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Intranodal cystic changes: A potential radiological signature/ biomarker to assess the HPV (human papillomavirus) status of cases with oropharyngeal malignancies

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Abstract

Objective—To determine, if lymph node imaging findings can predict human papillomavirus (HPV) positivity in oropharyngeal squamous cell cancers.

Methods and Materials—Pre-treatment post-contrast Neck CT of 49 patients (male = 35, female = 14; age range = 45–76) diagnosed with oropharyngeal malignancies, and with available HPV data, were retrospectively reviewed. Metastatic lymph nodes were identified based on standardly accepted size and morphologic criteria. Various lymph node parameters were studied, including presence of cystic foci in the metastatic lymph nodes, abnormal lymph nodes showing low attenuation foci, matted lymph nodes and morphologically normal smaller (<1.5cm) lymph nodes. These parameters were then independently correlated with the available HPV status of these patients. Finally, an extended criteria i.e. intranodal cystic changes in cases with morphologically normal small (<1.5cm) lymph nodes, was correlated with HPV status. Sensitivity, specificity, positive and negative predictive values were calculated.

Results—Of these 49 cases with oropharyngeal cancers, 27 were HPV positive (+ve) and 22 cases were HPV negative (–ve). 8 cases (3 HPV +ve and 5 HPV –ve) did not have metastatic lymph nodes. Of remaining 41 cases with metastatic abnormal lymph nodes, 26 were HPV +ve and 15 were HPV –ve. Of these 41 cases with metastatic lymph nodes, 14 had one or more lymph nodes with cystic foci. Ten of these 14 cases i.e. 71.4% were HPV +ve. Resultant sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of cystic foci for the presence of HPV status were 38.4%, 73.3%, 71.4% and 40.7% respectively. Intranodal cystic changes in cases with morphologically normal small (<1.5cm) lymph nodes were found in 5 cases; all 5 were HPV positive. Resultant accuracy was specificity and PPV of 100%, sensitivity of 19.2% and NPV of 41.6%.

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Conclusion—Intranodal cystic changes seen on the pretreatment post-contrast CT Neck of patients with oropharyngeal malignancies are radiological signatures strongly associated with the HPV status of the patient. The results in this initial study warrant larger prospective studies to determine if this finding may be used in addition to other molecular biomarkers to help identify those patients that may be amenable to the most appropriate treatment options.

Keywords

HPV; oropharyngeal cancer; cystic; lymph node

Introduction

HPV DNA is identified in 70% of the cases of oropharyngeal cancer (OPC), with known causal association.^{1, 2} It is described that oral infection with HPV 16 increases the risk of OPC, 14 times than in the general population.^{3, 4} It is also known that HPV positive cases of OPC show better response to treatment and even to organ preservation treatment with better survival rate compared to HPV negative cases of OPC.^{4–7} Several means to evaluate the HPV status of these cases are being evaluated. This is challenging on imaging as well. Our aim was to determine if the pre-treatment imaging of the lymph nodes could be correlated with HPV status of the patients.

Methods and Materials

Pretreatment post-contrast-neck CT of 49 patients diagnosed with oropharyngeal malignancies, were retrospectively reviewed. 35 were males and 14 were females. Our patients ranged from 45 to 76 years of age. HPV data was available for all the patients based on Multiplex PCR Mass spectroscopy analysis of DNA isolated from the tumor block⁸.

All patients had contrast enhanced neck CT with 1.25mm thick axial slices at 1.25mm interval and pitch of 0.97:1. Automatic exposure control was used during the CT scan with 140 kVp and 125–380 mAs. ASIR technique was used for noise reduction to achieve noise index of 26.4 and to allow reduction in radiation exposure. Metastatic lymph nodes were identified based on standardly accepted size and morphologic criteria.⁹ Various lymph node parameters were studied, including presence of cystic foci in the metastatic lymph nodes, abnormal lymph nodes showing low attenuation foci, and matting of lymph nodes (Tables 1–3). The presence of cystic focus in lymph node was defined as low attenuation area measuring less than 25 HU, with well-defined and smooth margin. If the low attenuation focus was too small to measure the attenuation, then it was considered cystic, if its more than 70% margin was well defined and smooth. If it had irregular margins, it was considered as necrotic focus. These parameters were then independently correlated with the available HPV status of these patients. To further improve on accuracy for prediction of HPV status of these cases, extended criteria was used. In the extended criteria, only those cases were included that had completely normal morphology of smaller (1.5cm or less) lymph nodes and also had cystic changes in the metastatic lymph nodes. Finally, the extended criteria i.e. intranodal cystic changes in cases with morphologically normal small (<1.5cm) lymph nodes was correlated with HPV status of the cases (Table 4). Sensitivity, specificity, positive predictive value and negative predictive value were obtained for each parameter as well as the extended parameter to detect HPV status of these cases.

Results

Of the 49 cases with oropharyngeal malignancies, 27 were HPV positive (+ve) and 22 cases were HPV negative (–ve). Eight cases (3 HPV +ve and 5 HPV –ve) did not show metastatic

lymph nodes. Of the remaining 41 cases with abnormal lymph nodes, 14 showed one or more lymph nodes with cystic foci. Of these, 10 were HPV +ve and 4 were HPV -ve. Thus, majority (10 of 14 = approx. 71.4%) of the cases with intranodal cystic changes were HPV +ve, with resultant sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 38.4%, 73.3%, 71.4% and 40.7% respectively (Table 1). Extended criteria i.e. intranodal cystic changes in cases with morphologically normal small (<1.5cm) lymph nodes was found in 5 cases (Table 4). All these were HPV positive. This resulted in improved accuracy for prediction of HPV status of OPC, with specificity and PPV of 100%, although sensitivity and NPV were still low i.e. 19.2% and 41.6 % respectively.

