2	13	3 21 PPV 43%
	3	4	2	1	1 8
		267	27	51	21 366
		Spec 60%	Sens 82%		

termination of pregnancy, birth of a baby and smoking on average more than 10 cigarettes a day.

There were no significant associations between disease variables and cervical HPV. The variables examined were history of vulval warts obtained from the questionnaire, genital warts found previously on clinical examination, past history of genital warts obtained from the clinical notes, verified diagnosis of genital herpes, vaginal candidosis or more than one sexually transmitted disease including anogenital warts. For example, 111 of 179 patients with cervical HPV gave a history of vulval warts on the questionnaire compared with 180 of 289 patients without cervical HPV ($p = 1.00$). A past history of genital warts was given by 22 of 179 patients with cervical HPV compared with 28 of 289 without ($p = 0.46$).

More significant associations between behavioural variables and CIN are shown in table 5, which also compares patients with high grade lesions (CIN 2 or 3) with patients who had no CIN. In respect of CIN of all degrees there were significant associations with coitarche before 16 years of age ($p = 0.02$), use of an IUCD ($p < 0.001$), termination of pregnancy ($p = 0.01$) and having given birth to a baby ($p < 0.0001$). High grade lesions were significantly associated with age over 25 years ($p < 0.05$) and with smoking more than 10 cigarettes per day ($p = 0.02$) but not with coitarche below 16 years of age.

The relationship between CIN and disease variables is shown in table 6. Highly significant

negative associations were found between CIN, including high grade lesions, and both a history of vulval warts ($p = 0.0001$) and the finding of anogenital warts on clinical examination ($p < 0.001$) but not with a past history of anogenital warts. There were no significant correlations with other sexually transmitted diseases apart from the presence of cervical HPV ($p < 0.001$).

Multivariate analysis

Variables examined by univariate analysis using the chi-square test were entered into a stepwise logistic regression model¹⁸ restricting removal and entry to significance at the 5% level ($p < 0.05$). Only IUCD use showed significant relative risk for HPV as the dependent variable ($RR = 3.49$; 95% CI 1.28 – 9.51).

The dependent variables used for CIN were all degrees of CIN and CIN 2 or 3 (table 7). Current IUCD use showed a high degree of risk both for all degrees of CIN ($RR = 7.75$) and for CIN 2 or 3 ($RR = 6.91$). Early coitarche below 16 years of age was an independent risk for CIN as a whole ($RR = 3.72$). However, the risk of CIN was reduced by history of vulval warts both for all degrees of CIN ($RR = 0.34$) and for CIN 2 or 3 ($RR = 0.34$).

In view of the unexpected nature of these findings, they were re-examined using cervical dyskaryosis determined by cytological examination in place of CIN determined by histological examination as the dependent variable. The result is shown in table 8. A history of

Table 5 Association with CIN: age and behavioural variables

Variables	No CIN	CIN	p	CIN2/3	p
Age > 25 years	127/369	44/99	0.09	34/72	< 0.05
Coitarche < 16 years	46/332	22/90	0.02	15/67	0.11
< 17 years	109/332	38/90	0.13	28/67	0.20
Partners > 5	168/325	50/88	0.46	38/65	0.39
Contraception – nil	73/365	14/98	0.25	11/71	0.47
– OC	191/365	49/98	0.78	32/71	0.32
– IUCD	20/365	16/98	< 0.001	12/71	0.002
TOP	70/369	31/99	0.01	22/72	0.04
Baby	43/369	31/99	< 0.0001	25/72	0.0001
Smoking > 10 cigarettes	69/212	18/42	0.27	16/28	0.02

