Comparison of prevalence of human papillomavirus antigen in biopsies from women with cervical intraepithelial neoplasia

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SUMMARY Human papillomavirus antigen was found in 39 (16%) of 253 colposcopic biopsies from a group of women at high risk for cervical cancer who had been examined in the early 1970s. Immunohistochemical evidence of papillomavirus infection was found in 20 (30%) of 67 cervical intraepithelial neoplasia lesions infected with wart virus from these patients. When these results were compared with results of a similar study carried out in the early 1980s there was no significant difference in the prevalence of human papillomavirus antigen in cervical intraepithelial neoplasia lesions infected with wart virus from women who had been examined over a decade apart.

Human papillomavirus has increasingly been implicated as a possible aetiological agent in cervical neoplasia. The presence of viral deoxyribonucleic acid sequences has been shown in over 60% of invasive and preinvasive cervical cancers.^{1,2} Baird recently presented serological evidence showing that 93% of women with invasive carcinoma had an IgG antibody to a group specific papillomavirus antigen in their sera.³ The carcinogenic potential of the papillomavirus is strongly supported by evidence from its role in the production of cancers in animals.⁴⁻⁶

Recent studies have suggested that a change has occurred over the past decade in the natural history of cervical neoplasia. Two such changes have serious connotations in respect of diagnosis and management. Firstly, there has been an increase of 117% in the number of cases of carcinoma in situ reported in England and Wales between 1973 and 1979, and secondly, the number of women under 35 years of age dying of cervical cancer has doubled during this period.⁷ Another disturbing feature is the increasing number of invasive lesions in young women that appear to have developed rapidly after a very short premalignant period.⁸ This changing pattern of disease could be due to the appearance of a new sex-

ually transmitted agent, human papillomavirus. Recently, there has also been a significant increase in the number of clinically recognisable genital warts, which have been associated with an increased recognition of preclinical cervical wart virus lesions visible only by colposcopy.^{9,10}

If human papillomavirus is responsible for the change in the natural history of cervical carcinoma then it would have to be shown that an increase in the prevalence of this infection has occurred among those women at risk for the development of cervical malignancy. An editorial discussing the histological evidence for the association of human papillomavirus with cervical intraepithelial neoplasia commented that, "although we have no figures for the before and after, it is hard to believe that until a few years ago such dramatic changes (ie, related to wart virus) were overlooked."¹¹

Recent immunohistological studies have shown that the prevalence of papillomavirus antigen in colposcopically directed biopsies of premalignant lesions and wart virus lesions among a group of women with abnormal cytology in the 1980s was 23%.¹² Another study conducted in the early 1970s obtained similar results from women at high risk of cervical carcinoma, the rate for histologically diagnosed premalignant lesions among them being among the highest reported—that is, 92/1000.¹³ We

have studied the material from this second group using similar immunohistochemical techniques and have obtained an estimation of the prevalence of human papillomavirus in cervical intraepithelial neoplasia in two groups of women separated by a decade. The groups were similar, in that the women in both were at risk for cervical cancer, but not directly comparable. Comparison may be made, however, between the expression of viral antigen in the histological material obtained from each group.

Material and methods

The cervical biopsies were obtained during a colposcopic study of cervixes of women confined to a penal institution (HM Prison, Holloway, London) in the early 1970s, the behavioural characteristics of whom have been discussed elsewhere.¹⁴ The study group was randomly selected from two groups of women, who were either short or long term stay prisoners. They were then invited to attend the colposcopy clinic in the prison. Most of the women examined were short stay prisoners (average four months); their ages ranged from 16 to 55 years (mean age 25).

Colposcopically directed biopsies were taken from multiple areas within the atypical transformation zone. From the 304 cervical biopsy specimens collected, 253 biopsy blocks were recovered that had sufficient tissue left for immunohistochemical study. Sections stained with haematoxylin from these biopsies were examined and graded, using the same criteria as in the previous study.¹² Sections (4 μ) were taken from each block and stained using an indirect immunoalkaline phosphatase technique.¹⁵ Antiserum for this technique was rabbit antipapillomavirus (Dako Corporation, United States), kindly provided by Professor M Nadji, University of Miami, United States. The presence of papillomavirus antigen was seen by a deep red colouration within the nuclei of cells showing koilocytotic atypia.

Results

The table shows the prevalence of positive staining biopsies, according to the histological diagnosis in the two groups studied in the early 1970s and 1980s. The overall prevalence of the papillomavirus antigen in the biopsies was 16% in the earlier group and 20% in the later group. In the abnormal lesions showing cervical intraepithelial neoplasia grades I, II, and III, and on histological evidence of wart virus infection, papillomavirus antigen was found in 20 (30%) of 67 lesions in the 1970s and 26 (23%) of 112 lesions studied in the 1980s. The χ^2 test indi-

Prevalence of human papillomavirus antigen staining in both study groups according to histological diagnosis

Diagnosis	1970-2 n	group No (%) antigen positive	1981-2 n	group ¹² No (%) antigen positive
Metaplastic or native squamous epithelium	186	19 (10)	25	2 (10)
Cervical intraepithelial neoplasia: Grade I, or infected with wart virus	33	8 (24)	28	10 (36)*
Grade II	11	6 (55)	20	5 (25)
Grade III	23	6 (26)	64	11 (17)

*Lesions reported previously as being infected with wart virus alone have not been included in this category as it is not possible to make a complete distinction between these abnormalities histologically.

cated no difference in the prevalence of papillomavirus antigen in those lesions between the two groups ($p > 0.25$).

Discussion

There was no significant difference in the prevalence of human papillomavirus antigen in the cervical intraepithelial neoplasia tissue we studied from two groups of women separated by at least a decade. There was also no difference in the prevalence of papillomavirus antigen in the category of metaplasia and native squamous epithelium.

The absence of a difference in the prevalence of papillomavirus infection between these two study groups suggests that the increase in the association reported between human papillomavirus and cervical intraepithelial neoplasia may be due to an improvement in the techniques for identification of infection with human papillomavirus rather than a true increase in the prevalence of the virus among women at risk of cervical carcinoma. Thus the recent changes in the natural history of this cervical disease—that is, the increase in prevalence of cervical intraepithelial neoplasia and the appearance of a neoplasm of seemingly rapid onset—cannot be explained by the appearance of human papillomavirus as a new agent.

An alternative hypothesis might be that though the role of this antigen has not changed, there may have been changes in the cofactors needed to operate with it for a neoplastic transformation: smoking and herpes simplex virus have been suggested as possible cofactors.¹⁶ Smoking among women is increasing and is now cited as a major factor in association with sexual behaviour in the aetiology of cervical carcinoma.¹⁷ The relation between herpes simplex virus and cervical carcinoma is well documented.¹⁸⁻²⁰ The role of oral contraceptive

steroids in the aetiology of cervical neoplasia remains unclear; it is known, however, that steroids increase viral replication in tissue culture,²¹ and the growth of genital warts during pregnancy under the influence of sexual hormones is well known.

We suggest that whereas human papillomavirus remains a catalyst for the aetiological development of cervical carcinoma, the recent changes in the pattern of this disease reflect other changes in the incidence of certain cofactors that may be required for the virus to exhibit its malignant potential rather than a direct carcinogenic effect of the virus itself.

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