*Q J Med* 2010; **103**:229–236
doi:10.1093/qjmed/hcp176 Advance Access Publication 16 December 2009

# Review

QJM

# Human papillomavirus-associated oropharyngeal cancer: a new clinical entity

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## Summary

The incidence of oropharyngeal cancers is rising worldwide in both nonsmokers and nondrinkers. Epidemiology studies suggest a strong association between human papillomavirus (HPV) 16 infection, changing sexual behavior and cancer development. Despite initial presentation with locally advanced disease and poorly differentiated histology, HPV-associated oropharyngeal carcinoma is associated with a good prognosis because its response to chemotherapy and radiation. Clinicians should be aware of the risk of oropharyngeal cancer in young people to avoid unnecessary delay in diagnosis and treatment. A history of oral sex should be elicited in young patients with enlarged neck nodes and/or tonsillar masses.

## Introduction

Oropharyngeal carcinoma prevalence is rising steadily in the United States and Western Europe despite successful effort to control smoking and drinking.[1–3] A report from the Surveillance, Epidemiology, and End Results (SEER) database demonstrated a statistically significant increase of oropharyngeal cancers affecting young people between the age of 20- and 44-years old.[4] There was a strong association between human papillomavirus (HPV) 16 and oropharyngeal malignancy which affected all ethnic groups in the US.[5,6] A recent public health study projects an increased incidence of HPV infection of epidemic proportion in young adults because of changing sexual habits.[7] Thus, the cost to society would be unacceptable in terms of loss of life because of the expected rise in pharyngeal malignancy.[8] It is important for primary care physicians to recognize this new clinical entity to avoid delays in diagnosis that can lead to poorer outcomes.[9] This review describes the mechanism of HPV infection in oropharyngeal cancers, current treatment options and prognosis following treatment.

## Materials and methods

This systematic review was designed to investigate the rising incidence of oropharyngeal carcinoma in the young. A search was undertaken from 1990

when the first cases of HPV 16 DNA integration into the tonsillar carcinoma genome was reported, until 2009. Searches were based on PubMed, Embase and Google Scholar electronic databases.

The following terms were explored and used for each database search: oropharyngeal carcinoma, young, HPV 16, treatment and prognosis. Searches for additional publications were conducted using the references lists of applicable articles.

## Epidemiology of HPV 16 infection and oropharyngeal cancer

Tobacco and alcohol abuse used to be the strongest predictors of developing oropharyngeal carcinoma in old individuals (over 60).[10,11] Most patients presented with locally advanced stages at diagnosis. Despite aggressive treatment with postoperative radiation or chemoradiation, survival remained poor because of the high rates of recurrences[12–19] (Table 1). Recent epidemiologic studies revealed an increased incidence of oropharyngeal carcinoma in young people with no smoking or drinking history.[1,20,21]

A viral etiology for oropharyngeal cancer similar to cervical cancer has been postulated as sexual partners of patients with HPV infection developed a higher risk of second head and neck primaries.[20] Recently, HPV 16 DNA with identical sequences was isolated in tonsillar carcinoma of two non-smoking, non-drinking couples highlighting the infectious nature of the disease.[22] Young patients with multiple sex partners and oral–genital sex are at an increased risk of developing HPV-associated oropharyngeal cancers with HPV 16 as the predominant type.[23] Polymerase chain reaction (PCR) has demonstrated HPV DNA presence in cancer cells but not in the adjacent normal epithelium.

**Table 1** Survival of locally advanced head and neck cancer following post-operative radiotherapy or chemoradiation in randomized studies

| Study | Site | Survival (%) (years) |
|---|---|---|
| Kramer *et al.*[11] | All sites | 36% (5) |
| Denis *et al.*[12] | Oropharynx | 22% (5) |
| Fallai *et al.*[13] | Oropharynx | 40% (5) |
| Staar *et al.*[14] | Oropharynx | 60% (2) |
| Semrau *et al.*[15] | Oropharynx | 22.9% (5) |
| Bensadoun *et al.*[16] | Oropharynx | 54% (2) |
| Posner *et al.*[17] | All sites | 62% (3) |
| Brizel *et al.*[18] | All sites | 55% (3) |
| Adelstein *et al.*[19] | All sites | 57% (3) |

