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Pathology in Focus

An aggressive and invasive growth of juvenile papillomas involving the total respiratory tract

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Abstract
A malignant course of juvenile laryngeal papillomatosis has rarely been reported. In the present case the patient had had laryngeal papillomas since the age of three years. The papillomas gradually spread to the entire respiratory system, and during 30 years the patient was operated on more than 80 times. At present an invasive tumour spreading from the tongue into the parapharyngeal space, extending to the cranial base, has been demonstrated by magnetic resonance imaging (MRI).

Intralesional therapy with Cidofovir, a promising antiviral drug against human papillomavirus (HPV) infection, was started with some clinical effect, although only on the superficial tumour growth. Histology of removed tumour tissue has demonstrated a mixture of exophytic and inverted growth pattern, and has mainly been interpreted as benign, in spite of a focally high mitotic index and an intermittent lack of maturation in the epithelium. In the most recent biopsies a verrucous carcinoma has been diagnosed. Expression of p53 was noted to increase in papillomas with time. All samples have been shown to harbour HPV 11, but no other HPV types.

Key words: Papilloma; Laryngeal diseases; Papilloma virus; Carcinoma; Immunochemistry

Introduction

Juvenile laryngeal papillomatosis is usually diagnosed between the ages of 0.5–10 years (Strong *et al.*, 1979; Cohen *et al.*, 1980; Mounts and Shah, 1984; Abramson *et al.*, 1987). The incidence in Denmark is 3.6 per million per year (Lindeberg and Elbrønd, 1990). The route of infection is controversial, but most likely the infection is acquired during or even before birth from mothers with genital HPV infections.

The lesions are aggressive and can occur throughout the respiratory tract, although it is most commonly seen in the glottis. Human papillomavirus (HPV) type 6 and 11 is known to be the main cause of the disease (Steinberg *et al.*, 1983; Lindeberg and Elbrønd, 1990; Pignatari *et al.*, 1992; Buchwald *et al.*, 1995).

The tumours have a low malignant potential, although they often display some degree of nuclear atypia and mitotic activity (Quick *et al.*, 1979). Carcinomas arising in respiratory papillomas have been reported in a number of cases, and an elevated risk has been demonstrated following irradiation (Lindeberg *et al.*, 1989; Lindeberg and Elbrønd, 1991). Duration of the disease and recurrence rate is unpredictable. We report a case of juvenile laryngeal papillomatosis with an unusual aggressive and protracted course, with a focally dysplastic growth pattern leading to the dubious diagnosis of invasive cancer on two occasions with an interval of 13 years and most recently to the diagnosis of verruous carcinoma, involving most of the upper airways and leading to death.

Case report

The patient is a 34-year-old heterosexual non-smoking male, the second of two children. An older sister shows no signs of the disease. The mother had no known HPV-caused disease and birth was at term and uncomplicated. The patient was first admitted to hospital at three and a half years of age, where an adenotonsillectomy was performed because of recurrent episodes with tonsillitis and catarrh. Four months later the patient was readmitted with stridor, and papillomas localized to the vocal folds and subglottis were removed. From early childhood to puberty the papillomas recurred mainly in the larynx, the trachea and at one occasion in the bronchial tree. In puberty no new papillomas were diagnosed. After puberty the papillomas recurred and spread to the nasopharynx and the nasal cavity. After 30 years duration of disease the patient has been admitted more than 80 times and has had large masses of papillomas removed from the following localizations: larynx, trachea, bronchi, nasopharynx, a fistula between trachea and skin (due to a transient tracheostomy), cavum nasi, right tonsil and the right side of the tongue root. The patient has been thoroughly examined, and no complicating disease or immunodeficiency has been detected. Histopathological evaluation

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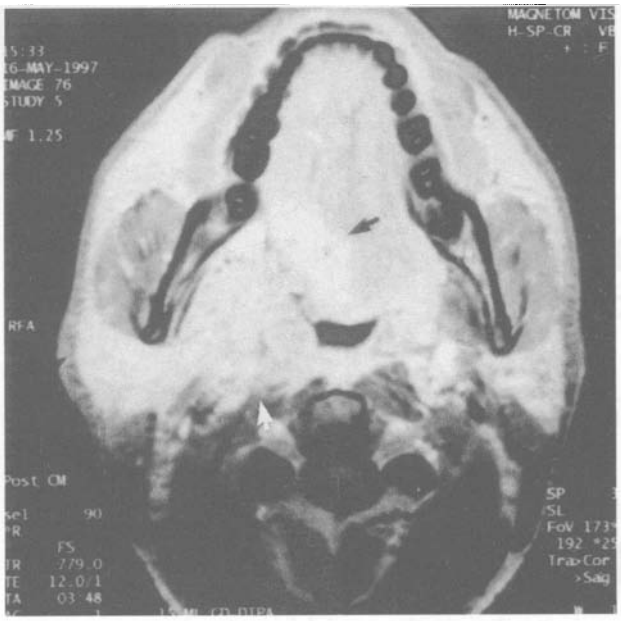

