


## BRIEF REPORT

# Cytokeratin7 expression in histologic and cytologic specimens of cystic neck metastasis from HPV positive squamous cell carcinoma of the tonsil: A case report

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The majority of cystic squamous cell carcinomas (SCCs) of the neck have been shown to be metastatic tumors from tonsillar SCCs associated with high-risk human papillomavirus (HR HPV). Recent studies have demonstrated cytokeratin (CK)7 involvement in the development of HPV positive SCC, but no report has been issued on its simultaneous expression in primary tonsillar and metastatic tumor with cystic change. We present a case of HPV positive tonsillar SCC of a 42-year-old male that initially manifested as a cystic neck mass expressing CK7, CK19, and p16 in primary and metastatic tumors. Immunohistochemical examination revealed diffuse CK19 and p16 expression, and patchy CK7 expression in the solid components of primary and metastatic tumors. However, in cystic components of metastatic tumors the expression of CK7 and CK19 was preserved but p16 expression was absent, which was consistent with immunocytochemical findings of fine-needle aspirates from cystic neck mass. In immunocytochemistry performed on aspirates of a branchial cleft cyst for the comparison of cystic SCC and benign cyst, CK19 staining was positive but CK7 and p16 staining was negative. These results suggest that CK7 immunocytochemistry on aspirated material from cystic neck mass may be a useful adjunct for distinguishing cystic metastasis of tonsillar SCC from branchial cleft cyst, although a larger scale study would be required.

## KEYWORDS

CK7, cystic neck metastasis, HPV, immunocytochemistry, squamous cell carcinoma

## 1 | INTRODUCTION

Oropharyngeal squamous cell carcinomas (OPSCCs) have unique clinicopathologic features, such as, a causative association with high-risk human papillomavirus (HR HPV), frequent neck lymph node metastasis with cystic change, and better survival than squamous cell carcinoma (SCC) arising in a site other than oropharynx.<sup>1–3</sup> The palatine tonsil is the most common site for OPSCC.<sup>3,4</sup>

The tonsil is a lymphoid organ covered by stratified squamous epithelium and have multiple crypts lined with reticulated epithelium, and tonsillar crypts are considered to be where HPV integration and carcinomatous change initiate.<sup>5,6</sup> Cytokeratin (CK)7 is a

crypt epithelial marker and its overexpression is associated with HR HPV infection and tonsillar SCC with a non-keratinizing morphology.<sup>7</sup> CK19 is also expressed in crypts<sup>8</sup> and CK19 expression is upregulated in HPV positive oral/OPSCC (O/OPSCC) compared with HPV negative O/OPSCC.<sup>8</sup> p16 is a surrogate marker of E7 oncoprotein of HR HPV and p16 staining is used to detect cystic node metastasis from HPV positive OPSCC because p16 and HPV status remains unchanged after metastasis.<sup>2</sup>

In this study, we report expressions of CK7, CK19, and p16 in the tissues and aspirates of metastatic tonsillar SCC presenting as cystic neck mass, and comparative immunocytochemical findings of these molecules in cystic SCC and branchial cleft cyst.

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**FIGURE 1** Contrast-enhanced computed tomography scan shows a left-side enhancing mass with internal necrotic areas measuring 33 mm  $\times$  27 mm, located at left level II area posterior to left submandibular gland

## 2 | CASE REPORT

A 42-year-old male patient presented with a palpable left submandibular area mass that had been detected two years previously and had increased in size with mild intermittent pain over 6 weeks prior to presentation. Physical examination revealed a firm, fixed, and nontender mass. Contrast-enhanced computed tomography demonstrated a necrotic enhancing mass measuring 33 mm  $\times$  27 mm, posterior to left submandibular gland (Figure 1).

