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Main Articles

The incidence and detection of HPV in the upper aerodigestive tract using brush and biopsy techniques

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Abstract

The association between human papillomavirus (HPV) and squamous carcinoma is well established. In the polymerase chain reaction (PCR) we have an effective technique for detecting small quantities of viral DNA, but the assay requires tissue taken at endoscopy to reveal the presence or absence of HPV. Brushings have been used effectively elsewhere in the body to obtain cytological material. This study set out to ascertain if sufficient viral DNA could be obtained, using a laryngeal brushing and the PCR, to detect the presence or absence of HPV.

Six patients with squamous carcinoma of the larynx and seven controls who were having laryngoscopy for other reasons underwent laryngeal biopsy. In addition, in the patients with carcinoma, biopsies were taken at the tumour margins and brushings from both sites. The samples were tested for the presence of HPV types 6, 11, 16, 18 and 31 by means of the PCR. The distribution of HPV types was as expected in the biopsy specimens, but only one brushing detected any HPV type at all. We conclude that laryngeal brushing is an inadequate technique for assessing the presence of HPV in the larynx.

Key words: Human papillomavirus; Polymerase chain reaction; Carcinoma, squamous cell; Biopsy; Brushing

Introduction

Viruses have been implicated as carcinogenic agents for many years. More recently the human papillomavirus (HPV) has been implicated in the aetiology of premalignant epithelial conditions (Fuchs *et al.*, 1988; Lehn *et al.*, 1988). HPV is an epitheliotropic DNA virus of the Papovaviridae family and has consistently been identified in squamous cell carcinoma of the female and male genital tract (Zur Hausen, 1987). There is increasing evidence of a causal link between HPV and cervical cancer. However, the role of HPV in carcinoma of the upper aerodigestive tract remains unclear. Previous studies have shown conflicting results. Older studies were inconsistent because of a lack of a sensitive method of detecting HPV which is very species specific. More recent studies utilizing the polymerase chain reaction (PCR) amplification assay method have been criticized because of small numbers and lack of suitable controls. Others were retrospective analyses of paraffin blocks, lacking clinical details. The problem of obtaining controls from normal subjects involves ethical issues, i.e. the potential risk of biopsies of the laryngopharynx in normal individuals. In a recent study by Jalal *et al.* (1992) the PCR method was used to detect HPV type 16 in oral scrapings rather than biopsies. This was possible because the PCR method is extremely sensitive, requiring very little cellular material to detect viruses. Our study set out to ascertain whether the technique of brushing would provide sufficient cellular material, comparable to biopsies, for the detection of HPV. This would obviate the risks of biopsies of the laryngopharynx in normal subjects, allowing properly controlled studies of the HPV in the upper aerodigestive tract.

Patients and methods

The study group consisted of patients undergoing direct laryngoscopy for various reasons at the Royal National Throat, Nose and Ear Hospital. Patients with squamous cell carcinoma had two biopsies taken, one from the tumour itself and one from the

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macroscopically normal laryngeal margin. At the same time brushings were taken from the same places as the biopsies. All specimens were preserved in sterile 50 per cent alcohol and processed and typed by the PCR amplification assay. Also an equivalent number of controls undergoing direct laryngoscopy for non-neoplastic disease were biopsied and the specimens processed in an identical manner. samples were tested blindly for human papillomavirus types 6, 11, 16, 18 and 31.

Results

Six patients with carcinoma of the larynx and seven benign controls were studied. The controls were aged between 16–83 years old, three were female and four male, and only one of the patients was a smoker. Two patients had benign vocal fold nodules, two vocal fold polyps, one bilateral vocal fold abductor palsy, one unilateral vocal fold palsy and one a post-intubation subglottic stenosis.

The patients with known carcinoma of the larynx were all male, all smokers and had an age range of 51–73 years. Three had had radiotherapy previously. Three patients had T4 disease, two T3 and one T1. Three patients had neck nodes.

No patients tested positive for HPV 18, 11 or 31. Four of the patients with carcinoma (66 per cent) had HPV 16 detectable in one or more of the tissue samples. Three of the patients with benign pathology (43 per cent) had HPV 6 detectable in the biopsies, although one (23-year-old female with vocal fold nodules) tested positively for HIV 16. Only one brushing yielded any viral material at all and this correlated with positive biopsies from both the tumour and the tumour-free margin, although even in this patient the tumour margin brushing was negative. Interestingly, in two patients both HPV 6 and 16 were detected. These results are summarized in Table I.