Among all the head and neck sites, tonsillar carcinoma had the highest prevalence of HPV 16 infection suggesting that the virus had a special affinity for tonsillar epithelium.[24,25] There was a strong correlation between the patient age and the prevalence of HPV 16 in the biopsy specimen. El Moffty *et al.*[26] reported a 91% rate of HPV 16 infection in tonsillar carcinoma of patients <40- years old. The prevalence of HPV 16 DNA diagnosed in tonsillar carcinoma specimens by in situ hybridization or PCR ranged from 50% to 84% for patients with median age in the fifties.[27–32] For patients with median age in the sixties, HPV infection rate ranged from 21% to 46%. It is currently unclear why younger patients are more at risk for HPV 16-associated oropharyngeal cancer. One possible explanation is the vulnerability of the tonsillar epithelium to HPV infection as observed in cervical carcinoma.[33] In the US, oral sex begins early among middle and high school students exposing their oropharynx to HPV virus infection.[34,35] Among students who practiced oral sex, up to two-thirds had more than one partner.[34] Condoms are seldom used for oral sex.[36] Thus, viral-induced oropharyngeal carcinoma may reach epidemic proportion because of the change in sexual behavior. As an illustration of this phenomenon, the proportion of HPV 16-associated tonsillar carcinoma has steadily increased in Sweden with rates of 68%, 77% and 93% for the periods of 2000–2002, 2003–2005 and 2006–2007, respectively ($P < 0.0001$).[27] The prevalence of HPV 16 negative tumors also decreased in the same period. Multiple studies have corroborated the relationship between sexual behavior, risk of developing HPV infection and subsequent development of oropharyngeal cancer in young patients which is now of epidemic proportion worldwide.[3,4,37–41]

## Postulated mechanism of HPV 16 infection and oropharyngeal cancer

The mechanism of HPV 16 infection leading to the development of oropharyngeal carcinoma is unclear. HPV is a small double-stranded virus with special affinity for the skin and mucosa. Transfection of human primary epithelial cell lines derived from normal tonsils with HPV 16 produced immortal cell lines.[42] All transformed cells contained abnormal chromosomal abnormalities (breakage, condensation, dicentric and acentric chromosomes). Viral DNA was integrated into the genome of transfected cells with a particular predilection for chromosome 7q31 and 9q34. Even though transformed cells retained the morphology of normal tonsillar cells,

they were poorly differentiated with less cytokeratin expression.[42] Integration of viral DNA in host chromosome 7q31 induced alteration of E2 gene which normally downregulates E6 and E7 genes.[43] Tonsillar carcinoma cells infected with HPV 16 demonstrated high expression of E6 and E7 messenger RNA.[44] As a result, oncoproteins E6 and E7 accumulated inside infected cells.[45] Oncoprotein E6 induced the degradation of tumor suppressor p53 and reduced the infected cell ability to undergo apoptosis. Oncoprotein E7 degraded the retinoblastoma protein (pRb) and prevented it from inhibiting of the cell cycle leading to uncontrolled cell proliferation. Consistent with this hypothesis, tonsillar carcinoma associated with HPV 16 demonstrated downregulation of pRB and cyclin D1.[46] Inhibition of E6 and E7 by short hair-pin RNA retroviruses induced the functional restoration of p53 and pRB functions in HPV 16-associated oropharyngeal squamous cell lines.[47] However, even though the overexpression of oncoproteins E6 and E7 are essential steps for malignant transformation because of continued cellular proliferation, it remains unclear how other genes may interact with these oncoproteins to transform the infected cells into an immortal state. A potential synergistic role for ras oncogene has been suggested with E6 and E7 in transgenic mice to produce ear and mouth tumors, but more work needs to be done to elucidate the mechanism of cancerization.[48]

The P16 protein (p16) is a cyclin-dependent kinase (CDK) inhibitor that is normally inhibited by pRB. Loss of pRB function by HPV 16 virus incorporation into host genome leads to overexpression of p16.[49,50] Increased p16 expression is often associated with poorly differentiated tumor and locally advanced (T4,N2-3) HPV-associated oropharyngeal carcinoma at diagnosis.[50]