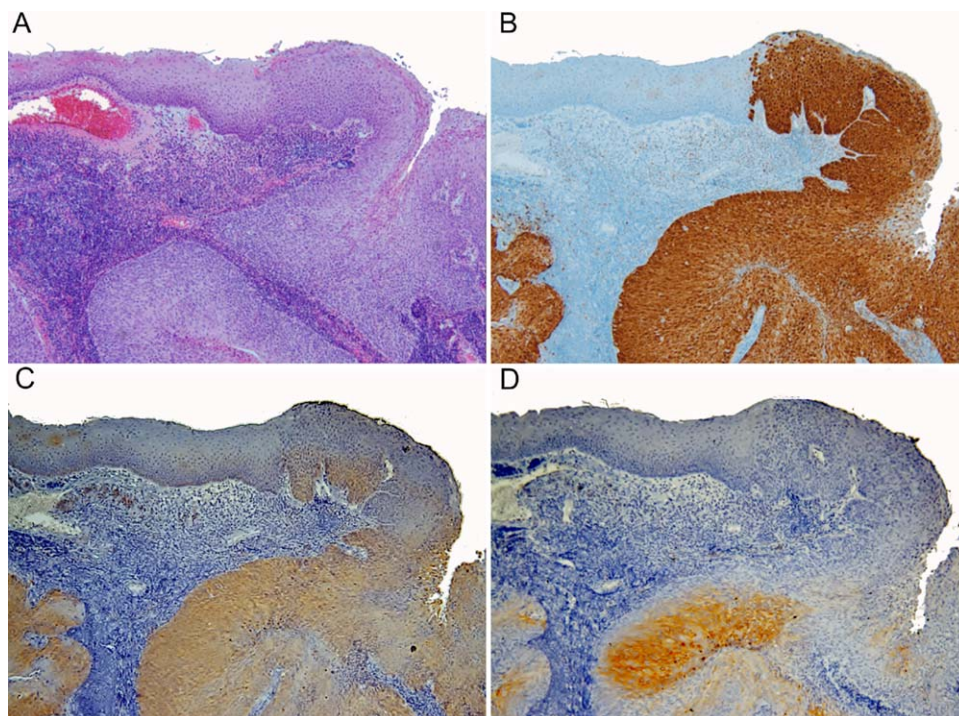
Fine-needle aspiration cytology (FNAC) was performed and a finding of a few squamous cells with inflammatory and necrotic debris raised suspicion of a cystic lesion, such as, a branchial cleft cyst. However, the possibility of malignancy could not be entirely excluded and biopsy was recommended.

The patient was taken to the operating room for excisional biopsy of the mass. Severe adhesion was present between the mass and adjacent structures such as sternocleidomastoid and omohyoid muscles. Intraoperative frozen section biopsies resulted in a finding of SCC with extension to adjacent skeletal muscles. Left neck dissection was performed under a diagnosis of metastatic cervical carcinoma of unknown primary origin, and bilateral tonsillectomy was done to find the primary site of malignancy.

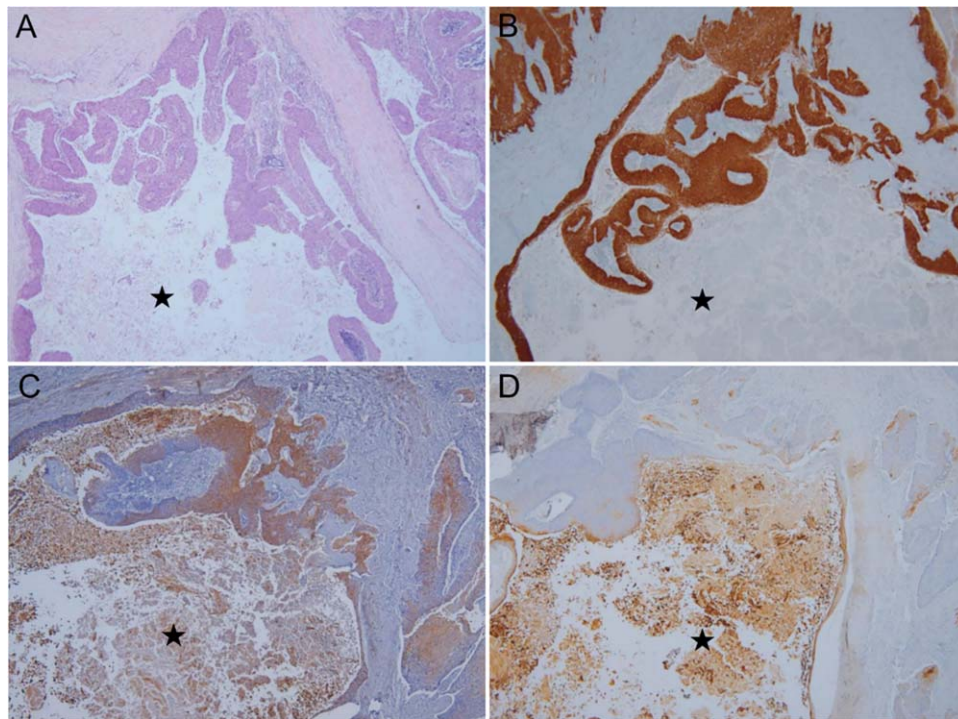
Grossly, both tonsils were not enlarged and symmetric (both measured 33 mm  $\times$  25 mm). The right tonsil was unremarkable, but the left tonsil was whitish gray and had a firm cut surface. The neck dissection specimen was composed of ill-defined, whitish gray solid mass, which adhered to lymph nodes and exhibited cystic necrosis. The mass measured 55 mm in aggregate.