Discussion

The technique of brushing has been widely used to acquire material for cytological analysis. It has been described as a useful tool in the diagnosis of bronchial carcinoma (Debeljak *et al.*, 1994), gastric tumours (Sherman *et al.*, 1994), pancreaticobiliary tumours (Ryan and Baldauf, 1994), cervical carcinoma (Covell and Frierson, 1992) and the investigation of benign lung diseases such as asthma (Gibson *et al.*, 1993). In all these accounts it is emphasized that the technique has a high degree of specificity but low sensitivity. This is highlighted by Popp *et al.* (1992) who point out that, in the diagnosis of bronchial carcinoma, with a single brushing, sensitivity may be as low as 68 per cent, though this figure may be improved to a maximum of 89.6 per cent by using up to five separate brushings.

TABLE I

THE RESULTS OF THE PCR ASSAY PERFORMED ON THE BIOPSY AND BRUSHING SPECIMENS.

Case no.	Site	HPV 6	HPV 11	HPV 16	HPV 18	HPV 31
1	Larynx	Negative	Negative	Negative	Negative	Negative
2	Larynx	Negative	Negative	Negative	Negative	Negative
3	Larynx	Positive	Negative	Negative	Negative	Negative
4	Larynx	Positive	Negative	Negative	Negative	Negative
5	Larynx	Positive	Negative	Negative	Negative	Negative
6	Larynx	Negative	Negative	Negative	Negative	Negative
7	Larynx	Negative	Negative	Positive	Negative	Negative
8	TcBx	Negative	Negative	Positive	Negative	Negative
8	TxBr	Negative	Negative	Negative	Negative	Negative
8	Mar Bx	Positive	Negative	Negative	Negative	Negative
8	Mar Br	Negative	Negative	Negative	Negative	Negative
9	TxBx	Negative	Negative	Negative	Negative	Negative
9	TxBr	Negative	Negative	Negative	Negative	Negative
9	Mar Bx	Negative	Negative	Negative	Negative	Negative
9	Mar Br	Negative	Negative	Negative	Negative	Negative
10	TxBx	Negative	Negative	Negative	Negative	Negative
10	TxBr	Negative	Negative	Negative	Negative	Negative
10	Mar Bx	Negative	Negative	Positive	Negative	Negative
10	Mar Br	Negative	Negative	Negative	Negative	Negative
11	TxBx	Positive	Negative	Positive	Negative	Negative
11	TxBr	Negative	Negative	Positive	Negative	Negative
11	Mar Bx	Negative	Negative	Negative	Negative	Negative
11	Mar Br	Negative	Negative	Positive	Negative	Negative
12	TxBx	Negative	Negative	Positive	Negative	Negative
12	TxBr	Negative	Negative	Negative	Negative	Negative
12	Mar Bx	Negative	Negative	Negative	Negative	Negative
12	Mar Br	Negative	Negative	Negative	Negative	Negative
13	TxBx	Negative	Negative	Negative	Negative	Negative
13	TxBr	Negative	Negative	Negative	Negative	Negative
13	Mar Bx	Negative	Negative	Negative	Negative	Negative
13	Mar Br	Negative	Negative	Negative	Negative	Negative

Patients 1–7 had benign pathology and underwent one laryngeal biopsy. patients 8–13 had squamous cell carcinoma of the larynx and underwent biopsies from the tumour (TxBx) and the tumour margin (Mar Bx), and brushings from the tumour (TxBr) and the tumour margin (Mar Br).

The technique has been used by Sonnex *et al.* (1991) to detect HPV, though not in the larynx. This group used brushings in conjunction with DNA hybridization to detect anal canal HPV infection. They report a sensitivity for HPV types 6, 11, and 16 of between 83–98 per cent according to site, and credit their improved detection rate compared to other studies to the use of brushings to obtain their samples.

We had hoped that the technique would also prove to be a useful tool in the detection of HPV in the larynx. In combination with the PCR method of DNA amplification it was thought that adequate cellular material could be obtained to get an accurate assessment of HPV presence. This would overcome ethical concerns regarding biopsies in patients with no laryngopharyngeal lesions, and considerations when taking biopsies from the normal larynx. This in turn would dramatically increase the number and range of subjects who could be studied and thereby increase our knowledge and understanding of patterns of HPV infection in the macroscopically normal larynx. This would negate the obvious criticism of the only study currently looking specifically at HPV in the normal larynx (Nunez *et al.*, 1994), namely the use of autopsy specimens.

In his study the presence of HPV in the ratio predicted by Morgan *et al.* (1991) is confirmed by the tumour and margin biopsies. HPV seems closely associated with malignant disease as predicted (Kashima *et al.*, 1986; Dekmezian *et al.*, 1987; Brandsma and Abramson, 1989; Kashima *et al.*, 1990). Unfortunately, only in one patient did the brushing correspond to the biopsy. The inference from this is that the viral load in patients with laryngeal carcinoma is low and brushing appears to pick up insufficient viral DNA in most cases for the reliable detection of HPV. Certainly Dekmezian *et al.* (1987) confirms that the epithelial layers contain both infectious particles and viral DNA, therefore if these were present in sufficient quantities they should be detected by the use of brushing and the PCR amplification technique.

