CASE REPORT

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IN VIVO REAL-TIME DIAGNOSIS OF NASOPHARYNGEAL CARCINOMA IN SITU BY CONTACT RHINOSCOPY

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Accepted 16 April 2005
Published online 30 August 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/hed.20281

Abstract: Background. Nasopharyngeal dysplasia or nasopharyngeal carcinoma in situ (NPCIS) lesions have rarely been reported. Timely diagnosis of the preinvasive lesion may improve prognosis. Contact endoscopy has been documented to accurately differentiate normal cells of the nasopharynx from malignant cells and allows a real-time diagnosis of primary and recurrent nasopharyngeal carcinoma (NPC) in a clinical setting. However, the role of contact endoscopy in the diagnosis of NPCIS is unknown.

Methods. The superficial cells of the nasopharynx in a patient with NPCIS were examined in vivo under local anaesthesia by use of a contact rhinoscope. The contact endoscopic findings were correlated with the histologic findings of the biopsy.

Results. The atypical cells of the lesion were magnified and visualized under contact endoscopy. Histopathologic analysis of the biopsied tissue confirmed the presence of NPCIS staining positively for Epstein-Barr virus (EBV)–encoded RNA (EBER). No cell-free EBV DNA was detected in the sera of the patient.

Conclusions. Contact endoscopy can accurately identify the atypical cells of a tiny preinvasive lesion in the nasopharynx in a clinical setting, which may not be evident in routine imaging examination. © 2005 Wiley Periodicals, Inc. Head Neck 27: 1008–1013, 2005

Keywords: nasopharyngeal carcinoma; carcinoma in situ; diagnosis; contact endoscopy; in-situ hybridization Epstein-Barr virus–encoded RNA

Nasopharyngeal carcinoma (NPC) represents a major cancer killer in the Cantonese Chinese population not only in southern China but wherever this population has settled.1−4 Endemic NPC in our locality is typically undifferentiated carcinoma (World Health Organization type III), and almost all, if not all, harbor Epstein-Barr virus (EBV) in tumor tissues.5 Unlike other epithelial carcinomas in the head and neck region, nasopharyngeal dysplasia or nasopharyngeal carcinoma in situ (NPCIS) has rarely been documented. Most of the reported cases of NPCIS

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are associated with invasive carcinoma. Pure preinvasive lesions seem to be extremely rare, but the true incidence is uncertain. In our experience, the transformation period for a preinvasive lesion to become invasive NPC is 4 to 5 years. Early recognition of a preinvasive lesion facilitates timely treatment.

First introduced by Hamou in 1979, the contact endoscope was designed so that its tip could make direct contact with the living tissue surface and visualize its superficial cells at high magnifications, thereby allowing real-time, in vivo and in situ examination of epithelial cellular morphology. This technique has been used in the larynx and nose using the contact laryngoscope (Karl Storz, Tuttingen, Germany, 8715AA, 8715BA). The advent of recently designed contact rhinoscopes (Karl Storz, Tuttingen, Germany, 7215AA, 7215BA), with a diameter of 4 mm, allow in vivo examination of the nasopharynx through the nasal cavity with the patient under local anesthesia.

Since January 1999, we have used contact rhinoscopes to examine patients with normal nasopharynges and NPC in the clinic of Prince of Wales Hospital (The Chinese University of Hong Kong). We found that contact endoscopy can accurately differentiate normal cells of the nasopharynx from malignant cells and allows an in vivo diagnosis of primary and recurrent NPC in a clinical setting. However, its efficacy in the diagnosis of preinvasive lesions of the nasopharynx has not been established.

In this study, our experience in the diagnosis of a preinvasive lesion of the nasopharynx by contact endoscopy is reported. We believe it is the first report of contact endoscopic findings of NPCIS in the literature.