Histologically, the left tonsil revealed SCC with a non-keratinizing basaloid feature centered on tonsillar crypts (Figure 2A). In the metastatic SCC, which presented as a neck mass, tumor bands were narrowed and central cystic spaces expanded (Figure 3A). In cystic spaces, sloughed tumor cells, necrotic debris, and inflammatory cells, which included neutrophils, eosinophils, macrophages, and lymphoid cells, were observed. Cystic spaces were ruptured and liquefactive necrosis was seen in an affected lymph node (Figure 4A).

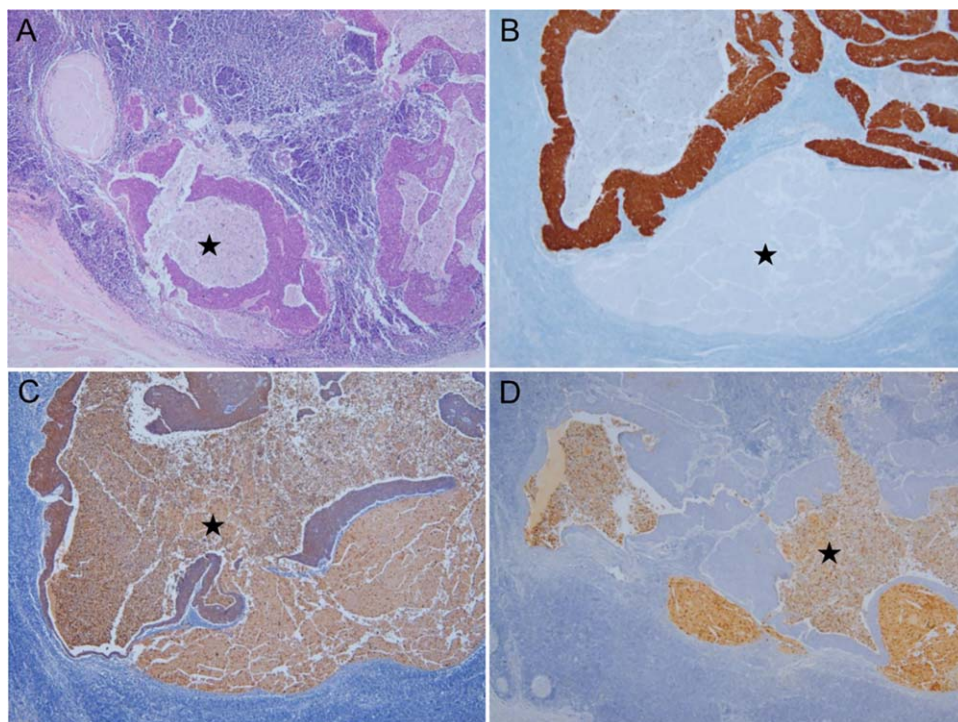
Immunohistochemical staining was carried out for CK7 (Dako, Carpinteria, CA, 1:100), CK19 (Dako, 1:100), and p16 (Dako p16INK4a kit)



