Managing women with human papillomavirus changes in cervical cytology

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SUMMARY Of 86 women with cervical cytological evidence of human papillomavirus (HPV) infection who underwent colposcopy, 55 yielded histological confirmation of HPV infection and 17 of them had cervical intraepithelial neoplasia (CIN). Of 51 women followed up six months after cryotherapy of their cervical lesions, 46 showed no evidence of HPV infection in their cervixes. Of the patients with CIN lesions, only one out of the 16 followed up had persistent CIN II six months after treatment, which gave a clearance rate of 94% for early CIN lesions treated by this method. Of 62 sexual partners examined, 48 had sexually transmitted infections, 38 of them genital warts. The use of colposcopy with directed punch biopsy is a practical way of managing patients whose cervical smears suggest HPV infection. Cryotherapy is effective in treating histologically confirmed cervical HPV and associated minor CIN lesions. Contact tracing and screening for other sexually transmitted diseases is an integral part of managing genital warts.

The prevalence of genital warts has rapidly increased in the last decade, and sexually transmitted disease (STD) clinics increasingly manage problems associated with genital warts. Genital human papillomavirus (HPV) (DNA types 6, 11, 16, and 18) has been associated with preinvasive and invasive neoplasia of the female genital tract, particularly the cervix.¹⁻⁷ It is therefore important to detect HPV infections of the cervix in women attending STD clinics, who form a population at risk for the infection. Although most cervical HPV infections are subclinical, Papanicolaou smears and changes in the colposcopic appearance of the cervix can suggest their presence.^{8,9} It is impractical, however, to examine by colposcopy every woman attending a large STD clinic. The Papanicolaou smear is commonly performed in STD clinics and can alert doctors to the presence of HPV infection of the cervix. Patients with positive results can then be investigated further.

The managing of patients with cervical cytological evidence of HPV infections should be directed at confirming the infection and associated dysplastic change. Identifying the DNA type can be useful, as

infection with types 16 and 18 seems to have a worse prognosis of progression to malignancy than infection with types 6 and 11. As routine DNA typing is impractical, however, and as all cervical condylomata and any associated dysplastic lesions of the cervix should be treated irrespective of the DNA types,¹⁰ this procedure remains essentially a research tool. Treatment should also prevent the spread of infection to non-infected and new sexual partners.

The aims of this study were to investigate women with cervical cytology results suggestive of HPV infection, using colposcopy and biopsy for histological confirmation of the HPV infection and to diagnose associated CIN changes, to treat histologically confirmed HPV infections and associated CIN changes with cryotherapy, and to assess the efficacy of the treatment.

Patients and methods

Women attending the Whitechapel Clinic of this hospital routinely had cervical cytology smears performed, which were interpreted using standard criteria.¹¹ We studied those showing evidence of HPV infection. Cytological identification of HPV infection relied predominantly on the observation of both koilocytes and parakeratosis in a Papanicolaou stained smear. We excluded from the study women in whom cervical condylomata were suspected on naked eye examination. We also excluded three women whose initial

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cervical cytology results showed CIN changes in addition to HPV infection. They were referred for gynaecological evaluation.

Patients were examined with the Zeiss OPMI-1 colposcope. After initial inspection under magnification $\times 6$ and $\times 10$, the cervix was washed with 5% (v/v) acetic acid and re-examined. Minibiopsies were taken from representative acetowhite areas using a Gasteyer punch biopsy forceps that had been modified by shortening the lower jaw and having a distal cutting edge. This reduced the cutting area of the forceps to 6 mm $\times$ 3 mm. We have found that this modification was minimally traumatic, caused little discomfort or bleeding, and enabled us to take multiple samples if sites were wide or if many acetowhite areas showed up. The tissues were fixed in formalin and sent for histological studies. The fixed tissues were embedded in paraffin, serially sectioned, and stained with haematoxylin and eosin. Histological evidence of HPV infection included acanthosis, koilocytosis, dyskeratosis, papillomatosis, parakeratosis, hyperkeratosis, basal cell hyperplasia, binucleation, and nuclear degeneration.¹² Several features usually coexist, although on rare occasions koilocytosis with degenerative nuclear changes alone were accepted as evidence.

