

## RESEARCH ARTICLE

# Significance of p16 Positive Expression in Oropharyngeal Cancers

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### Abstract

**Background:** The involvement of HPV in oral and oropharyngeal carcinogenesis was first proposed in 2004, based on epithelial HPV tropism and detection of HPV genotypes in oral squamous cell carcinoma samples. While 60-70% of oropharynx tumors may be HPV-positive, only 10 to 19% of tumors of the oral cavity, larynx and hypopharynx appear to have HPV infection. The aim of the study was to evaluate HPV infection associated with oropharyngeal cancer. **Materials and Methods:** Seventy-eight cases were selected for p16 immunoreaction, and demographic data were collected for comparisons. **Results:** Most patients were over 60 years old, and 64.1% were smokers. Immunohistochemistry results showed that 86.3% of cases stained positive for p16 protein. **Conclusion:** The oropharyngeal cancer profile at Erasto Gaertner Hospital presented a high index of smokers over 60 years as well a high number of p16+ tumors, for what we can not determinate the main etiologic factor, but can be aware of the number of patients that presented HPV infection. Since prevention is still the best way to deal with cancer disease, it is important to analyze the interaction of these two etiologic factors and how to detect lesions at an early stage.

**Keywords:** Oropharynx cancer - HPV - p16 expression - prevention

*Asian Pac J Cancer Prev*, 15 (23), 10289-10292

### Introduction

Cancer is a major public health problem worldwide. Currently, in Brazil, it is the second cause of death, and over 14,000 cases of head and neck cancer were estimated for 2012, with 45% of death due the disease (Jemal et al., 2011). Tobacco, alcohol, and recently, HPV infection are major risk factors associated with oral cancer. Worldwide, tobacco is responsible for 42% of deaths from mouth cancer (Jemal et al., 2011; Mishra and Meherotra, 2014). However, between 15-20% of cases of head and neck cancer can not be related to exposure to tobacco and alcohol (Gillison and Shah, 2001).

An increase in the number of cases of squamous cell carcinoma of the oropharynx, specifically tonsils and base of tongue, has been noted worldwide, more frequently in patients aged 45 to 50 years (Marur et al., 2010; Elango et al., 2011; Tural et al., 2013). Most patients are leucoderma men with no history of tobacco and alcohol consumption.

The main characteristics of patients associated with a favorable head and neck cancer prognosis are related to lack of exposure to tobacco and alcohol, good general health and no comorbid associated. These characteristics are often associated with HPV-positive tumors. Retrospective studies suggest that individuals with

HPV-positive oropharyngeal cancer have a better response to chemotherapy and radiotherapy, with increased survival compared with those with tobacco-related tumors (Marur et al., 2010; Huang et al., 2012).

The treatment of choice for oropharyngeal cancers is surgery with or without chemotherapy and/or adjuvant radiotherapy for preservation of speech and swallowing, especially when it involves the tongue base. The increased prevalence of oropharyngeal cancer in young populations and improved prognosis is in contrast to the survival rate in older patients with comorbidities associated with tobacco and alcohol (Marur et al., 2010; Tural et al., 2013). The relation between oncogenic HPV and anogenital cancer is already well established in the literature, but only in the last decade this is associated with some types of head and neck cancer (HNC). To date, over 100 different types of HPV have been identified of which at least 15 have some oncogenic potential (Munoz et al., 2003). However, most cases of HPV- HNC are related only to HPV 16, the same type that causes the anogenital cancer (Jalilvand et al., 2014). Studies show that while it is possible to observe a slight decrease in cases of HNC associated with tobacco, the opposite happens with cases associated with HPV, reaching a younger age group. In Sweden, for example the number of cases of tonsil cancer linked to HPV, increased

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1-5 times for women and 2-6 for men times between the years 1970 and 2002 (Hammarstedt et al., 2006). In the United States, an increase was observed 1-3% of cancers of the tongue base and 0-6% of tonsillar cancer every year between 1973 and 2004 (Chaturvedi et al., 2008). The significant increase in cases of oropharyngeal cancer indicates HPV as the main cause of tonsillar malignancies in North America and Europe (Marur et al., 2010). Changes in sexual behavior among young people appear to be the main cause associated with increased HPV-induced malignancies.