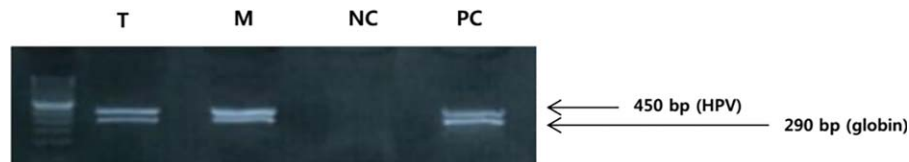
**FIGURE 2** Histologic finding of left tonsillar tumor (A). Non-keratinizing basaloid squamous cell carcinoma is seen at the tonsillar crypt (H&E, 100 $\times$ ). Immunohistochemical findings (B-D). Tumor cells show strong immunoreactivity for p16 (B, 100 $\times$ ), diffuse staining for CK19 (C, 100 $\times$ ), and patchy staining for CK7 (D, 100 $\times$ ). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 3** Histologic finding of metastatic squamous cell carcinoma presenting as a cystic neck mass (A). The tumor shows an enlarged cystic space (asterisk) containing sloughed tumor cells, inflammatory cells, and necrotic debris (H&E, 40 $\times$ ). Immunohistochemical findings (B-D). Tumor cells show strong p16 positivity in solid tissue and p16 negativity in the cystic space (asterisk) (B, 40 $\times$ ), CK19 positivity of variable intensity in both solid tissue and the cystic space (asterisk) (C, 40 $\times$ ), and patchy staining for CK7 in solid tissue and strong CK7 staining in the cystic space (asterisk) (D, 40 $\times$ ). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 4** Histologic finding of metastatic squamous cell carcinoma in a neck lymph node (A). The tumor exhibits ruptured cystic spaces and liquefactive necrosis (asterisk) (H&E, 40 $\times$ ). Immunohistochemical findings (B-D) reveal diffuse strong immunoreactivity for p16 in the tumor and p16 negativity in necrotic material (asterisk) (B, 40 $\times$ ), diffuse CK19 positivity in both tumor and necrotic material (asterisk) (C, 40 $\times$ ), and strong CK7 positivity in necrotic material (asterisk) (D, 40 $\times$ ). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 5** Detection of human papillomavirus (HPV) infection by real-time polymerase chain reaction in tonsillar and metastatic squamous cell carcinoma. T = tonsillar squamous cell carcinoma, M = metastatic cystic squamous cell carcinoma, NC = negative control, PC = positive control, 450 bp: amplification of HPV gene, 290 bp: amplification of the human  $\beta$ -globin gene used as internal control [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

using Dako Autostainer (DakoCytomation, Carpinteria, CA).<sup>9</sup> Immunohistochemistry revealed diffuse and strong p16 staining in both primary and metastatic tumors, but its absence in cystic spaces within the metastatic tumor nest (Figures 2B, 3B, and 4B). CK19 staining was consistently positive in primary and metastatic tumors including cystic spaces (Figures 2C, 3C, and 4C). CK7 staining was patchy in the solid tumor component but strongly positive in cystic spaces within the metastatic tumor nest (Figures 2D, 3D, and 4D). HPV DNA was detected in the tonsillar and metastatic SCC by real time polymerase chain reaction using formalin-fixed, paraffin-embedded tumor tissues after deparaffinization and DNA extraction (Figure 5).

Immunocytochemistry for CK7, CK19, and p16 was performed retrospectively on a cell block of FNAC specimen. As was observed immunohistochemically in the cystic component, CK7 and CK19 staining was positive but p16 staining was negative in aspirated material (Figure 6).

We additionally performed immunocytochemistry on FNAC specimen of a histologically confirmed branchial cleft cyst (a 35-year-old male) to compare CK7, CK19, and p16 staining findings of this cystic SCC with those of benign cyst. Branchial cleft cyst showed CK7 and

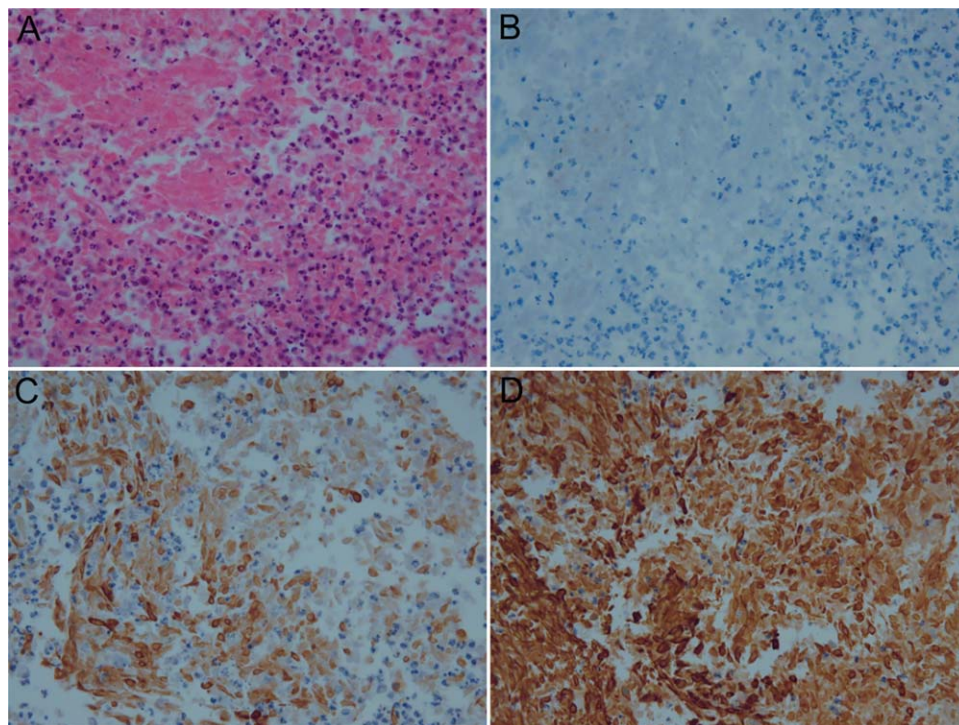
p16 negativity and CK19 positivity in scattered squamous cells (Supporting Information Figure 1).