After histological confirmation, the cervical lesions were destroyed by cryotherapy using the cryoprobe (Spemby) operated with standard non-syphon medical nitrous oxide. Freezing was continued until the area surrounding the lesions became white with ice up to a margin of 1–2 mm. This generally occurred within one and a half to two minutes of freezing if the cylinder pressure was kept at 600 to 900 pounds per square inch. Probe tips of various sizes were used to freeze small lesions individually, and conical tips were used to treat wide lesions around the cervical os and those associated with CIN changes. Freezing was repeated after allowing the frozen area to thaw completely. Patients were warned of probable increased vaginal discharge after treatment and asked to avoid sexual intercourse until they were reviewed after six weeks, when the cervix was checked for healing. Thereafter patients were advised to use condoms for protection during sexual intercourse. Associated genital warts were treated conventionally until all vaginal, vulval, anal, and perianal warts were cleared. The sexual partners of all patients were asked to attend the clinic for examination and treatment. Six months after treatment the patients were re-examined with the colposcope after a smear had been taken for cytological examination. If the squamocolumnar junction was above the external os, an endocervical smear was also taken. Persistent warty lesions were treated again with cryotherapy. Patients with persisting CIN changes were referred to a gynaecologist. All patients were further followed up with a cervical smear and

colposcopic examination one year after treatment. If lesion clearance was confirmed, they were advised to have a yearly cervical smear thereafter.

Results

Of the 86 patients studied (age range 17 to 30) 78 were 25 or younger; 68 were white, 17 Afro-Caribbean, and one Asian; 69 were nulliparous.

At colposcopy the cervixes of six patients had normal appearances and were not biopsied. The colposcopic findings in the other 80 patients were categorised, based on the major area of acetowhite change seen on the cervix, as having (a) papilliferous acetowhite areas; (b) flat acetowhite areas with or without fine punctation (the acetowhite epithelium was flush with the rest of the squamous epithelium) or (c) raised acetowhite areas with or without fine punctation (the surface contour of the acetowhite epithelium appeared distinctly raised from the rest of the squamous epithelium).

Acetowhite mosaic lesions were not categorised separately because they were present in only four patients, none of whom had associated areas of punctation. Three of these patients were in group (b) and the histology of the lesions showed HPV infection in two patients and normal squamous epithelium in one. The fourth patient was in group (c) and her lesion showed HPV infection on histology.

The table shows the overall results of histology correlated with the various categories of acetowhite areas of the cervix.

OTHER GENITAL WARTS AND ASSOCIATED INFECTIONS

Of the 86 women, 48 had extracervical genital condylomas and 44 had associated STD. *Neisseria gonorrhoeae* was isolated from six, an enzyme immunoassay for *Chlamydia trachomatis* (Chlamydiazyme, Abbotts Laboratories, Chicago, USA) from the cervix was positive in 14, *Trichomonas vaginalis* was found in nine, herpes simplex virus was isolated from three who presented with genital ulcers, *Candida albicans* was found in 24, and bacterial vaginosis was diagnosed clinically in 22. One patient had flat acetowhite epithelium on colposcopy and changes attributable to HPV infection on histology, which also showed inclusions typical of cytomegalovirus infection in the columnar epithelial cells of the cervix.

FINDINGS IN SEXUAL PARTNERS

Of 62 sexual partners seen, no STD was identified in 14. Of 38 with genital HPV infection, two had gonococcal and 14 non-gonococcal urethritis. Of 10 men without evidence of genital warts, six had non-

Table Results of histology correlated with colposcopic findings in 80 women biopsied

Histological results	Acetowhite area* on colposcopy			Total
	Papilliferous	Flat	Raised	
Human papillomavirus (HPV) infection	10	13	15	38
HPV + cervical intraepithelial neoplasia (CIN) type I	1	7	5	13
HPV + CIN II	1	1	2	4
Inflammatory cell infiltrate	0	5	13	18
Squamous metaplasia or normal squamous epithelium	0	7	0	7
Total	12	33	35	80

*Flat acetowhite areas (with or without fine punctuation) = acetowhite epithelium flush with rest of squamous epithelium; raised acetowhite areas (with or without fine punctuation) = surface of acetowhite epithelium raised from rest of squamous epithelium.

gonococcal and four had both gonococcal and post-gonococcal urethritis.

