

## RESEARCH ARTICLE

## Increase in the Rate of HPV Positive Oropharyngeal Cancers During 1996-2011 in a Case Study in Turkey

Deniz Tural<sup>1\*</sup>, Olgun Elicin<sup>2</sup>, Sebnem Batur<sup>3</sup>, Deniz Arslan<sup>1</sup>, Buge Oz<sup>3</sup>, Suheyla Serdengeçti<sup>4</sup>, Omer Uzel<sup>1</sup>

### Abstract

**Background:** Primary aim of this study is to assess whether or not there is an increase at rate of HPV positive oropharyngeal cancers during 1996-2011 in Turkey, for comparison with prior reports from Western countries. **Materials and Methods:** A total of 138 newly diagnosed patients with oropharyngeal cancer were identified, 39 of which had no primary tumor specimen available and 18 patients with invalid HPV status, therefore HPV status for remaining 81 patients was evaluated. The presence and type of HPV DNA were determined with formalin-fixed paraffin embedded specimens, using an HPV DNA-based multiplex PCR assay. Associations between HPV status and clinicopathological characteristics were evaluated using a two-sample t-test for the continuous variables and the categorical variables were compared by chi-square test. Overall survival (OS) periods were calculated with Kaplan-Meier method. **Results:** The proportion of HPV-positive cancer has continued to increase during 2004-2011 as compared with 1996-2003. Notably, 33% (6/18) of the cases were HPV-positive in 1996-1999, 43% (9/21) in 2000-2003, 55% (11/20) in 2004-2007 and 70% (16/23) in 2008-2011. Thus, when we compared the results obtained during the 2004-2011 with results of 1996-2003 period, we found that increase at HPV-positivity ratio was statistically significant (38% vs 64%  $p=0.012$ ). **Conclusions:** This study demonstrated that HPV positive oropharyngeal cancers are increasing in Turkish patients as in the Western world.

**Keywords:** Oropharyngeal cancer - HPV status - incidence - Turkey

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### Introduction

Epidemiologic studies have demonstrated that there has been a decrease in the incidence of oral cavity, hypopharyngeal and laryngeal cancers through the past two decades due to decrease in smoking which is the primary risk factor for these cancers (Sturgis et al., 2011). However, the incidence of oropharyngeal cancer increased due to increase at HPV related cancers at the tonsillar and base of tongue region (Kreimer et al., 2005; Hobbs et al., 2006; Chaturvedi et al., 2011; Sturgis et al., 2011). The main risk factors for developing tonsillar cancer are tobacco usage and alcohol consumption. However, past two decade trials have demonstrated that HPV is a risk factor for the development of oropharyngeal squamous cell carcinoma (Gillison et al., 2001; Klusmann et al., 2001; Mork et al., 2001; Smith et al., 2004; D'Souza et al., 2007). The way of transmission is still under debate, but it has been suggested that oropharyngeal HPV infection maybe acquired sexually (D'Souza et al., 2007, Smith et al., 2004). Cohort studies in the 1990s indicated that approximately 50% of oropharyngeal cancer was related to human papillomavirus (HPV), while recent studies

demonstrated that HPV may account as 70-80% of these cancer (Chaturvedi et al, 2011; Sturgis et al., 2011).

Moreover studies have indicated that important clinicopathological differences between HPV associated oropharyngeal cancer and HPV negative patients. Patients with HPV positive oropharyngeal cancer are approximately ten years younger than HPV negative patients (Näsman et al., 2009; Gillison et al., 2010; Chaturvedi et al., 2011). HPV positive oropharyngeal cancers seem to be presented more likely with a relatively early T stage, but relatively advanced lymph node involvement. However, despite aggressive feature of HPV positive cancers, these diseases appear to have better prognosis than oropharyngeal cancer with HPV negative patients. Retrospective analysis of two randomized studies demonstrated that the incidence of distance metastases was lower at HPV positive patients than HPV negative oropharyngeal cancer patients (Ang et al., 2010; Huang et al., 2012).