CASE REPORT
A 32-year-old Chinese man was initially seen with a painless mass beneath the angle of the left jaw. He had no other ear, nose, or throat symptoms. Physical examination revealed a 2-cm firm mass in the tail of the left parotid gland. Endoscopic examination of the nasopharynx was performed. A small nodule in the right fossa of Rosenmüller was noted (Figure 1).

MRI of the nasopharynx and neck showed subtle asymmetry of the nasopharynx with a slightly thicker enhancing mucosa on the right side of the posterior nasopharynx and a 2-cm cystic mass in the superficial lobe of left parotid gland (Figure 2).

In an attempt to identify the nature of the nasopharyngeal lesion, contact endoscopy of the nasopharynx was performed with the patient under local anesthesia, as previously described. Under high magnification, atypical cells were visualized in the superficial epithelium of the suspicious nodule, whereas normal respiratory epithelium was observed adjacent to the lesion (Figure 3). The atypical cells exhibited enlarged hyperchromatic pleomorphic nuclei in sharp contrast with those of the adjacent respiratory epithelial cells. The findings suggested the diagnosis of dysplasia or NPCIS. The lesion was subsequently biopsied. Histologic examination revealed atypical cells replacing the epithelium. Those atypical cells exhibited cellular features resembling undifferentiated carcinoma cells with pleomorphic nuclei, prominent nucleoli, and indistinct cytoplasmic boundaries. No invasive carcinoma was detected, and the diagnosis of NPCIS was confirmed. Positivity of nonisotopic in situ hybridization for EBV-encoded RNA (ISH EBER) was demonstrated (Figure 4). Reactive lymphoid hyperplasia was also noted in the biopsy.

The immunoglobulin A (IgA) titer against EBV viral capsid antigen (VCA) was elevated (1/20). Based on the method previously described, real-time polymerase chain reaction (PCR) detection of serum EBV DNA was performed and serum samples were processed as previously described.
Serum DNA was extracted using a QiaAmp Blood Kit (Qiagen, Hilden, Germany) with the “blood and body fluids protocol” as described by the manufacturer. Real-time quantitative PCR for the BamHI-W fragment was performed using the primers and fluorogenic probe with a 7700 Sequence Detector (Perkin-Elmer Applied Biosystems, Foster City, CA). All data were presented as copies of EBV genome per milliliter of serum as reported. The result showed that the serum EBV DNA concentration was 0 copies/mL.

Subsequently, left superficial parotidectomy was performed. Histologic analysis of the specimen showed features of benign cystic oncocytoma with negative EBER staining (Figure 5). To rule out the coexistence of invasive carcinoma, repeated nasopharyngeal biopsies were performed. The results again showed the presence of the NPCIS without any invasive components. In view of the persistent preinvasive lesions in the nasopharynx, the patient was treated with external...
irradiation to the nasopharynx and the neck. There was no evidence of recurrence 2 years later.

**DISCUSSION**

The first series of intraepithelial neoplastic changes in the nasopharynx of patients with NPC was reported by Teo in 1957. The occurrence of NPCIS of the nasopharynx has subsequently been sporadically reported. The prevalence of NPCIS was found to vary from 2% to 3.6% of nasopharyngeal biopsy specimens taken from symptomatic patients. In most of these cases, however, NPCIS was identified in areas adjacent to invasive carcinoma. Clinical cases of pure NPCIS lesions without the associated invasive component of NPC seem to be rare, reportedly occurring in 0.6% of nasopharyngeal biopsy specimens.

Cheung et al and Pak et al have identified patients with purely preinvasive NPC lesions without evidence of an invasive component. The presenting symptoms were reported as unilateral tinnitus and blood-stained postnasal drip, whereas the lesions were described as small protrusions in the nasopharynx. However, the clinical appearance of the nasopharyngeal lesions was not documented in those studies. In this described case, the preinvasive lesion was asymptomatic and documented as a small nodule in the nasopharynx without any associated symptoms in a patient with a parotid tumor and caught the attention of the clinician to observe a small mass in the contralateral side of the nasopharynx. The avenue to early diagnosis depends on a high index of clinical suspicion and the presence of elevated EBV serology.