FOLLOW UP AFTER TREATMENT

Of the 55 patients treated for cervical lesions, four did not attend for follow up. Of the 51 who were examined at six months, the HPV lesions had resolved in 46. Of the 17 patients with CIN lesions associated with HPV infection, 16 were followed up at six months. In one patient the CIN II lesion had persisted, but without evidence of residual HPV lesions. This gave a clearance rate of 94% for minor CIN lesions associated with HPV infection. We examined 48 treated patients (33 with HPV lesions alone and 15 with HPV lesions associated with CIN) one year after treatment; HPV lesions were still present in three, but none had evidence of dysplasia.

Discussion

Of 31 patients in the study with cytological evidence suggestive of HPV infection, six had a normal appearance on colposcopy, and in 25 the infection could not be confirmed by histology even after multiple sampling of all acetowhite areas and serial sectioning of biopsied tissues. As some of these patients had associated vaginal warts, it is possible that the original smears were contaminated with desquamated vaginal cells, rather than the subjective cytological criteria having been misinterpreted, or the infection could have been transient and had cleared at the time of colposcopy. Despite multiple minibiopsies, it is also possible that these missed representative HPV infected areas. Discrepancies between findings on cervical cytology and colposcopy or histology have been reported previously.¹³

Seventeen patients without cytological evidence of CIN showed changes of minor CIN on histology. The combination of cytological and histological investigations improves the accuracy of diagnosing cervical abnormality.¹⁴ In this study the overall incidence of histologically confirmed CIN in association with

cervical cytological smears showing HPV infection was 20% (17/86), but the association of CIN with histologically proved HPV infection was 31% (17/55). Other workers using cervical cytology and colposcopy alone in women with vulval warts have shown a high association of CIN with cervical infection with HPV.¹⁵

The colposcopic classification used was based on the nature of the largest acetowhite area seen in each patient, though in many cases isolated small areas showing other changes were seen and biopsied. As the histology of biopsy specimens taken from different representative areas did not alter the final outcome in any one case, this simple classification was retained. Of the three colposcopic findings, papilliferous acetowhite epithelium alone showed 100% (12/12) correlation with histological criteria of HPV infection, but this was only seen in 12 (22%) of 55 women with histologically proved HPV infection. The other two common colposcopic findings (flat and raised acetowhite epithelium) were also associated with histological changes of inflammatory cell infiltrate and squamous metaplasia or normal squamous epithelium. These changes are therefore unreliable in diagnosing HPV infection without histological confirmation. In our experience the most common vessel abnormality noted colposcopically was fine punctuation.^{12,16} Mosaic pattern was uncommon and was seen in only four patients who showed either evidence of HPV infection or normal squamous epithelium; none was associated with CIN. This may be due to selection criteria, as patients whose cervical cytology showed both CIN and HPV were excluded from the study and referred to gynaecologists. Of 17 women with histological evidence of CIN I or II, however, none had mosaic pattern on colposcopy. The criteria for distinguishing between HPV infection of the cervix and CIN using the colposcope have not been uniformly accepted,^{17,18} and in this study no particular colposcopic feature could be attributed to the associated minor CIN lesions. The CIN changes found on histology were not seen in the original cervical smears. Thus histological confirmation is essential before starting treatment. No patient in the study

showed histological changes of CIN III or invasive neoplasia.

The high incidence of other STDs in the women in the study group (51%) and their sexual partners with genital warts (37%) emphasises the need for adequate screening of patients with genital warts for other STDs. Proper contact tracing and screening of sexual partners is essential.

Because of the progressive potential of HPV infection¹⁹ and minor grades of CIN,²⁰ both conditions were treated in this study. Nash *et al* argued that, as only a third of untreated histologically proved HPV lesions of the cervix progressed to CIN during one year, these lesions are best treated only on evidence of progression.²¹ Such an argument is untenable, as it does not take into account the infective nature of these lesions to sexual partners and the uncertainty of patient compliance to follow up in the long term.

Colposcopy and biopsy of suspect cervical lesions for histological confirmation of HPV infection and CIN can be a practical procedure in many clinics dealing with this problem and can enable cervical HPV infection and associated CIN lesions to be treated effectively. Cryotherapy was chosen because it was readily available, economical, easy to use, and of minimal discomfort to patients. Cryotherapy in our hands gave results comparable with other methods, with a resolution of 90% of HPV lesions and 94% of CIN six months after treatment. The success of the regimen depends on all cervical lesions and extracervical genital warts being treated as well as the concomitant treatment of genital warts in sexual partners to prevent reinfection. Using condoms further minimises the risk of reinfection.

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