The biopsy positive to HPV 16 in a 23-year-old non-smoking female is curious, and previously unreported in a normal larynx. It is possible that this is a false positive generated by the over sensitivity of the PCR method as described by Bryan *et al.* (1990) although a more likely explanation is that this represents 'normal carriage' as seen in the genital tract. Further research on a larger scale is required on normal larynges, however this will require the taking of biopsies and not, as was hoped, merely brushings.

Conclusions

Controls seem to substantiate the concept that HPV6 is associated with normal larynges and HPV16 is a marker of malignancy. Unfortunately, presumably due to a low viral load in the tissues, brushing is an unreliable method of detection of HPV DNA in the larynx.

References

Brandsma, J. L., Abramson, A. L. (1989) Association of papillomavirus with cancer of the head and neck. *Archives of Otolaryngology, Head and Neck Surgery* **115:** 621–625.

Bryan, R. L., Bevan, I. S., Crocker, J., Young, L. S. (1990) Detection of HPV 6 and 11 in tumours of the upper respiratory tract using the polymerase chain reaction. *Clinical Otolaryngology* **15:** 177–180.

Covell, J. L., Frierson, H. F. (1992) Intraepithelial neoplasia mimicking microinvasive squamous-cell carcinoma in endocervical brushings. *Diagnostic Cytopathology* **8 (1):** 18–22.

Debeljak, A., Mermolja, M., Sorli, J., Zupancic, M., Zorman, M., Remskar, J. (1994) Bronchiolar lavage in the diagnosis of peripheral primary and secondary malignant lung tumours. *Respiration* **61:** 226–230.

Dekmezian, R., Batsakis, J., Goepfert, H. (1987) In situ hybridization of papillomavirus DNA in head and neck squamous cell carcinomas. *Archives of Otolaryngology, Head and Neck Surgery* **113:** 819–821.

Fuchs, P., Girardi, F., Pfister, H. (1988) Human papillomavirus DNA in normal, metaplastic, preneoplastic, and neoplastic epithelia of the cervix uteri. *International Journal of Cancer* **41:** 41–45.

Gibson, P., Allen, C., Yang, J., Wong, B., Dolovich, J., Denburg, J., Hargreave, F. (1993) Intraepithelial mast cells in allergic and non-allergic asthma: assessment using bronchial brushings. *American Review of Respiratory Diseases* **148:** 80–86.

Jalal, H., Sanders, C., Prime, S., Scully, C., Maitland, N. (1992) Detection of human papillomavirus type 16 DNA in oral squames from normal young adults. *Journal of Oral Pathology and Medicine* **21:** 465–470.

Kashima, H., Kutcher, M., Keiss, T., Levin, S., De Villiers, E., Shah, K. (1990) Human papillomavirus in squamous cell carcinoma, leukoplakia, lichen planus, and clinically normal epithelium of the oral cavity. *Annals of Otology, Rhinology and Laryngology* **99:** 55–61.

Kashima, H., Mounts, P., Kuhajda, F., Loury, M. (1986) Demonstration of human papillomavirus capsid antigen in carcinoma in situ of the larynx. *Annals of Otology, Rhinology and Laryngology* **95:** 603–607.

Lehn, H., Villa, L., Marziona, F., Hilgarth, M., Hillemans, H., Sauer, G. (1988) Physical state and biological activity of human papillomavirus genomes in precancerous lesions of the female genital tract. *Journal of General Virology* **69:** 187–196.

Morgan, D., Abdullah, V., Quiney, R., Myint, S. (1991) Human papillomavirus and carcinoma of the laryngopharynx. *Journal of Laryngology and Otology* **105:** 288–290.

Nunez, D., Astley, S., Lewis, F., Wells, M. (1994) Human papillomaviruses: a study of their prevalence in the normal larynx. *Journal of Laryngology and Otology* **108:** 319–320.

Popp, W., Merkle, M., Schreiber, B., Rauscher, H., Ritschka, L., Zwick, H. (1992) How much brushing is enough for the diagnosis of lung tumours? *Cancer* **70:** 2278–2280.

Ryan, M., Baldauf, M. (1994) Comparison of flow cytometry for DNA content and brush cytology for detection of malignancy in pancreaticobiliary strictures. *Gastrointestinal Endoscopy* **40:** 133–139.

Sherman, M., Anderson, C., Herman, L., Ludwig, M., Erozan, Y. (1994) Utility of gastric brushing in the diagnosis of malignant lymphoma. *Acta Cytologica* **38:** 169–174.

Sonnex, C., Schofield, J., Kocjan, G., Kelly, G., Whatrup, C., Mindel, A., Northover, J. (1991) Anal human papillomavirus infection: a comparative study of cytology, colposcopy and DNA hybridization as methods of detection. *Genitourinary Medicine* **67:** 21–25.

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