Postoperatively, the patient underwent combined radiotherapy (6000 cGy) and chemotherapy, and no recurrence occurred over 5 years of follow-up.

### 3 | DISCUSSION

Although a growing number of studies have reported a close link between CK7 or CK19 and HPV positive OPSCC, little is known about the roles played by CK7 and CK19 in the pathogenesis of HPV-related SCC.<sup>7,8,10</sup> However, a few studies have analyzed interactions between HPV16 E7 mRNA and CK7 and CK19 in cervical cancer cell lines.<sup>11–13</sup> In particular, in SiHa cells containing CK7/CK19, CK19 promotes HPV16 E7 oncoprotein expression by binding to the 6-mer peptide SEQIKA of CK7, which interacts with E7 mRNA and inhibits E7 mRNA translation.<sup>11–13</sup>

Tonsillar and uterine cervical SCCs share some histopathological and immunohistochemical features. They both arise in CK7 and CK19 positive squamocolumnar junctional cells infected by HR HPV and



**FIGURE 6** Cytologic finding (A) of aspirates from metastatic cystic squamous cell carcinoma showing necrotic material and inflammatory cells. (H&E, 400 $\times$ ). Immunocytochemical findings (B–D) reveal p16 negativity (B, 400 $\times$ ), CK19 positivity (C, 400 $\times$ ), and CK7 positivity in necrotic tumor cells (D, 400 $\times$ ). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

frequently accompany intratumoral cystic changes.<sup>4,5,7,9</sup> In an earlier study, we observed cystic change in a subset of uterine cervical SCC and topological differences between the expressions of CK7, CK19, and p16.<sup>9</sup> The present case exhibited a staining pattern similar to that of uterine cervical SCC, that is, patchy CK7 staining in the inner portions of cystic tumor nests, diffuse CK19 staining in cystic and solid tumor nests, and strong p16 staining in solid tumor component.

Nonetheless, tonsillar SCC has unique clinicopathologic features, such as, frequent neck node invasion and initial presentation as a cystic neck mass, which are associated with specialized tonsillar structure and direct lymphatic connection between the tonsil and neck nodes, including jugulodigastric lymph nodes.<sup>2,4,14</sup> Furthermore, the lymph node provides a familiar microenvironment for metastatic epithelium to interplay with lymphoid cells, recapitulating the parent crypt epithelium.<sup>4,14</sup> At about the 14th-15th embryonic weeks, tonsillar epithelial invagination occurs with canal formation and central necrosis,<sup>15</sup> and the necrotic substances produced play a role of local antigenic stimulation for the development of immature lymphoid cells.<sup>15</sup> HPV-infected tumor cells might augment this immune reaction, and the marked inflammatory response induced might accelerate tumor cell necrosis and formation of central cystic spaces.<sup>4,16</sup>

In the present case, metastatic SCC showed narrow tumor bands with expanded cystic spaces or liquefaction necrosis, resembling crypts containing inflammatory cells and necrotic material. Interestingly, in cystic components of metastatic tumors the expression of CK7 and CK19 was positive but p16 expression was negative, which was consistent with immunocytochemical findings of FNAC specimen from cystic neck mass. The comparative immunocytochemistry on the aspirates of branchial cleft cyst showed CK7 and p16 negativity and CK19 positivity. These findings show that the differential expression of CK7 in FNAC specimens from metastatic SCC and branchial cleft cyst may be helpful for distinguishing these two cystic lesions especially when the aspirated material contains rare tumor cells and extensive necrosis.

In conclusion, we observed consistent CK7 expression in cystic components of metastatic SCC in both histologic and cytologic specimens, which was in contrast to CK7 negativity in branchial cleft cyst. This observation suggests the possible use of CK7 immunocytochemistry in aspirated material from cystic neck mass as an adjunct to distinguish cystic metastasis of tonsillar SCC from branchial cleft cyst. However, we suggest a larger scale study be conducted to confirm these findings.

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This study was not supported by any grant or funding of any kind.

## CONFLICT OF INTEREST

None.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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