FIG. 1
MRI of head and neck showing tumour borders marked by black and white arrow.

revealed areas with dysplasia of the epithelium in many regions and an atypical growth pattern with inverted papillomatosis, in areas lacking a distinct basal cell layer, thus leading to the diagnosis of invasive carcinoma at the age of 20. At this time the diagnosis lacked clinical support, and no specific action was taken. In the last years the disease has progressed more rapidly, and after one transfacial approach to the nasal cavity and the nasopharynx, where large numbers of papillomas were resected, new therapeutic attempts were made. Treatment with interferon was without effect. Cidofovir R[(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, HPMPC] has been demonstrated to inhibit the growth of tumours induced by HPV (Snoeck *et al.*, 1995), and local treatment once a week, using Cidofovir R at a concentration of 2.5 mg/ml, 10-15 ml each time, was initiated. After two months, regression of superficial tumour growth into the pharyngeal space in the treated areas could be demonstrated, but the patient complained of progressing pain in the right side of the face and neck. MR-scanning of the pharynx (Figure 1) demonstrated tumour progression to the right side of the oropharynx with involvement of the tongue, the retromaxillary space, the parotid gland and involvement of basis cranii. The latest biopsies revealed a highly differentiated squamous carcinoma. Attempts to control the tumour progression by radiotherapy failed, and the patient died. No post mortem was performed.

Materials and methods

Immunohistochemistry

Six-μm sections were cut from formalin-fixed, paraffin-embedded tissue blocks and mounted on glass slides. Immunohistochemistry was performed using microwave pretreatment, anti-p53 (clone DO7, Dako, Denmark) and streptavidin-biotin-peroxidase methodology (Dako, Denmark).

PCR

From each selected tissue block, three to five sections were cut, the number of sections depending on the amount of tissue present in the individual blocks. The sections were placed in 1.5 ml standard tubes. Two empty tubes were marked as negative controls. Proteinase K (200 μlitre/ml in saline) was added and the tubes were placed at 65°C for two hours. After a short spin the tubes were placed at 37°C overnight. The paraffin, forming a lid on the top, was penetrated with a pipette tip and the DNA-solution transferred to a new tube and placed at 95°C for 15 minutes to inactivate the proteinase K. The polymerase chain reaction (PCR) was performed as described (Lindeberg *et al.*, 1989; Buchwald *et al.*, 1995) with a 3.3 μlitre DNA solution in a total volume of 33 μlitre, with type-specific primers against HPV6/11, 16, 18 and 31. Twelve μlitre of the amplified material was run on a four per cent submerged agarosegel (NuSieve 3:1) in TAE buffer and examined under ultraviolet light after staining with ethidium bromide. Appropriate positive and negative controls were used in all procedures. Details of procedures and precautions taken to avoid false positive results have been described elsewhere (Buchwald *et al.*, 1993; Hørding, 1994; Buchwald *et al.*, 1995; Husman *et al.*, 1995).

The amplified GP-product was spotted onto nylon filter and fixed, and hybridized with AP-labelled oligoprobes against HPV types 6/11 and 16. As HPV 6/11 had been demonstrated in all samples, we amplified the most recent sample with HPV consensus primers MY09/MY11. The expected 449 bp fragment was excised from the gel and the DNA recovered by electroelution and cut with the DNA restriction enzyme Pst 1. This enzyme does not cut the MY-fragment of HPV 6, while HPV 11 is cut into two fragments of 208 and 241 bp (Figure 2).

