Carcinomas occurring in papillomas of the nasal septum associated with Human Papilloma Virus (HPV)*

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INTRODUCTION

The most common benign sinonasal neoplasia is papilloma (Hyams, 1971). The incidence rate in a Danish sub-population has been estimated as 7.4 per million per year (Buchwald et al., 1995a). Histologically, the sinonasal papillomas are divided into 3 different subtypes: inverted papillomas, columnar cell papillomas, and exophytic papillomas (Shanmugaratnam and Sobin, 1991; Hellquist, 1990). Exophytic papillomas are predominantly localised on the nasal septum while the others almost always occur on the lateral nasal wall or in the adjacent sinuses. Inverted papilloma has in several reports been associated with squamous cell carcinoma. The malignant component may be present at the first presentation or it may appear in a recurrent lesion. The incidence of carcinomatous transformation or concurrent malignancy in inverted sinonasal papillomas is approximately 10%. The columnar cell papilloma associated with squamous cell carcinoma has rarely been reported. Exophytic papillomas associated with malignancy have apparently not been reported until now (Hyams, 1971; Hellquist, 1990; Kashima et al., 1992; Buchwald et al., 1995a).

The frequency of HPV in sinonasal papillomas has been found to vary from 10% to 75% (Furuta et al, 1991; Kashima et al., 1992; Wu et al., 1993; Tang et al., 1994; Beck et al., 1995; Buchwald et al., 1995b). The diversity in the reported frequencies is a major problem, and at present the role of HPV in relation to sinonasal papillomas is unclear. The reasons for the variation in the reported frequency of HPV in sinonasal papillomas may be the result of different techniques and different histological definitions of the subtypes of sinonasal papillomas. In addition, geographical differences may exist. Similar variations in the reported results on the role of HPV are found in publications on oral and laryngeal carcinomas. A discussion of these important issues is outside the scope of the present study, but a few comments on the techniques may be appropriate.

PCR is the most sensitive method for the demonstration of HPV-DNA. A consequence of this high sensitivity is the risk of false-positive results, due to contamination with minute amounts of HPV-DNA during the collection of samples, or in the PCR laboratory. DNA in situ hybridisation, on the other hand, is less sensitive and may yield false-negative results.

SUMMARY

Carcinomas arising in pre-existing sinonasal papillomas of the nasal septum are rare. To our knowledge only one case has been reported. We report two cases of carcinomas occurring in septal papillomas. In the first case a carcinoma developed in an exophytic papilloma 16 years after the first operation for a papilloma. In the second case a carcinoma was present at the first presentation within an inverted papilloma, and a metastasis had also developed. In the first case HPV type 6/11 was demonstrated by in-situ hybridisation and PCR in the original papilloma as well as in the recurrent papilloma and in the carcinoma. In the second case HPV type 18 was found in the nasal lesion as well as in the metastasis. All samples were examined for Epstein-Barr virus (EBV) by PCR, but with negative results. We believe that case one is the first reported case of carcinomatous transformation within an exophytic septal papilloma.

Key words: sinonasal papilloma, nasal carcinoma, HPV, EBV

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However, the interpretation of results of in situ hybridisation may imply subjectivity when the positive reaction is weak and only comprises a small number of “positive” cells. In order to avoid false-positive results it seems prudent not to record uncritically such weak results as positive.

Recently, the presence of another oncogene virus, Epstein-Barr virus (EBV), has been demonstrated in inverted papillomas (McDonald et al., 1995).

We here present two cases of carcinoma occurring in papillomas of the nasal septum and discuss the possible viral aetiology.

MATERIAL AND METHODS

Clinical record of Case 1

A 41-year-old male was admitted with a tumour involving the right nasal septum and roof of the mucosa-lining roof of the vestibulum nasi. A biopsy had shown an exophytic papilloma with areas of carcinoma in situ. The patient explained that he had been operated upon 16 years ago for three minor benign papillomas localised in the same anatomical area as the present lesion. This was confirmed by reviewing the surgical and pathological files (Figure 1a).

Two months after the primary surgery a recurrence on the nasal septum had been visible by self-inspection. During the following years he had observed minor changes in the size of the lesion but he did not find re-examination important. We had a CT scan performed which showed thickening of the septal mucosa on the right side. The anterior nasal roof and the right septal mucoperichondrium were excised en bloc after an alar rhinotomy, and the defect was covered by a split-thickness skin graft. The patient recovered uneventfully. Histological examination of the tumour revealed an exophytic papilloma with squamous cell carcinoma (Figure 1b). After surgery, the patient underwent radiotherapy. This combined treatment did not prevent recurrences, and within the following 18 months the patient was re-operated twice. Currently, six months after the last surgical interventions, he is without recurrence. A surgical procedure is planned to reconstruct the external nose.

Clinical record of Case 2

A 42-year-old female presented with a 1-year history of intermittent blood-stained nasal discharge and a 4-month history of a swelling on the upper left part of the neck. Clinical examination revealed a papillomatous tumour based on the left nasal septum and a 2\times3\times3 cm mass in the neck below the left angle of the man-
A biopsy from the nasal lesion showed an inverted papilloma with mild dysplasia. A CT scan showed a tumour of the left nasal septum extending to the lamina cribrosa (Figure 2). A frozen biopsy of the mass in the neck revealed a metastatic carcinoma. The septal lesion was re-biopsied, and at this time histological evaluation showed an inverted papilloma with invasive squamous cell carcinoma (Figures 1c-d). The patient underwent radiation therapy. Currently, one year after radiotherapy, she is without signs of recurrence.