In western country the incidence of HPV related oropharyngeal cancer increased. Primary aim of this study is to assess whether or not there is an increase at rate of HPV positive oropharyngeal cancers during 1996-2011 in the Turkey, in comparison with prior reports in western countries.

<sup>1</sup>Akdeniz University, Medical Faculty, Department of Internal Medicine, Division of Medical Oncology, Antalya, <sup>2</sup>Department of Radiation Oncology, <sup>3</sup>Department of Pathology, <sup>4</sup>Department of Internal Medicine, Division of Medical Oncology, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey \*For correspondence: deniztural@gmail.com

## Materials and Methods

### Patients

The medical records of patients who were treated for oropharyngeal squamous cell carcinoma between December 1996 and December 2011 were retrospectively analyzed. A total of 138 newly diagnosed patients with oropharyngeal cancer were identified, 39 of which had no primary tumor specimen available, 18 patients with invalid HPV status, therefore remaining 81 patients' HPV status were evaluated. Patients with a histological diagnosis of oropharyngeal squamous cell carcinoma provided from the tumor that was located in oropharyngeal site of head and neck (Palatal tonsil, base of tongue, soft palate, back wall of oropharynx) were included in the study. Patients are excluded from the study if they had metastatic disease. Demographical data including age at diagnosis, gender, tobacco exposure were obtained from medical records. Tumor site, stage, grade were determined from reviews of radiology, surgery, pathology, medical oncology, radiation oncology reports. Tumor stage was determined according to the 2010 American Joint Committee on Cancer (AJCC) staging criteria. Patients were classified as never smoker or smoker. Patients are undergoing treatment based on stage as primary surgery with or without adjuvant radiotherapy or definitive radiotherapy or definitive concurrent chemo radiotherapy. Written informed consent of the patients or their next of kin were obtained prior to the study.

### HPV assessment

**Sample preparation:** paraffin-embedded tissues which were in 1.5 ml microcentrifuge tubes were isolated using the Instruction for Invisorb® Spin FFPE Tissue Kit (Germany). Tissues in the tubes were rinsed off paraffin by treating with xsilene (1ml) and alcohol (0.5ml). Lysis is obtained by leaving them in the thermoshaker with the kit solutions (400µl Lysis Buffer G ile 0,4µl DTT ve 40µl Proteinase K) for 4 hours at 52°C. A 50µl Elution Buffer D is prepared as the final sample volume. PCR procedure is started with the 2µl of the prepared sample. The per protocol amplification procedure of the isolated samples is initiated with the amplification mix used for INFINITI® HPV Genotyping Assay. After the PCR stage, the samples are analyzed with the AUTOGENOMICS INFINITI® Analyzer.

**PCR Amplification:** the presence and type of HPV DNA were determined at 5mm sections of formalin-fixed paraffin embedded cancer tissue, using an HPV DNA-based multiplex PCR assay (M-PCR) by AutoGenomics. This assay simultaneously detects and identifies 26 HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 73, 82, 6, 11, 30, 34, 70, 85) that are designed to amplify the E6/E7 genes. A 5µL volume of extracted DNA was added to 15µL of master mix. Amplification was done in a 24-well plate in a GeneAmp® PCR System 9700 (Applied Biosystems).

**L1 Gene Amplification and Sequencing:** the universal primers MY11 and MY09 were used to amplify the L1 gene of HPV. PCR was performed in a LightCycler (Roche) and melting curves were compared to HeLa cell and human genomic DNA (hgDNA) controls, and to no

template controls (NTC). Samples with melting patterns similar to the hgDNA and NTC were not able to be sequenced. All other samples were sequenced by standard sequencing methods in a 3730 DNA Analyzer (Applied Biosystems) using the MY11/MY09 primers. HPV-DNA testing of paraffin sections is especially vulnerable to cross-contamination. This was monitored by incorporating negative paraffin controls as well as cervical cancer paraffin specimens as positive controls with every staining procedure performed.