Paradoxically, despite advanced stages at diagnosis, overexpression of P16 is a strong predictor for survival advantages independent of TNM staging for HPV-associated oropharyngeal carcinoma.[50–60] There is a strong correlation between P16 expression and response of oropharyngeal tumors to radiotherapy.In a prospective study of 156 head and neck cancers treated with radiotherapy alone, 35 patients were reported to have increased P16 in the biopsy specimen.[57] Twenty-four of these patients (69%) had oropharyngeal cancer. The 5-year loco-regional control was 58% and 28% for p16 positive and negative tumors, respectively ($P = 0.0005$). Corresponding values for survival were 62% and 26% ($P = 0.0003$), respectively. Increased radiosensitivity of p16 positive tumors was also corroborated in another study. Complete response rate to radiotherapy alone was respectively 86% and 33% for high and low expression p16 tonsillar cancers.[53] P16 is also a biomarker for tumor chemosensitivity. Kumar *et al.*[52] reported 50 patients with locally advanced oropharyngeal cancer treated by induction chemotherapy followed by chemoradiation and postoperative radiotherapy for responders and non-responders, respectively. There was a statistically significant correlation between p16 expression and response to chemotherapy which translated into better survival for patients with high P16 expression.[52] Thus, survival for patients with locally advanced oropharyngeal cancer and high P16 expression was significantly increased with chemoradiation because of the tumor radio- and chemosensitivity.[55,56] Nichols *et al.*[56] reported a 3-year survival of 89% and 65% for P16 positive and negative oropharyngeal tumors, respectively following concurrent chemotherapy and radiotherapy. Other studies have also corroborated the excellent survival associated with high expression of P16 following chemoradiation for oropharyngeal cancers.[52,55] One possible explanation for the radiosensitivity of HPV 16-associated oropharyngeal tumors is the upregulation of RBBP4, a gene that has been shown to induce radiosensitivity, in head and neck cancer cell lines infected with HPV 16.[61] In addition to a favorable response rate to treatment, patients with HPV-associated oropharyngeal cancers also have a very low rate of second malignancies and distant metastases which may account for their long-term disease-free survival.[62] Both long-term survival and disease-free survival correlated with viral load. The 4-year survival was respectively 64% and 100% for viral copies less than 50 and more than 500.[63] Other studies also corroborated the beneficial effect of increased viral load in the tumor specimen suggesting that the biology of HPV-positive tumors is less aggressive compared to HPV-negative tumors.[29,54]

The molecular biology of HPV 16-associated oropharyngeal carcinoma is markedly different from HPV-negative oropharyngeal carcinoma. HPV-negative oropharyngeal carcinoma occurs in older individuals with a long history of smoking and drinking.[64] Thus, they are at risk of field cancerization in contrast to HPV 16 which has a special predilection for tonsillar crypts.

HPV-negative tonsillar carcinoma are associated with a high expression of p53 and cyclin D1, which is minimal or absent in HPV-positive tumors.[45,65] HPV-negative tumors are also associated with well-differentiated squamous histology in contrast to the poorly differentiated or basaloid histology of HPV-positive tumors.[45] Epidermal growth factor receptor (EGFR) is strongly expressed in

**Table 2** Characteristic differences between HPV 16-positive and -negative oropharyngeal cancers

| | Positive | Negative |
|---|---|---|
| Age | Younger (30–50s) | Older (60–70s) |
| Life style | Oral sex | Smoking, drinking |
| Field cancerization | No | Yes |
| Predilection | Tonsils | No |
| Histology | Poorly differentiated Basaloid | Well differentiated |
| Biomarkers | P 16 | EGFR, p53, cyclin D, survivin |
| Chromosomal alterations | Less frequent | Frequent |
| Prognosis | Excellent | Poor |
| Distant metastases | Rare | Frequent |
| Second malignancies | Rare | Frequent |

HPV-negative tumor and is absent or minimally expressed in HPV-positive Tumors.[49] High expression of EGFR is usually associated with a poor prognosis because of high loco-regional recurrences rates and distant metastases.[66,67] Licitra *et al.*[59] suggested that low EGFR levels may be the cause for improved survival in HPV-positive oropharyngeal cancers treated with surgery alone. However, other biomarkers for poor survival such as survivin are also elevated in HPV-negative oropharyngeal cancers and may account for the poor prognosis observed.[68] HPV-positive and negative oropharyngeal tumors are also characterized by distinct genetic signatures.[69,70] Chromosomal alterations and amplifications are more frequent in HPV-negative tumors and are associated with worse survival.[69] HPV-negative and positive tumors have distinct sets of upregulated and downregulated genes involved in cell proliferation, transcription, apoptosis and DNA repair.[70]

Table 2 summarizes characteristics of HPV 16-positive and -negative oropharyngeal tumors. Table 3 summarizes survival difference between HPV-positive and negative oropharyngeal cancers.

**Table 3** Survival difference between HPV 16-positive and -negative oropharyngeal cancers

| Study | Patient No | Treatment type | Survival HPV+ (%) (years) | Survival HPV− (%) (years) |
|---|---|---|---|---|
| Chung *et al.*[28] | 46 | CRT | 86 (5) | 35 (5) |
| Hafkamp *et al.*[46] | 77 | NS | 69 (5) | 31 (5) |
| Kumar *et al.*[52] | 50 | CRT | 80 (5) | 40 (5) |
| Weinberger *et al.*[54] | 107 | RT PostopRT | 79 (5) | 20 (5) |
| Nichols *et al.*[56] | 44 | CRT | 89 (3) | 69 (3) |
| Lassen *et al.*[57] | 156 | RT | 62 (5) | 26 (5) |
| Reimers *et al.*[60] | 97 | S RT CRT | 73 (5) | 63 (5) |
| Fakry *et al.*[71] | 62 | CRT | 78 (5) | 50 (5) |

CRT, chemoradiation; NS, not specified; RT, radiotherapy; S, surgery; postopRT, postoperative radiation.