**HPV and EBV studies**

Tissue blocks of formalin-fixed and paraffin-embedded papillomas and carcinomas, removed during surgery, were investigated for HPV-DNA by *in situ* hybridisation. Using PCR the samples from the blocks were also examined for HPV- and EBV-DNA.

**In situ hybridisation**

*In situ* hybridisation was performed with HPV wide-spectrum biotin-labelled DNA probes for detection of the vast majority of HPV types of mucosal origin (pan-HPV; Krea-Tech Diagnostic, The Netherlands). HPV-positive sections were subsequently examined with HPV small-spectrum DNA probes including HPV groups 6/11, 16/18 and 31/33/51 (Enzo Diagnostic). *In situ* hybridisation was performed as previously described (Buchwald et al., 1995b).

**Polymerase chain reaction**

Two 10-µm sections were cut from each tissue block and prepared for PCR as described. In order to ensure the presence of amplifiable DNA, each sample was initially amplified with primers against a part of the human β-globin gene (Buchwald et al., 1993), and then examined for the presence of HPV by amplification with HPV-consensus primers targeting the E1 region (Smits et al., 1992; Tieben et al., 1993). The amplificates were examined by gel electrophoresis, stained with ethidium bromide and viewed by ultraviolet-light transillumination. Samples showing the 188-bp HPV band were further amplified with HPV type-specific primers against HPV types 6/11, 16, 18 and 31. The presence of an amplified DNA band of the expected size was interpreted as the presence of HPV of the specific type. In addition, the samples were amplified with EBV primers against a section of the reiterated BamHI-W fragment (Hörding et al., 1994). Appropriate positive and negative controls were included in each run.

**RESULTS**

**HPV and EBV studies**

Case 1: *In situ* hybridisation demonstrated the presence of HPV type 6/11 (Figure 3a) in the original papilloma. In the recurrent tumour the architecture of an exophytic papilloma was still present, in addition to areas of invasive carcinoma. HPV type 6/11 was demonstrated in the papillomatous parts of the tumour as well as in the carcinoma (Figure 3b). The presence of HPV types 6/11 was confirmed by PCR (Figure 4), while EBV was not detected (Data not shown).
Case 2: HPV was not demonstrated by *in situ* hybridisation, neither in the inverted papilloma, nor in the carcinoma, nor in the metastasis. However, PCR demonstrated HPV type 18 in the nasal tumour as well as in the metastasis of the neck (Figure 4). EBV was not demonstrated (Data not shown).

**DISCUSSION**

Primary carcinomas of the nasal septum are rare, comprising approximately 10% of all malignant lesions of the nasal cavity, and less than 350 cases have hitherto been reported (Sim et al., 1989; Ang et al., 1992; Fradis et al., 1993). In only one of these cases was a co-existing septal papilloma and carcinoma described (Ang et al., 1992; Fradis et al., 1993). Thus, the two presented cases brings the total number to three. Furthermore, Case 1 comprises the first reported case of a squamous cell carcinoma arising in an exophytic sinonasal papilloma. In a previous report we found HPV in 69% of exophytic papillomas, compared with only 6% in the inverted papillomas. The predominating HPV type was 6/11, which was present in every specimen. We also demonstrated HPV in 2 out of 5 inverted papillomas associated with carcinomas; HPV type 6 in one case and HPV type 18 in the other (Buchwald et al., 1995b). HPV types 6 and 11 are considered low-risk types according to their low-transforming potential, while HPV types 16 and 18 and others are considered high-risk types as they are associated with carcinogenesis, i.a. in the uterine cervix (Muñoz et al., 1994). Thus, the finding of HPV 6/11 in a benign exophytic papilloma (Figure 1a) in Case 1 is expectable. It is not possible to determine if HPV 6/11 had any causative role in the development of malignant lesion. It should, however, be noted that HPV 6/11 has been found in a number of carcinomas arising from pre-existing HPV-6/11-positive laryngeal papillomas, which suggests that this HPV type may not always be innocent (Lindeberg et al., 1989; DiLorenzo et al., 1992).

In Case 2, HPV type 18 was solely demonstrated by PCR in samples from the nasal lesions as well as in the metastasis. As *in situ* hybridisation failed to demonstrate HPV in the nasal lesions, it was not possible to determine if HPV 18 was present in the benign papillomatous part or in the malignant part or in both. The negative result may be explained by the lower sensitivity of *in situ* hybridisation. Corresponding to the high risk of malignant transformation of HPV-18-positive lesions of the uterine cervix, it appears likely that HPV 18 in Case 2 played an important role in the development of the carcinoma, as well as the metastasis (Schneider, 1994). However, definite proof cannot be provided. Assuming that the lesion started as a purely benign papilloma, HPV typing at an earlier stage might have warned of the malignant potential.

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**REFERENCES**


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