### Statistical methods

Associations between HPV status and clinicopathological characteristics were evaluated by using a two-sample t-test for the continuous variables and categorical variables were compared by using chi-square test. HPV status were calculated for 2-year period and from 1996-2003 to 2004-2011. Overall survival (OS) periods were calculated with Kaplan-Meier method. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, SA) software. The statistical level of significance was defined as  $p < 0.05$ .

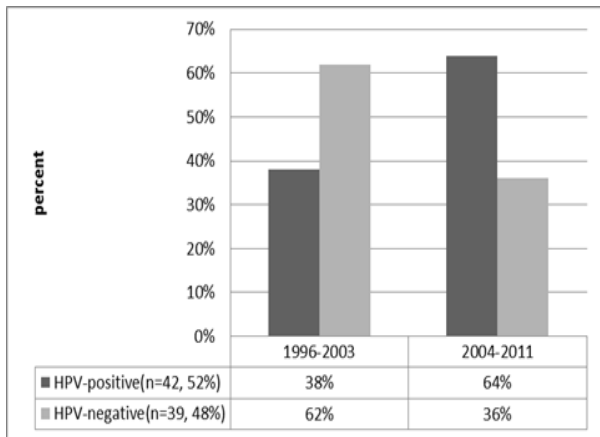
## Results

### Patients and tumor characteristics

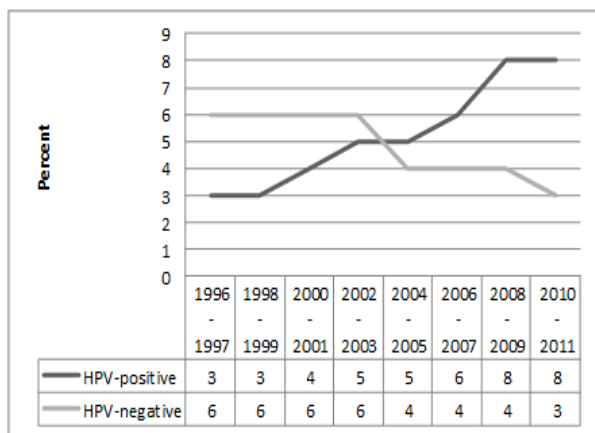
Fifty-two percent (42/81) of the tumor samples were HPV positive. HPV16 was detected in 36 patients' (86%) tumor samples by multiplex and type-specific PCR. Five patients and 1 patient were confirmed as HPV18 (12%) and HPV33 (2%) by multiplex PCR, respectively. Comparing the results obtained during 2004-2011 period

**Table 1. Characteristics of 138 Patients with Oropharyngeal Squamous Cell Carcinoma**

	1996-2003 64 (47%)	2004-2011 74 (53%)	p value	
Age in years, (median, SD)	61.2 ±13.4	55.4 ±10.2	0.015	
Sex				
	Males	47 (74)	56 (77)	0.6
	Females	17 (26)	17 (23)	
Clinical T stage				
	T1	11 (17)	13 (18)	0.3
	T2	26 (40)	32 (43)	
	T3	9 (14)	11 (15)	
	T4	11 (18)	12 (16)	
	Unknown	7 (11)	6 (8)	
Clinical N stage				
	N0	32 (50)	35 (47)	0.4
	N1	14 (23)	16 (22)	
	N2	13 (20)	18 (24)	
	N3	3 (4)	3 (4)	
	Unknown	2 (3)	2 (3)	
Stage				
	I/II	28 (44)	31 (42)	0.2
	III/IV	32 (50)	38 (51)	
	Unknown	4 (6)	5 (7)	
Tumor differentiation				
	Well or moderate	39 (61)	40 (54)	0.04
	Poor	22 (34)	31 (42)	
	Unknown	3 (5)	3 (4)	
Tumor localization				
	Base of tongue	22 (34)	24 (32)	0.01
	Tonsil	25 (39)	38 (52)	
	Other subsides	17 (27)	12 (16)	
Tobacco exposure history				
	Smoked	37 (58)	24 (33)	0.02
	Never smoked	22 (34)	44 (59)	
	Unknown	5 (8)	6 (8)	
HPV-status (determination of 81 patients)				
	Positive	15 (38)	27 (64)	0.012
	Negative	24 (62)	15 (36)	