EBV is associated with NPC in both preinvasive and invasive phases. It is recognized that the clonality of EBV DNA in NPC arises from the clonal expansion of a single EBV-infected cell, and the pattern of expression of EBV genomes in the preinvasive lesions is identical to that of the invasive NPC. Most patients with invasive NPC have high titers of IgA antibodies against EBV VCA. In accordance with previously reported cases, we have found that IgA antibodies against EBV VCA are elevated not only in patients with invasive lesions but also preinvasive lesions. This study further supports the view that IgA serologic testing can be used as a screening tool for preinvasive lesions of NPC.

Recently, cell-free EBV DNA has been detected in the serum of patients with NPC and used as indicator of recurrent disease. Using real-time quantitative PCR, the median plasma EBV DNA concentration was found to be elevated in patients with recurrent disease and decreased in patients who remained disease free. In this study, we analyzed the cell-free EBV DNA concentration in this patient’s serum at the time when the preinvasive lesion was identified. However, no serum EBV DNA signal was detected. One explanation could be the low tumor cell load in the patient. A second possibility is that preinvasive and invasive lesions differ in their intrinsic behavior with regard to the release of tumor-related DNA into the circulation. This latter issue is unclear and deserves further study.

The histologic findings of preinvasive NPC are characteristic. Characteristically, atypical epithelial cells are present, demonstrating loss of polarity, large vesicular nuclei, prominent eosinophilic nucleoli, and scanty indistinct cytoplasm. The neoplastic cells tend to scatter throughout the epithelium and are interspersed among an intraepithelial lymphoplasmacytic infiltrate. Because latent EBV infection can be identified in NPCIS by the ISH EBER, positive staining for EBER is useful in confirming the diagnosis for this rare condition.

Under contact endoscopy, the atypical cells of this preinvasive lesion were visualized. They were characterized by the presence of pleomor-
aphic cells containing enlarged nuclei with an increased nuclear/cytoplasmic ratio. This text seems to be the first description of contact endoscopic findings of NPCIS. Previously, we have shown that contact rhinoscopy can make an accurate real-time diagnosis of nonirradiated and recurrent NPC. This case further illustrates that contact endoscopy can accurately identify the atypical cells of a tiny focal lesion in the nasopharynx and establish the diagnosis of NPCIS, which may not be readily seen on routine endoscopic examination and MRI.

Although preinvasive lesions of the nasopharynx cannot be easily identified by routine endoscopy, through the real-time identification of the cellular morphology in all accessible areas in the nasopharynx of suspected patients, contact endoscopy can accurately localize the abnormal tissue not otherwise identifiable and direct biopsies.

In our experience, contact endoscopy may have the following technical shortcomings in its application to diagnose preinvasive lesions of the nasopharynx. First, it is not easy to examine the fossa of Rosenmüller of the nasopharynx, which is the most common site of origin of NPC, by the 0° endoscope. This problem can be overcome by using a 30° endoscope. Second, it takes time for an endoscopist to recognize the atypical cells in the background of normal cells. It is essential for the endoscopist to work closely with a pathologist in the initial phase of the learning curve.

Contrary to the examination of the invasive carcinoma, contact bleeding is not a great hurdle to the contact endoscopy of the preinvasive lesions.

Furthermore, there have been some concerns about the potential hazard of phototoxic reactions of methylene blue, a vital stain, to the living tissue of the nasopharynx. However, to our knowledge, there has been no solid evidence to suggest its adverse effects toward the intact skin under a brief exposure of light at wide wavelengths.

This case report and our previous experiences support our belief that contact endoscopy can be used as a screening method to allow in vivo diagnosis of NPC at different stages in high-risk populations.

REFERENCES