Discussion

Although environmental exposures such as tobacco and alcohol are the well-known risk factors for head and neck cancers^{10–13}, 15–20% of head and neck cancers occur in nonsmokers and nondrinkers. Epidemiologic and molecular data suggest that HPV infection of the upper airway may also promote head and neck tumorigenesis.^{11, 12} HPV has been shown to be associated with many oropharyngeal cancers^{13–15}, particularly tonsillar and tongue base cancers.^{1, 2, 4, 12, 16, 17}

The association has been found particularly strong in younger patients, nonsmokers and nondrinkers.^{1, 2, 4, 12, 13, 17, 18} Mostly, HPV 16 and 18 are high risk HPVs and are known to be tumorigenic in human epithelial tissues.^{4, 12}

It is proposed that E6 protein in the HPV binds to and inactivates the tumor suppressor protein p53, while the E7 protein of HPV does the same to retinoblastoma protein (pRB), leading to malignant transformation of HPV infected cells.^{4, 12, 14, 19} It has also been proved that HPV positive status of OPC is strongly associated with better therapeutic response and hence the better survival rate compared to HPV negative cases of OPC.^{5–7, 12, 18} HPV positive OPC tend to be poorly differentiated cancers and metastasize early compared to HPV negative cancers.²⁰ But, they have half the risk of death from head and neck squamous cell cancers and lower recurrence rates compared to HPV negative cases and overall have better prognosis.^{4, 12, 20} Favorable results in OPC cases undergoing organ preservation chemoradiation protocol treatment are reported in the literature with >80% disease free survival of these cases at the end of 2 and 3 years following treatment.⁵ It is also well known that HPV positive OPC cases respond better to nonsurgical organ preservation treatment compared to HPV negative cases.^{4, 6, 7, 18, 21, 22} Hence overall, there is more potential for organ preservation treatment in HPV positive OPC cases. However, detecting HPV status noninvasively is challenging.

In situ hybridization or fluorescence in situ hybridization (FISH) are useful tests to confirm the diagnosis of HPV within tumor cell nuclei, however these are available only at limited tertiary centers. Real time PCR may be performed on microdissected tumor. p16 immunohistochemistry may be a reasonable marker, as correlation has been seen between cytoplasmic p16 staining, in situ hybridization and PCR based assays. Other possible tests include detection of HPV16 DNA in the tumor or plasma, serum reactivity for E6 and E7 proteins of HPV etc.^{4, 20} Highly sensitive FISH enables HPV DNA detection up to the level of a single copy per cell nucleus, and allows discrimination between replicative (episomal) and integrated virus on the basis of the nuclear staining pattern.²⁰ We used Multiplex PCR Mass spectroscopy analysis of DNA isolated from the tumor block, to detect the HPV status. This assay can detect 15 high risk HPV types with a high sensitivity and high specificity.⁸

It is well recognized that cancers arising from Waldeyer's ring has strong association with cystic lymph node metastasis (33–50%).¹ Goldenberg et al. showed 87% (13/15 cases) sensitivity when they used intranodal cystic changes as a radiological parameter to predict the HPV positivity. However, they had only 4 HPV negative cases of oropharyngeal malignancies as the control¹, which we think is concern for bias. We have more (20) HPV–OPC (vs. 29 HPV+ OPC) cases in our study, with somewhat unbiased results.

Overall, we found good correlation between intranodal cystic foci and HPV positivity of the cases with oropharyngeal malignancies. Extended criteria, i.e. “Intranodal cysts with morphologically normal smaller (<1.5 cm) lymph nodes”, has a stronger association with HPV+ oropharyngeal cancer (specificity & PPV = 100%). It may serve as one of the important radiological signatures for assessing the HPV status of these cases. Favorable results in this initial study, warrant larger prospective study. If the results are consistent, this finding may be used in addition to other molecular biomarkers to help identify those (HPV positive) patients that may be amenable to Non-Surgical Organ Preservation treatment options.

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Figures 1a & b.

Intranodal cystic changes: Axial postcontrast CT scan of the neck in 2 different cases with HPV positive oropharyngeal malignancies. Note the well-defined low attenuation (attenuation was <25 HU) with smooth margins in figure 1a, consistent with intranodal cystic focus. In figure 1b, the low attenuation focus is too small to measure the attenuation, but majority ($>70\%$) of its wall is smooth consistent with a cystic focus.

Figure 2. Intranodal necrosis: Axial postcontrast CT scan of the neck in a case with HPV negative oropharyngeal (right tonsillar) malignancy. Note the irregular margin of the low attenuation focus consistent with necrosis within the metastatic lymph node.

Table 1

Correlation of HPV status of OPC cases with intranodal cystic changes on pretreatment neck CT

Cystic foci within lymph nodes	HPV status	
	+	–
+	10	4
–	16	11

Table 2

Correlation of HPV status of OPC cases with matting of lymph nodes on pretreatment neck CT

Matted lymph nodes	HPV status	
	+	–
+	14	9
–	12	6

Table 3

Correlation of HPV status of OPC cases with intranodal low attenuation foci on pretreatment neck CT

Smaller (<1.5cm) lymph nodes with low attenuation focus	HPV status	
	+	–
+	9	12
–	17	3

Table 4

Correlation of HPV status of OPC cases with extended criteria on pretreatment neck CT

Intranodal cystic focus (with morphologically normal smaller lymph nodes)	HPV status	
	+	–
+	5	0
–	21	15