The p16 is a tumor suppressor protein encoded by the CDKN2A gene, frequently mutated in different types of tumor. The immunoeexpression of this protein in squamous cell carcinoma is strongly associated with HPV infection, and therefore it can be a valuable guide for deciding between the regimes of concomitant treatment. Studies show that immunohistochemical detection of p16 protein has a higher sensitivity compared to in situ hybridization and can be an alternative when it can not perform detection with the use of PCR (Kong et al., 2007). In addition, expression of p16 protein is also used as an initial approach to indicate the presence of the virus, followed by PCR analysis to identify the type of HPV present.

The aim of the study was to evaluate the prevalence of HPV infection associated with oropharyngeal cancers at Erasto Gaertner Hospital and assess patient's risk factors profile.

## Materials and Methods

### Samples

Patients with squamous cell carcinoma of the oropharynx (SCCO) treated at Erasto Gaertner Hospital between the years 2005 to 2009 were selected.

Medical records were consulted to collect data such as age, sex, occupation, diagnosis, time of evolution, family history, treatment, risk factors and prognosis.

All patients with SCCO as primary tumor that have not been subjected to previous antineoplastic treatment of any kind were included in the study. Patients who underwent previous antineoplastic treatment, or patients who had other previous malignancy were excluded.

### Immunohistochemical reaction

For all samples selected, histopathological slides were revised for diagnosis confirmation.

For immunohistochemical staining, 3  $\mu$ m slides were taken. The slides were incubated overnight at 4°C with a primary antibody, p16 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) diluted 1:400. After primary antibody exposure, the slides were washed and treated with biotinylated antibody for 30 min. Antigen visualization was achieved using ENVISION FLEX (DAKO, Carpinteria, CA, USA) for 30 min followed by diaminobenzidine chromogen (DAKO Liquid DAB+, K3468). Slides were counter-stained with haematoxylin.

Sections were considered positive when more than 5% of the tumor cells presented nuclear immunostaining.

The results were analyzed using chi-square test. For all tests, p value was <0.05.

## Results

Seventy-eight patients were selected for these study, 71 male and 7 female, most of them leucoderma (73%). Fifty percent of patients were in the 6°C decade of life. Most patients were between 51 and 65 years old (50%), and 24.6% had less than 50 years old. Twenty-one patients reported familial cancer, including stomach, breast, oral cancer and oropharynx.

Risk factors included tobacco and alcohol consumption. Nineteen patients (24.3%) were considered heavy drinkers (average 1L per day), and 39 patients (50%) were former drinkers. Among tobacco users, 50 patients (64.1%) were smokers and 22 (28.2%) were former smokers.

Tumor location considered only oropharynx sites, and most frequent was the base of the tongue (38.4%), followed by tonsillar pillar (32%) and soft palate (25.6%). Most of patients presented stage II tumor (42.6%) followed by stage IV (35.9%), stage III (20.5%) and stage I (5.8%) (Table 1).

The most frequent treatment of choice was surgery (42%), and 94% were associated with adjuvant therapy -chemotherapy, radiotherapy and combination of both. Cases which surgery was not possible or indicated, patients were submitted to chemotherapy, radiotherapy or combination of both.

After treatment, 6.4% of patients were free of the disease, 19.2% presented residual disease or metastasis, 8.9% had second primary tumor, 6.4% presented a tumor recidive and 50% did not finish treatment for unknown reasons. Follow-up of these patients showed that 16.6% died from the disease, 11.5% died from other causes, and 34.6% died from complications due the disease.

### Immunohistochemistry analysis

Immunohistochemistry results showed that 86.3% of cases stained positive for p16 protein. Comparing these results with clinical data, only 6% of patients are alive free of the disease and all of them were p16+. Forty-nine patients died due disease complications, being 69% p16+. There was no correlation between p16 expression and overall survival in this group (p=0.25).