**Figure 1. The Changes of the Annual HPV Status of Oropharyngeal Cancers between 1996 and 2011 Shown as 2 Year Intervals**



**Figure 2. Comparing the HPV Positive and Negative Patients' Results Obtained during 1996-2003 Period and 2004-2011 Period**

with results for 1996-2003 period, we found that median age decreased (55.4 vs 61.2,  $p=0.015$ ), tonsillar subsite of oropharyngeal squamous cell carcinoma increased (52% vs 39%,  $p=0.01$ ), poor tumor histology ratio increased (42% vs 34%,  $p=0.04$ ) and rate of patients who smokes decreased (33% vs 58%,  $p=0.02$ ) (Table 1). The proportion of HPV-positive cancer has continued to increase during 2004-2011 as compared with 1996-2003. Notably, 33% (6/18) the cases were HPV-positive in 1996-1999, 43% (9/21) in 2000-2003, 55% (11/20) in 2004-2007 and 70% (16/23) in 2008-2011. Thus, comparing the result obtained during the 2004-2011 with result for 1996-2003, we found that increase in rate of HPV-positive subjects is statistically significant (38% vs 64%  $p=0.012$ ) (Figure 1 and 2).

## Discussion

This study confirmed that the rate of HPV-positive oropharyngeal squamous cancer in the Turkey increased during 1996-2011 period. We have been demonstrated a continuous increase in the proportion of HPV positive oropharyngeal squamous cancer from 1996-1999 period (33%) to 2008-2011 period (70%). Results of this study are consistent with prior studies that were held in western

countries (Romanitan et al., 2008; Näsman et al., 2009; Chaturvedi et al., 2011). In current study HPV16 was most common subtype, detected in 86% of tumor samples, HPV18 (12%) and HPV33 (2%) are the other common subtypes detected by multiplex and type-specific PCR. Consistently with the literature; HPV-16 is the dominant subtype at our patients as western countries. Chaturvedi et al. (2011) who used Surveillance, Epidemiology, and End Results (SEER) database, indicated that prevalence of HPV positivity increased from 1984-1989 (16%) period to 2000-2004 (72%) period in USA (Chaturvedi et al., 2011). Näsman et al. (2009) demonstrated that HPV positive tonsillar cancer were 23% in 1970, while it was detected 79% at 2000-2007 period in Sweden (Näsman et al., 2009). Also a Greece study indicated that HPV positive tonsillar cancer prevalence was 17% in 1992-1998 and 50% between 2000-2007. Western country cohort studies at 1990s indicated that approximately 50% of oropharyngeal cancers were related to human papillomavirus (HPV), while recent studies demonstrated that HPV may account up to 70-80% of these cancers (Chaturvedi et al., 2011; Sturgis et al., 2011). In past two decades increase in HPV positive cancer prevalence appears to be related with certain sexual behaviors, such as oral sex and increasing numbers of sexual partners (Gillison et al., 2001; D'Souza et al., 2007). However, in mainland China, Huang et al demonstrated that HPV positivity was detected 16.7% of all oropharyngeal cancers specimens. Chinese people have significantly different sexual lifestyle compared to the westerners; this may explain the lower overall infection rate of HPV (Huang et al., 2012).

The main limitation of this study is that the study includes small size of patients. Despite these limitations, this study demonstrated that HPV positive oropharyngeal cancers increased in Turkish patients same as western countries.

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