Association between human papillomavirus and carcinoma of the oesophagus in South African blacks

A histochemical and immunohistochemical study

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Summary

Twenty specimens from patients who had undergone oesophagectomy for invasive squamous carcinoma of the oesophagus were examined for morphological evidence of human papillomavirus infection; it was found in 13 specimens. Nineteen specimens showed focal epithelial hyperplasia of the non-neoplastic mucosa. The material was also submitted to immunoperoxidase and modified Feulgen staining to detect viral antigen. Positive Feulgen staining was detected in the superficial layers of the squamous mucosa in 15 specimens, while immunoperoxidase was entirely negative. This demonstrates a possible association between human papillomavirus and oesophageal carcinoma and that the modified Feulgen method may be more sensitive than immunoperoxidase for the detection of viral antigen. Electron microscopy and molecular hybridisation would have to be used for confirmation.

The human papillomaviruses (HPVs) are a heterogeneous group of DNA viruses that have been characterised into over 30 different serotypes. HPVs are considered unique in that they produce tumours in their natural host, the majority of lesions being benign. Some serotypes, however, appear to play a part in the development of malignant lesions, particularly carcinoma of the cervix. Viral condylomatous change has been observed in up to 60% of patients with cervical intra-epithelial neoplasia.\(^1\)

Viral koilocytic changes have been described in the mucosa of the oesophagus,\(^2\) and similar changes have been noted in black South Africans.\(^3\)

Material and methods

The material used in the study was derived from the files of the histopathology laboratory of the South African Institute for Medical Research at Baragwanath Hospital, Johannesburg, and comprised 20 oesophagectomy specimens removed during surgery for squamous carcinoma from 1983 to 1988. Nine of the specimens came from male patients aged 38 - 70 years, and 11 from women aged 29 - 66 years. Sections containing non-neoplastic epithelium were stained routinely with haematoxylin and eosin, and the modified Feulgen method.\(^4\) They were also stained with polyclonal antibody to HPV (Dako B580; Dakopatts, Denmark). HPV antigen was identified by means of an avidin and biotinylated horse-radish peroxidase complex (ABC technique, Vector laboratories). A verruca vulgaris was used as control material for the detection of viral product. All specimens were examined independently by two observers for the presence of koilocytosis in the non-neoplastic epithelium.

Results

Thirteen of the 20 specimens (65%) exhibited at least one area of koilocytosis, which in all instances were flat lesions (Fig. 1). The condylomatous changes were situated adjacent to infiltrating carcinoma in 11 specimens. Six of these 13 specimens also showed condylomatous change distant from the carcinoma. In 19 of the 20 resections the squamous epithelium showed diffuse alteration of the natural orientation and polarisation of the superficial layers resulting in a haphazard arrangement of the nuclei, foci of parakeratosis and focal epithelial hyperplasia (Figs 2 and 3). In some cases intracytoplasmic keratohyaline granules were seen.

Fig. 1. Oesophageal mucosa showing condylomatous atypia accompanied by acanthosis and basal cell hyperplasia (H and E \(\times 250\)).

Intra-epithelial carcinoma was seen in 5 of the 20 specimens of invasive carcinoma; in 4 instances this was associated with condylomatous atypia. With the Feulgen stain, 15 specimens showed intranuclear Feulgen staining, 3 were equivocal and 2 were negative. Feulgen positivity always conformed with the epithelial alterations described above (Fig. 4). Immunohistochemistry for HPV antigen was negative in all cases.
Syrjanen's study with demonstration of papillomatous tumour in Camer squamous cell carcinomas. Evidence of material Geschwulsforsch canal of cattle. Recognition of alimentary control. Histological changes identical to those of condylomatous morphological oesophageal Syrjanen. Clinicopathologic orders, and more recently have been closely linked to cervical intra-epithelial neoplasia. HPV's have been associated with a variety of epithelial disorders, and more recently have been closely linked to cervical intra-epithelial neoplasia. After Syrjanen's recognition of condylomatous changes in the oesophagus, other investigators have also demonstrated morphological and immunohistochemical evidence supporting HPV infection in this site. An animal model also exists in the form of papillomavirus-induced fibropapillomatosis in the upper alimentary canal of cattle. Focal epithelial hyperplasia and disturbance of epithelial maturation has been associated with morphological evidence of HPV infection of the oesophagus but, to our knowledge, has not been described in South African blacks. The Feulgen positivity in our 15 specimens conforms to these areas of abnormality, suggesting that this is representative of viral infection. Lucia et al. also demonstrated superficial Feulgen positivity in a variety of HPV-induced lesions and were able to correlate these areas with ultrastructural demonstration of viral particles. We have attempted to do likewise, but have failed for technical reasons. Intranuclear Feulgen positivity was identified in the superficial cells of the control material suggesting that the stain detects fully assembled viral particles. The Feulgen stain was positive in the infiltrating tumour in several specimens. It may be that in these areas the presence of abundant intracytoplasmic keratin may lead to protection of nuclear DNA from hydrolysis by DNAse and a false-positive result.

The low detection rate of HPV antigen using immunohistochemical investigations has been commented on by others. A possible reason for reduced positivity is that commercial antisera are obtained from papillomavirus types 1, 2 and 4, while types 6 and 11 are associated with condylomas. The presence of immunoreactivity may also be directly related to a high number of viral DNA copies in the cell.

This study has provided preliminary evidence of a possible HPV relationship with carcinoma of the oesophagus in black patients. The precise role of HPV is indefinite, since it is a ubiquitous agent and may be acting as a co-carcinogen. HPV infection appears to be present in oesophageal mucosa of patients with both benign and malignant conditions. Koilocytosis, however, appears to be a focal phenomenon that may not be detected in biopsy specimens. Immunoperoxidase techniques seem to have a low sensitivity for the detection of HPV DNA. However, if Feulgen staining can be demonstrated to be representative of the virus, its diffuse distribution may make it a valuable indicator of viral infection.

**REFERENCES**

2. Syrjanen KJ. Histological changes identical to those of condylomatous lesions found in oesophageal squamous cell carcinomas. Arch Geschwulsforsch 1982; 52: 283-292.