**Table 1. Cohort Profile**

Tumor site	No. of Cases (%)	p16+ (%)	Stage (%)	Status
Soft Palate	35.8	75	I-3.6	
			II-7.2	A-28.5%
			III-32.1	R-7.14%
			IV-57.1	D-64.2%
Base of the Tongue	41	65.6	I-9.4	
			II-9.4	A-18.75%
			III-28.1	R-12.5%
			IV-53.1	D-68.75%
Tonsillar pillar	19.2	73.3	I-6.7	
			II-6.7	A-26.6%
			III-46.6	R-26.6%
			IV-40	D-46.8%
Uvula	1.28	100	IV-100	A- -
				R- -
Others	2.56	100		D-100%
			IV-100	A-50%
				R- -
				D-50%

\*A: alive, free of the disease; R: disease recurrence; D: death due the disease

Considering patients under 50 years old, 70% showed p16 positive expression and from that 35% are alive, free of the disease. All of these patients were smokers.

## Discussion

The involvement of HPV in oral and oropharyngeal carcinogenesis was first proposed by Syrjanen et al. (2004), followed by other authors, based on epithelial HPV tropism, morphological similarities between oropharyngeal and genital epithelium, ability of immortalization of human oral keratinocytes in vitro, etiologic role of high-risk HPV in cervical cancer, and finally detection of HPV genotypes in oral squamous cell carcinoma samples (Syrjanen et al., 2004; Pannone et al., 2011). While 60-70% of oropharynx tumors are HPV-positive, only 10 to 19% of tumors of the oral cavity, larynx and hypopharynx have HPV infection related (Kreimer et al., 2005).

HPV is a sexually transmitted infection that affects mainly specialized reticular epithelium covering the tonsil crypts. Once integrated into the host cell genomic DNA, it deregulates expression of E6 and E7 oncoproteins. The E6 protein, in turn, induces the degradation of p53, leading to a loss of its activity, compromising their ability to activate repair mechanisms and apoptosis, generating genomic instability. The E7 protein binds the pRB protein, leading the cell to enter the S phase of the cell cycle, causing unregulated proliferation and malignant transformation (Marur et al., 2010). Once the retinoblastoma tumor suppressor gene (RB) is a negative regulator of the P16 gene, the tissues infected by HPV, frequently have high expression of p16. Thus, p16 immunopositivity can indicate HPV-infected cell rather than the presence of the virus (Pytynia et al., 2014).

Although most of cases of our study were p16+, tobacco was also a significant risk factor to be analyzed. It was not well established in the literature, how long the exposure to tobacco would take to induce a malignant transformation, as a general rule, the longer the exposure, higher the risk. The group of patients aged between 35 to 50 years old had 100% of smokers and 70% of p16+ tumors. Forty percent of patients of this group are alive which 62.5% showed p16 positive expression; thus, although risk factors, HPV infection and tobacco exposure could have act synergistically, it seems that HPV had a more prevalent role on carcinogenesis.

Results showed a high mortality rate that can be related to late diagnosis, since 35.9% of patients were diagnosed at stage IV. Late diagnosis with advanced-stage disease is the main cause of head and neck cancer morbidity and mortality (Mignogna et al., 2004). Although the oral cavity is an easy site for physical exam, often a delay in seeking medical care leads to the advanced-stage of disease at time of diagnosis (Ragin et al., 2007).

The identification of HPV related tumors has great clinical relevance, favoring more specific treatment and improving patient's quality of life (Huang et al., 2012). Given the clinical importance of HPV-related tumor identification, several methods have been proposed, but the immunopositivity of p16 protein seems to have a

good cost-effective result, and can indicate the need of PCR analysis for HPV- type identification. El-Naggar and Westra (2012) (El-Naggar and Westra, 2012) proposed that uniform p16 staining in all or most cancer cells of oropharyngeal carcinoma might substitute for other HPV testing. Thus, it is important that HPV identification in OC became a routine, so patients can receive better treatment.

OC profile at Erasto Gaertner Hospital presented a high index of smokers over 60 years as well a high number of p16+ tumors, for what we can not determinate the main etiologic factor, but can be aware of the number of patients that presented HPV infection. Since prevention is still the best way to deal with cancer disease, it is important to analyze the interaction of these two etiologic factors and how to detect the lesions at early stage.

Although we cannot state that our cohort was composed of HPV-related tumor, it showed a high prevalence of HPV, showing that the virus is present in great part of patients. More studies must be done to investigate the interaction of tobacco and HPV on oral carcinogenesis.

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