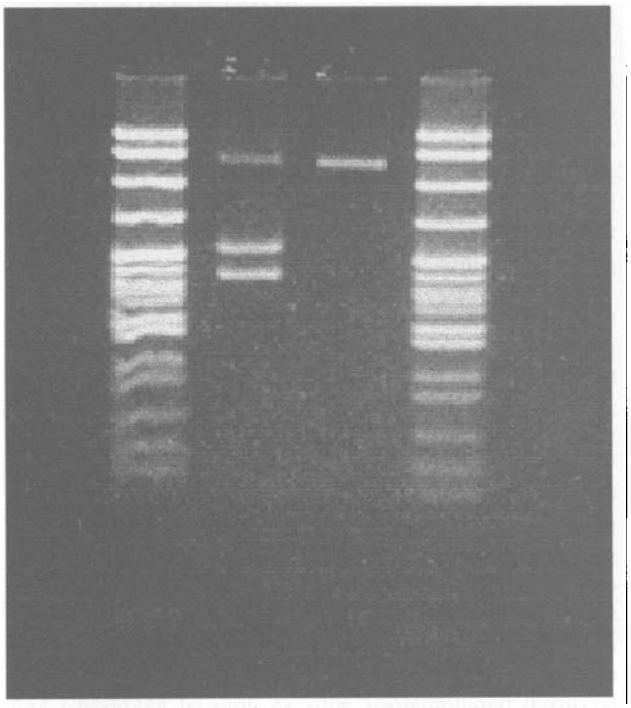

FIG. 2
Lanes 1 & 4: Size markers (pBR322/Msp I digest).
Lane 2: The amplified MY09/MY11-fragment of 449 bp.
Lane 3: Pst digest of the MY09/MY11 fragment showing 2 bands of 247 and 202 bp.

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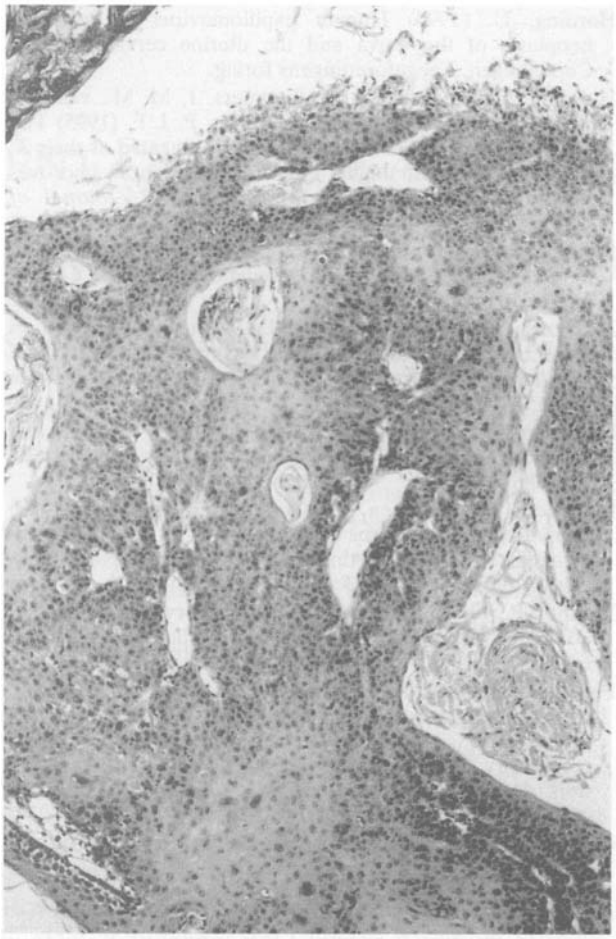

FIG. 3
Area showing pronounced cellular atypia in exophytic part of papilloma (H & E; × 100).

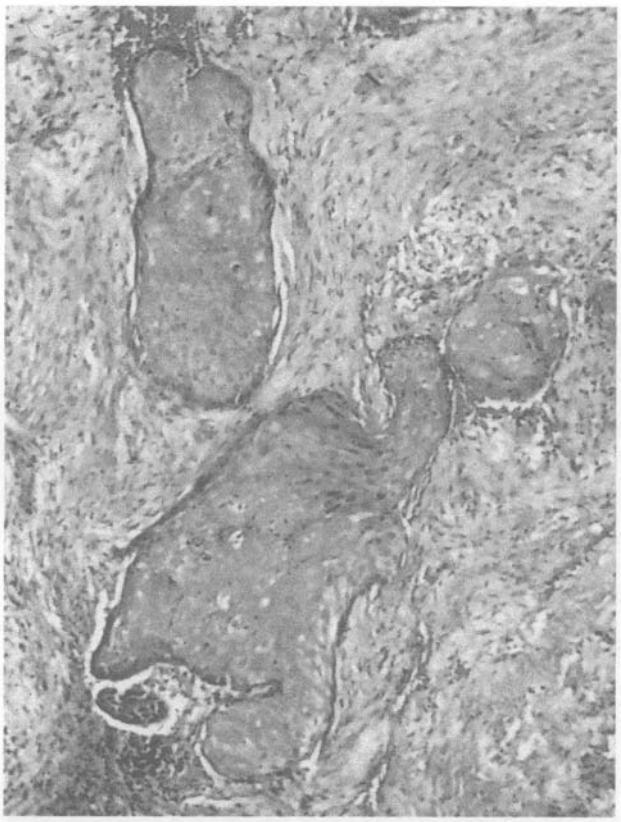

FIG. 4
Atypically inverted growth pattern with areas of invasion (H & E; × 40).

