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TP53 gene expression in HPV-positive oral tongue SCC and its correlation with nodal metastasis

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ABSTRACT

In this study, we investigated the prevalence of human papilloma virus (HPV) infection and TP53 expression in patients with squamous cell carcinoma (SCC) of the tongue and, subsequently, its significance in cervical lymph node metastases and tumor differentiation.

Sections of formalin-fixed, paraffin-embedded tissue blocks from 94 histologically confirmed tongue SCC cases were investigated in this study. Immunohistochemistry was used to study TP53 expression, and polymerase chain reaction (PCR) was performed for the detection of high risk HPV types (16 and 18).

The frequency of HPV-16 and HPV-18 infection was 10.6% and 16%, respectively. Overexpression of TP53 was observed in 70.2% of patients. Young patients (aged below 45 years) comprised 20% of all patients. There was no significant association between TP53, HPV-16, or HPV 18 presence and higher stages of the tumor, tumor differentiation, or presence of nodal metastasis.

Although an association between head and neck SCC and HPV infection is being recognized and reported, our data implicate that HPV infection or TP53 expression does not play a significant role in oral tongue SCC pathogenesis, differentiation, or metastasis, as seen in our patients.

Introduction

Oral cavity cancer is the eighth most common cancer in the world and the third most common in developing countries. Among oral cancers, the oral tongue is the most common subsite involved. Oral cavity cancer has a geographic predilection, with a higher incidence reported in Southeast Asia and Brazil [16–18]. The high incidence in these areas is due to the habitual usage of chewing tobacco and betel nut, which have a significant influence on malignant degeneration in the upper aero-digestive tract. In recent years, we have increasingly seen young patients who are non-smokers and non-drinkers, but suffer from oral cavity SCC. For these patients, other etiological factors, such as human papilloma virus (HPV) infection, may be considered [3,16]. HPV 16 and 18 have oncogenic activities, where their role in genital cancers is well established. The HPV proteins E6 and E7 inactivate TP53 and pRb genes, respectively, and can produce malignant degeneration through this process. However, regarding oral cavity malignancy, there have been many different studies with a wide range of HPV prevalence (2–98%) [9].

In this study, we investigated the HPV infection profile of 94 oral tongue SCC cases in Iran and determined its relationship with TP53 expression and cervical lymph node metastasis.

Materials and methods

Ninety-four histopathologically confirmed formalin-fixed, paraffin-embedded tissue blocks of patients suffering from oral tongue SCC were retrieved from the pathology archives of Tehran University of Medical Sciences and Mashhad University of Medical Sciences hospitals. From April 2001 through March 2008, these patients had undergone tumor resection and cervical lymph node dissection as the first treatment. Age, gender, TNM stage, histological differentiation grade, and alcohol or tobacco exposure profiles were obtained from the medical records database. Tumor differentiation grades were reported according to the WHO guidelines and TNM stage classification in accordance with the American Joint Committee on Cancer (AJCC Sixth Edition, 2002). Approval for this study was given by the investigation review board and

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medical ethics committee at the Tehran and Mashhad Universities of Medical Sciences.

Immunohistochemistry

Four micrometer thick paraffin-embedded tissue sections were cut and mounted on polylysine-coated slides and were then dried at 56 °C for 30 min. Specimens were de-waxed with xylene and rehydrated in serial graded ethanol solutions. Target antigen retrieval was performed with 95 °C solution of 0.01 M sodium citrate buffer (pH = 6).

For 15 min, hydrogen peroxide 0.1% was employed to block endogenous peroxidase activity. After rinsing with TBST (Tris buffer saline with Tween 20, Dako Cytomation, Denmark), sections were incubated with primary antibody against TP53 (mouse monoclonal anti-TP53, clone DO-7, Dako) at a concentration of 11.9 g total protein/l (diluted 1:30) for 30 min at room temperature. True negative and true positive staining was confirmed by the lack of staining in the absence of primary antibody and colon adenocarcinoma, respectively. Staining was considered positive if >15% of cells were stained for TP53.

DNA extraction

Ten micrometer sections from all formalin-fixed, paraffin-embedded tissue blocks were obtained using sterile microtome blades. All instruments used in extraction were heated and cleansed with xylene and alcohol before each sectioning to avoid HPV contamination. The extraction of DNA from these micro-dissected formalin-fixed, paraffin-embedded tumor tissues was performed using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions.

Detection of HPV DNA

β-Globulin gene served as an indicator of amplifiable DNA in every sample. FWD 5′ CAACTTCATCCACGTTCACC 3′ and REV 5′ GAAGAGCCAAGGACAGGTAC 3′ were used as the primer set. Samples with amplifiable DNA were subsequently analyzed by PCR for the identification and typing of two high risk human papilloma viruses (18 and 16). Amplification was performed with the human papilloma virus typing kit (DNA Technology, Moscow, Russia), according to the manufacturer's instructions. Amplicons were analyzed by electrophoresis on a 2% agarose gel followed by ethidium bromide staining.

Statistical analysis

Association of HPV infection with tumor nodal metastasis, tumor differentiation, TP53 expression, and tumor stage was analyzed by cross-tabulations using the chi-square or Fisher's exact test. The relationship between the patients' age group, smoking or alcohol use, and TP53 expression with the tumor characteristics was also analyzed by cross-tabulation.

Results

Ninety-four patients were eligible to enter this study. Patients' characteristics and tumor features are shown in Table 1. Nineteen subjects (20%) were less than 45 years old and therefore were considered as young patients. We did not find any significant association between cigarette smoking or alcohol use and tumor differentiation, the presence of nodal metastasis or tumor stage. In older patients (>45 years), there was no association between age and lymph node involvement, tumor differentiation, or tumor stage.