## Management of HPV 16-associated oropharyngeal carcinoma

Most patients with HPV 16-associated oropharyngeal carcinoma present with locally advanced disease at diagnosis.[52,54] Resectable tumors can be treated with either surgery followed by postoperative radiotherapy or concurrent chemoradiation with similar outcome.[72] Unresectable disease are usually treated with concurrent chemoradiation because of superior survival rates compared to radiotherapy alone.[12–19] However, survival is usually poor because of high rates of loco-regional recurrences and distant metastases. Three-year survival rates ranged from 55% to 62% and decreased to 22% at 5 years. The observed low survival rate was similar to patients with HPV 16-negative tumors (Table 3) and reflected a different patient population consisting of older, smokers and drinkers, and most likely EGFR positive and P16 negative subjects. HPV oropharyngeal cancers represent a distinct population of patients with excellent survival (5 year survival in the 80 percent range) regardless of the type treatment.

Given the fact that most HPV-positive patients are young, treatment selection should take into consideration the mortality and morbidity associated with the selected treatment. Surgery of the oropharynx is associated with significant alteration of

speech and swallow because of resection of critical muscles essential for these functions.[73,74] Chemoradiation offers several advantages including anatomic organ preservation and speech conservation. In addition, surgery is associated with a higher mortality rate compared to radiotherapy.[75] Mortality rates and serious complications were 3.2% and 23%, respectively for surgery compared to 0.8% and 6% for radiotherapy.[75] Thus, concurrent chemoradiation is usually selected in most institutions for locally advanced oropharyngeal cancers. However, with early detection, HPV-positive patients may not require the combined modality as surgery or radiotherapy alone provide excellent survival with less morbidity.[75,76] Patients with HPV associated oropharyngeal cancers are often initially misdiagnosed with upper aero-digestive infections because of their young age and lack of information by the primary care physicians about this clinical entity. These patients are usually treated with prolonged courses of antibiotics thus delaying their cancer diagnosis.[77] Oropharyngeal cancer patients with early diagnosis have been shown to have less advanced stages at diagnosis, resulting in improved survival.[78] Treatment with radiotherapy alone for early stages instead of concurrent chemoradiation for locally advanced diseases is less costly for society.

It is estimated that for each head and neck cancer patient treated with radiotherapy alone, 10 000 dollars is saved compared to chemoradiation due to the cost of chemotherapy and radiotherapy- related complications.[79] A history of oral sex should be obtained in sexually active young people with a sore throat and should raise a red flag for a possible underlying malignancy. Early referral to Ear, Nose and Throat surgeons will decrease treatment morbidity and treatment cost. Thus, primary care physicians will play a major role in the management of oropharyngeal cancer. As HPV 16-associated oropharyngeal cancers carry an excellent prognosis, new treatment protocols are under consideration for selection of patients with advanced stages who may benefit from radiotherapy alone instead of the combined modality. Patients presenting with small tumors, high expression of p 16, minimal or absent EGFR expression and high viral load in the tumor specimen have excellent survival despite the presence of neck nodes.[29,30,52,54,58,60,63] Patients fitting these criteria may be enrolled in a protocol study with radiotherapy alone to decrease treatment morbidity and to improve quality of life. This treatment approach should be considered experimental as concurrent chemoradiation or postoperative radiotherapy remains the standard of care for locally advanced head and neck cancer.

## Prevention of HPV 16 infection

As the mean time between HPV infection and cancer development is about 12 years, the number of young adults developing oropharyngeal cancer is expected to rise steadily in the years to come.[80] Vaccination must be started prior to sexual puberty to be effective as adolescents consider oral sex and deep kissing as safe alternatives to avoid unwanted pregnancy and venereal diseases. HPV 16 infection currently affect all ethnic groups in the US because of the changing attitude toward sex.[5] Vaccination should target both males and females along with public education as most adolescents have little knowledge about the risks involved with sexual behavior.[81,82] Unless physicians take an active role to educate the public and promote clinical trials for vaccination, we will witness a tidal wave of young people with head and neck cancer in the next decades.

## Conclusions

HPV-associated oropharyngeal cancers is a complete clinical entity distinct from the traditional head and neck cancer that affects young people and is related to sexual behavior. Despite excellent prognosis, physicians should refer patients early on to avoid delay in diagnosis and to reduce treatment cost and morbidity related to chemoradiation-induced complications. Vaccination against HPV 16 should be considered in future clinical trials.

*Conflict of interest*: None declared.

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