Table 6 Association with CIN: disease variables

Variables	No CIN	CIN	p	CIN2/3	p
History of vulval warts	245/369	45/99	0.0002 (neg)	29/72	0.0001 (neg)
Warts on examination	238/369	46/99	< 0.001 (neg)	31/72	< 0.001 (neg)
PH of warts	41/369	9/99	0.69	5/72	0.40
Genital herpes	27/369	6/99	0.83	4/72	0.78
Vaginal candidosis	141/369	35/99	0.69	22/72	0.27
Other STDs	220/369	66/99	0.25	45/72	0.75
Cervical HPV	126/369	53/99	0.0007	34/72	< 0.05

Table 7 Relative risks by stepwise logistic regression: CIN

Variables	All CIN			CIN 2/3		
	Step	RR	95% CI	Step	RR	95% CI
IUCD	1	7.75	(2.63–22.89)	1	6.91	(2.11–22.64)
Coitarche < 16	2	3.72	(1.51–9.20)			
History of vulval warts	3	0.34	(0.15–0.75)	2	0.34	(0.13–0.84)

Table 8 Relative risks by stepwise logistic regression: dyskaryosis

Variables	All dyskaryosis			Moderate/severe dyskaryosis		
	Step	RR	95% CI	Step	RR	95% CI
History of vulval warts	1	0.24	(0.13-0.45)			
Cervical HPV	2	2.49	(1.33-4.63)			
IUCD	3	4.79	(1.45-15.84)			
Anogenital warts on examination				1	0.2	(0.04-0.41)
Age > 25 years				2	3.95	(1.21-12.90)

vulval warts showed an equally protective effect against cervical dyskaryosis (RR = 0.24) and current IUCD usage similarly increased the likelihood of dyskaryosis (RR = 4.79). For moderate and severe dyskaryosis, the regression model selected the clinical finding of anogenital warts as the more strongly related variable (RR = 0.2) and this was linked with age over 25 years (RR = 3.95).

Discussion

We have analysed the results of colposcopic referral because of cytological evidence of HPV or CIN, or because of anogenital warts elicited by history or examination. The findings of colposcopic examination and cervical biopsy have been correlated with behavioural and sexually transmitted disease (STD) data derived from a standardised questionnaire and from the clinical records. This provided an opportunity to assess and review the indications for referral and to determine the risk factors for cervical HPV and CIN among women attending a genitourinary medicine clinic. Previous studies have reported CIN in 28 to 30% of patients with anogenital warts.¹⁹⁻²¹ These patients were drawn largely from STD clinics but they were not compared with any other clinical grouping to act as controls from within the same population. Anogenital warts may thus have acted as a surrogate marker for sexual behaviour. Moreover, biopsy was preceded by cervical scraping for cytological samples, which would have removed layers of superficial epithelial cells and created a histological artefact of CIN. Later reports have quoted much lower percentages similar to the present study.²²⁻²⁴ More recent work has found the relationship between anogenital warts and cervical cancer not to reach significance.²⁵

Histological evidence of HPV is less sensitive than DNA/DNA hybridisation or PCR methods of detection but does establish viral expression, which is a likely promoter of CIN. Thus, although insensitive, this finding is specific and relevant. Cervical HPV correlated only with young age (< 25 years) and IUCD usage; the latter may well itself promote viral replication. The lack of association with sexual behaviour, anogenital warts or other STDs suggests that subclinical cervical HPV is epidemiologically a distinct and separate entity, which may also be transmitted by nonsexual routes.²⁶⁻²⁸

By contrast, CIN showed well established links with early intercourse, pregnancy and smoking but not with numbers of sexual partners. This may be a reflection of the study

sample from a high risk group of women in which a higher proportion had had multiple partners. Other studies have shown trends to commence at only two sexual partners. For example, in a case-control study of risk factors for CIN, Cusick *et al* found that 9% of cases had only one sexual partner compared with 19% of controls.⁶ In our study overall, this figure was 7% and in our 1987 sexual behaviour study it was 9%.¹⁷ An earlier study by Rotkin found that 30% of cervical cancer cases reported only one sexual partner compared with 49% of controls.¹ Our study looks at risks within a high risk group, much as case-control studies among gay men attending STD clinics have defined specific risky sexual behaviours for HIV infection with controls drawn from the same population as the cases.

