J Clin Pathol 1985;38:855–857

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

A SINGER,* J WILTERS,* P WALKER,* D JENKINS,† G SLAVIN,‡ H COWDELL,‡* A TO,** O HUSAIN**

*From the *Department of Gynaecology, Royal Northern Hospital, London, the †Department of Histopathology, Whittington Hospital, London, the ‡Department of Pathology, St Bartholomew's Hospital, London, the ‡*Department of Pathology, John Radcliffe Hospital, Oxford, and the **Department of Cytology, Charing Cross Hospital, London.*

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 sexually 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

Accepted for publication 12 April 1985

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 (4μ) 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 indicated no difference in the prevalence of papillomavirus antigen in those lesions between the two groups ($p > 0{\cdot}25$).

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

Diagnosis	1970–2 group n	1970–2 group No (%) antigen positive	1981–2 group[12] n	1981–2 group[12] No (%) antigen positive
Metaplastic or native squamous epithilium	186	19 (10)	25	2 (10)
Cervical intraepithilial 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.

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.

This study was financially supported by the Helena Tomkinson award from the British Medical Association. We thank Mr Ron Yabsley and the technical staff of the pathology department, Whittington Hospital, for their help in preparing slides.

Dr Margureta Stevenson (gynaecologist, HM Prison, Holloway) obtained some of the biopsy material used in the 1970s study, and Dr Peter Smith of the London School of Hygiene and Tropical Medicine was responsible for the original documentation of the data from Holloway Prison.

References

[1] Durst M, Gissman L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Natl Acad Sci USA* 1983;**80**:3812–15.

[2] McCance D, Walker P, Dyson J, Coleman D, Singer A. Presence of human papillomavirus DNA in cervical intraepithelial neoplasia. *Br Med J* 1983;**287**:784–8.

[3] Baird P. Serological evidence for the association of papillomavirus and cervical neoplasia. *Lancet* 1983;ii:17–18.

[4] Jarrett WFH. Papillomavirus in benign and malignant tumours of cattle. In: Essex M, Todaro G, zur Hausen H, eds. *Viruses in naturally occurring cancers*. Vol A. Cold Springs Harbor: Lab Press, 1980:215–22.

[5] Ford J, Jennings P. Evidence for papillomavirus in ocular lesions in cattle. *Res Vet Sci* 1982;**32**:257–9.

[6] Vanselow B, Spraifrow P. Papillomaviruses, papillomas and squamous cell carcinomas in sheep. *Vet Rec* 1982;**110**:561–2.

[7] Roberts A. Cervical cytology in England and Wales 1965–80. *Health Trends* 1982;**14**:41–3.

[8] Bamford PN, Beilly JOW, Steele SJ, Vlies R. The natural history of cervical intraepithelial neoplasia determined by cytology and colposcopic biopsy. *Acta Cytol* (Baltimore) 1983;**27**:482–4.

[9] Oriel J. Condyloma acuminata as a sexually transmitted disease. *Dermatologic Clinics* 1983;**1**:93–102.

[10] Walker P, Singer A, Dyson J, Shah K, Wilters J, Coleman D. Colposcopy in the diagnosis of papillomavirus infection of the uterine cervix. *Br J Obstet Gynaecol* 1983;**90**:1082–6.

[11] Anonymous. Cervical intraepithelial neoplasia [Editorial]. *Lancet* 1982;iii:365–7.

[12] Walker P, Singer A, Dyson J, Shah K, Coleman D. The prevalence of human papillomavirus antigen in patients with cervical intraepithelial neoplasia. *Br J Cancer* 1983;**48**:99–101.

[13] Singer A. A study of the cervix uteri of women in prison. Oxford: University of Oxford, 1973. (PhD thesis.)

[14] Singer A. The uterine cervix from adolescence to the menopause. *Br J Obstet Gynaecol* 1975;**82**:81–99.

[15] To A, Dearnley D, Ormerod M, Canti G, Coleman D. Indirect immuno-alkaline phosphatase staining of cytological smears of serous effusions for tumour marker studies. *Acta Cytol* (Baltimore) 1983;**27**:109–117.

[16] zur Hausen H. Human genital cancer: synergism between two virus infections or synergism between a virus infection and initiating event? *Lancet* 1982;ii:1370–2.

[17] Trevathan E, Layde P, Webster L, Ory H. Cigarette smoking and dysplasia and carcinoma in situ on the uterine cervix. *JAMA* 1983;**250**:499–502.

[18] McDougall J, Galloway D, Fenaglio C. Cervical carcinoma: detection of herpes simplex virus RNA in cells undergoing neoplastic change. *Int J Cancer* 1980;**25**:1–8.

[19] Eglin R, Sharp F, MacLean A, MacNab J, Clements J, Wilkie N. Detection of RNA complementary to herpes simplex virus DNA in human cervical squamous cell neoplasia. *Cancer Res* 1981;**41**:3597–603.

[20] Maitland N, Kinsons J, Busuttil A, Ludgate S, Smart G, Jones K. The detection of DNA tumour virus-specific RNA sequences in abnormal human cervical biopsies in situ hybridization. *J Gen Virol* 1981;**55**:123–37.

[21] Ringald G, Sharls P, Yamamoto K. Production of integrated mouse mammary tumour virus DNA in infected rat hepatoma cells in a secondary action of dexamethasone. *J Virol* 1978;**26**:93–102.

Requests for reprints to: Dr A Singer, Department of Histopathology, Whittington Hospital, St Mary's Wing, Highgate Hill, London N19 5NF, England.