Results

Seven tissue blocks, ranging from biopsies taken at onset to biopsies taken after treatment with Cidofovir, were selected after examination of the haematoxylin and eosin stained tissue sections. Representative tissue blocks were selected: from the time of debut, from the first dubious diagnosis of invasive cancer, from later recurrences of exophytic/atypical inverted papillomas (Figure 3), and from the latest recurrence showing squamous carcinoma (Figure 4). All samples showed the expected 140 bp band after PCR with HPV 6/11 primers and after RFLP they were verified to be HPV 11 only (Figure 2). All samples were negative with primers against HPV 16, 18 and 31. By immunohistochemistry all sections show positive reaction to anti-p53, most pronounced in the parabasal and basal cell layer. The expression was stronger in recent biopsies, and a consistent high expression has been found in areas with dysplasia (Figure 5).

Discussion

Malignant change of laryngeal papillomatosis is rare, but has been reported (Lindeberg *et al.*, 1989; Gayliss and Hayden, 1991; Guillou *et al.*, 1991; Lindeberg and Elbrønd, 1991; Simma *et al.*, 1993). The protracted and aggressive course of disease described in the presented case of human papillomatosis is unusual, as well as the localization in the nasopharynx. In a number of reports HPV has been demonstrated in laryngeal carcinomas, most often HPV 16 (Brandsma *et al.*, 1986). However, the number of HPV-positive laryngeal carcinomas in published reports shows an unacceptable variation of 3.3–85 per cent (Lindeberg and Kroghdahl, in press). Only HPV type 11 could be demonstrated in the present case. Nuclear expression of p53 has been shown to be increased in the more dysplastic lesions, but is present in normal and benign papillomatous epithelium as well. A recent paper (Tan *et al.*, 1996) postulates that the seemingly increased expression of p53 is a manifestation of wild type protein more often found in the basal cells of the respiratory epithelium. Others have described an association between the transforming gene product HPV E6 and p53. The E6 gene product binds and inactivates p53, resulting in an abolition of the tumour suppression performed by wild type p53 (Stoppler *et al.*, 1994). The inactivation of p53 will lead to an accumulation of p53 intracellularly, making the immunohistochemical detection of the protein possible. The presence of HPV in non-diseased epithelium in patients with juvenile laryngeal papillomatosis (Pignatari *et al.*, 1992) can explain the expression of p53 in non-neoplastic epithelium found both in our study and in that of Tan *et al.* (1996).

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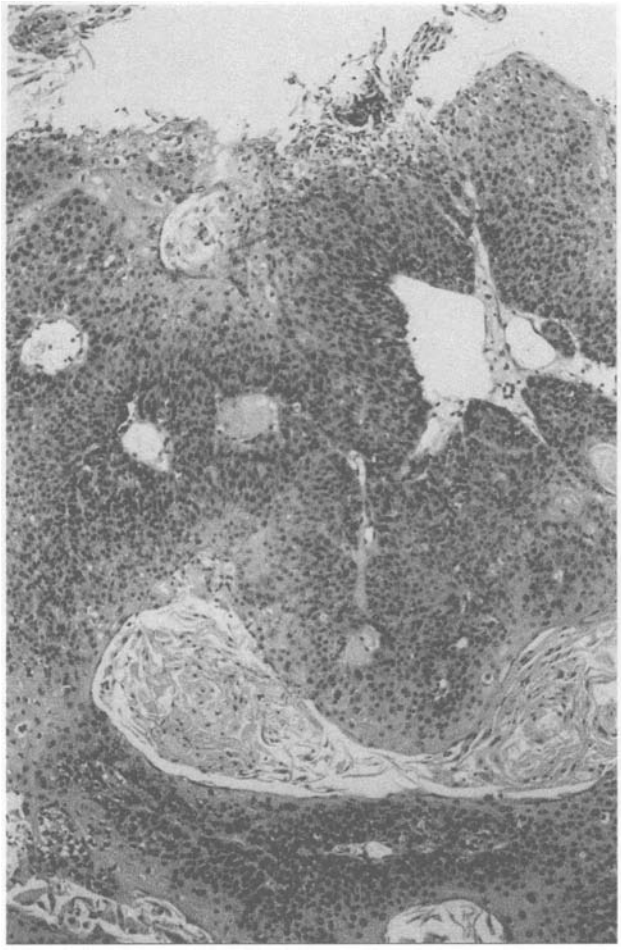

FIG. 5

Strong expression of p53 in dysplastic papillomatous epithelium (P53; × 100).

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