Previous case-control studies have not found a high risk of CIN for IUCD users. Our findings may therefore reflect a different study population which has not previously been the subject of investigation. If the effect of IUCD is dependent on the presence of HPV it could be masked by a control population with little or no HPV but a similar rate of IUCD use. The effect of IUCDs may well disappear with removal and permit spontaneous regression long before the peak age incidence for cervical carcinoma. Nevertheless, IUCD usage by younger women with cervical cancer merits further examination.

The negative correlation with anogenital warts was an unexpected finding, which has not been reported previously. On the one hand, anogenital warts may be a surrogate marker for behaviourally determined STD susceptibility in general. On the other hand, different diseases have emerged as more significant predictors when other STDs have been introduced into the analysis of risks.⁷ In our study group, there were no increased risks associated with other STDs, which had a similar frequency pattern to the clinic population generally and were therefore not a source of selection bias. No other study has drawn its controls from a population otherwise defined in the same way as its cases.²⁹

In contrast to external exophytic warts, cervical HPV was strongly associated with all degrees of CIN. Numerous studies have shown that the type of HPV found in exophytic warts (HPV 6 and 11) is generally different from that found in cervical HPV and CIN (HPV 16, 18, 31, 33, 35). Our study therefore supports cervical HPV as a risk factor for CIN and suggests that exophytic anogenital warts have a protective effect in contrast to other STDs. Our data also raise the possibility that this is age-related with cervical HPV in younger

women and CIN in older women. A possible mechanism for this effect may be a humerally mediated immune response to massive virus replication in exophytic lesions which has a suppressive effect on HPV-dependent CIN. Papillomaviruses have not been grown effectively in vitro so that it has not been possible to produce HPV type-specific antigens in sufficient quantities to develop appropriate detection systems. However, bovine papillomaviruses (BPV) have yielded group-specific antigens which have been used to detect high titres of antibodies both in patients with anogenital warts and patients with cervical cancer.¹¹

Nevertheless, this study has shown that the presence of anogenital warts is not an indication for colposcopy and that dyskaryotic changes on cervical cytology remain the primary reason for this investigation.

- 1 Rotkin ID. Sexual characteristics of a cervical cancer population. *Am J Public Health* 1967;57:815-29.
- 2 Martin CE. Marital and coital factors in cervical cancer. *Am J Public Health* 1967;57:803-14.
- 3 Harris RWC, Brinton LA, Cowdell RH, Skegg DCG, Smith PG, Vessey MP, Doll R. Characteristics of women with dysplasia or carcinoma in situ of the cervix uteri. *Brit J Cancer* 1980;42:359-69.
- 4 Terris M, Fitzpatrick W, Nelson JH. Comparative epidemiology of invasive carcinoma of the cervix, carcinoma in situ, and cervical dysplasia. *Am J Epidemiol* 1980;112:253-7.
- 5 Buckley JD, Doll R, Harris RWC, Vessey MP, Williams PT. Case-control study of the husbands of women with dysplasia; or carcinoma of the cervix uteri. *Lancet* 1981;ii:1010-5.
- 6 Cuzick J, Singer A, De Stavola BL, Chomet J. Case-control study of risk factors for cervical intraepithelial neoplasia in young women. *Eur J Cancer* 1990;26:684-90.
- 7 Herrero R, Brinton LA, Reeves WC. Sexual behaviour, venereal diseases, hygiene practices and invasive cervical cancer in a high-risk population. *Cancer* 1990;65:380-6.
- 8 Meisels A, Fortin R, Roy M. Condylomatous lesions of the cervix. II Cytologic, colposcopic and histopathologic study. *Acta Cytologica* 1977;21:379-90.
- 9 Zur Hausen H. Human genital cancer: synergism between two virus infections or synergism between a virus infection and initiating events. *Lancet* 1982;ii:1370-2.
- 10 Zur Hausen H. Papillomaviruses in anogenital cancer as a model to understand the role of viruses in human cancers. *Cancer Res* 1989;49:4677-81.
- 11 Baird PJ. Serological evidence for the association of papillomavirus and cervical neoplasia. *Lancet* 1983;ii:17-8.
- 12 Husain OAN, Butler EB, Evans DMD, McGregor JE, Ule R. Quality control in cervical cytology. *J Clin Pathol* 1974;27:935-44.
- 13 Lacey CJN, Mulcahy FM, Sutton J. Koilocyte frequency and prevalence of cervical human papillomavirus infection (letter). *Lancet* 1986;i:557-8.
- 14 Walker EM, Dodgson J, Duncan ID. Does mild atypia on a cervical smear warrant further investigation? *Lancet* 1986;ii:672-3.
- 15 Singer A, Walker P, Tay SK, Dyson J. Impact of introduction of colposcopy to a District General Hospital. *BMJ* 1984;289:1049-51.
- 16 National Cancer Institute Workshop. The 1988 Bethesda System for reporting cervical/vaginal cytologic diagnoses. *JAMA* 1989;262:931-4.
- 17 Evans BA, McCormack SM, Bond RA, MacRae KD. Human immunodeficiency virus infection, hepatitis B virus infection, and sexual behaviour of women attending a genitourinary medicine clinic. *BMJ* 1988;296:473-5.
- 18 Dixon WJ. *BMDP Statistical Software Manual Vol 2*, 1990. University of California Press, Berkeley.
- 19 Walker PG, Singer A, Dyson JL, Oriel JD. Natural history of cervical epithelial abnormalities in patients with warts. A colposcopic study. *Br J Venereal Dis* 1983;59:327-9.
- 20 Walkinshaw SA, Dodgson J, McCance DJ, Duncan ID. Risk factors in the development of cervical intraepithelial neoplasia in women with vulval warts. *Genitourin Med* 1988;64:316-20.
- 21 Campion MJ, Singer A, Clarkson P, McCance DJ. Increased risk of cervical neoplasia in consorts of men with penile condylomata acuminata. *Lancet* 1985;i:943-6.
- 22 Becagli L, Manfe M, Dalla-Pria S, et al. Genital HPV infection: relation between vulval and cervicovaginal localisation. *Minerva Ginecol* 1990;42:381-5.
- 23 Law CL, Merianos A, Grace J, et al. Clinical and virological associations between external anogenital warts and cervical HPV infection in a STD clinic. *Int J of STD AIDS* 1991;2:30-6.
- 24 Schauburger CW, Rowe N, Gundersen JH, et al. Cervical screening with cervicography and the Papanicolaou smear in women with genital condylomata. *J Reprod Med* 1991;36:100-2.
- 25 Sigurgeirsson B, Lindelof B, Eklund G. Condylomata acuminata and risk of cancer: an epidemiological study. *BMJ* 1991;303:341-4.
- 26 Kiviat NM, Koutsky LA, Paavonen JA, et al. Prevalence of genital papillomavirus infection among women attending a college student health clinic or a sexually transmitted disease clinic. *J Infect Dis* 1989;159:293-302.
- 27 Kjaer SK, Engholm G, Teisen C, et al. Risk factors for cervical human papillomavirus and herpes simplex virus infection in Greenland and Denmark: a population-based study. *Am J Epidemiol* 1990;131:669-82.
- 28 Sun-Kuie T, Tew-Hong H, Soo-Kim LT. Is genital human papillomavirus infection always sexually transmitted? *Aust NZ J of Obstet Gynaecol* 1990;30:240-2.
- 29 Munoz N, Bosch X, Kaldor JM. Does human papillomavirus cause cervical cancer? The state of the epidemiological evidence. *Brit J Cancer* 1988;57:1-5.