Table 1
Patients' characteristics and tumor features of the tongue squamous cell carcinoma study population.

Patients' characteristic	
Characteristics	*N* (%)
Age (years)	
Mean	57.88 ± 15.11
Range	22–84
Sex	
Male	51 (54.2)
Female	43 (45.8)
Risk factor (alcohol use or cigarette smoking)	
Positive	25 (26.5)
Negative	69 (73.5)
Stage	
I	24 (25.6)
II	25 (26.5)
III	29 (30.8)
IV	16 (17.1)
HPV 16	
Positive	10 (10.6)
Negative	84 (89.4)
HPV 18	
Positive	15 (16)
Negative	79 (84)
TP53 mutation	
Positive	66 (70.2)
Negative	28 (29.8)

Twenty-five patients (26.6%) were infected with either HPV 18 or HPV 16. No sample showed a coexisting HPV 16 and 18 infections. HPV infection was more prevalent among male patients compared to females (33.3% vs. 16.3%, *P*-value < 0.05). Overexpression of TP53 was observed in 70.2% of patients (Fig. 1). The relationships between patient and tumor characteristics and TP53 expression and HPV infection are summarized in Table 2. The frequency of HPV infection was higher in well-differentiated tumors than in moderately or poorly differentiated ones. However, the differences were not statistically significant (40.7% vs. 22.8%, *P*-value = 0.196). There was no significant association between HPV positivity and higher stages of the tumor or the presence of nodal metastasis.

No statistically significant association was found between TP53 expression and tumor stage, tumor differentiation, or lymph node involvement. TP53 and HPV infection profiles of our study population are shown in Table 3.

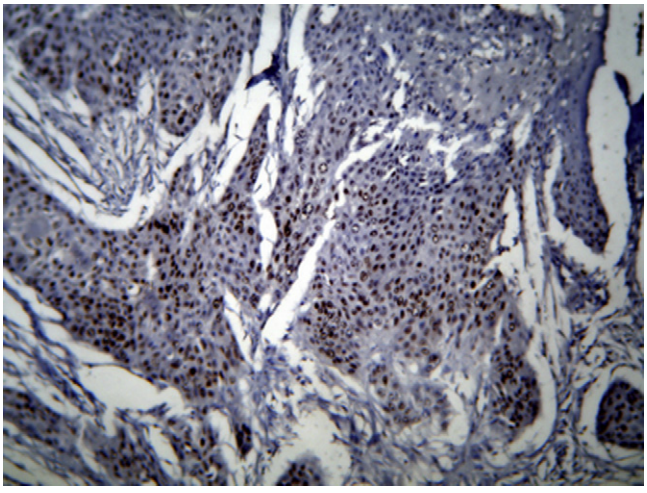

Fig. 1. Strong nuclear staining of TP53 in 80% of cells (mouse monoclonal anti-TP53, clone DO-7, 100×).

Table 2
Patients and tumor characteristics classified by TP53 mutation and HPV infection.

Characteristic	TP53 mutation			HPV		
	Positive	Negative	*P*-Value	Positive	Negative	*P*-Value
Age			1.000			1.000
≤45	14 (73.7)	5 (26.3)		5 (26.3)	14 (73.7)	
≥45	53 (70.6)	22 (29.4)		20 (26.7)	55 (73.3)	
Sex			0.816			0.061
Male	37 (72.5)	14 (27.5)		17 (33.3)	34 (66.6)	
Female	30 (69.7)	13 (30.3)		7 (16.3)	36 (83.7)	
Smoking history or alcohol use			1.000			1.000
Positive	20 (80)	5 (20)		4 (16)	21 (84)	
Negative	53 (76.8)	16 (23.2)		14 (20.2)	45 (79.8)	
Differentiation			0.160			0.147
Well	38 (64.4)	21 (35.6)		24 (40.7)	35 (59.3)	
Moderate–Poor	28 (80)	7 (20)		8 (22.8)	27 (77.2)	
T stage			0.864			0.141
I	17 (65.4)	9 (34.6)		5 (19.2)	21 (80.8)	
II	25 (69.4)	11 (30.6)		14 (38.9)	22 (61.1)	
III	17 (68)	18 (32)		3 (12.0)	22 (88)	
IV	6 (85.7)	1 (14.3)		2 (28.5)	5 (71.5)	
N stage			0.281			0.365
X	4 (50.0)	4 (50.0)		5 (62.5)	3 (47.5)	
0	36 (69.2)	16 (30.8)		14 (26.9)	38 (73.1)	
1	16 (66.7)	8 (33.3)		4 (16.7)	20 (83.3)	
2	8 (80)	2 (20)		2 (20)	8 (80)	
Stage			0.636			0.435
I	15 (62.5)	9 (37.5)		5 (20.8)	19 (79.2)	
II	16 (64)	9 (36)		10 (40)	15 (60)	
III	21 (72.4)	8 (27.6)		6 (20.7)	23 (80.3)	
IV	12 (80)	3 (20)		4 (26.7)	11 (73.3)	

Table 3
HPV positivity and TP53 expression cross-tabulation.

P-Value = 0.850	TP53	
	Overexpression *N* (%)	Normal
HPV+	17 (68)	8 (32)
HPV−	49 (71)	20 (29)

Discussion

In recent years, there has been an impressive and hopeful progress in cancer treatment. However, for head and neck cancers, poor outcomes due to loco-regional relapse, even in early stages, are not unexpected.

Most patients with oral SCC (OSCC) are male, above 60 years old, and have a history of smoking and alcohol drinking. Over the past decades, the increase in the number of young patients or in the patients without any risk factors for OSCC calls for a new method of considering prognostic factors other than the TNM classification and histological grade [3,14]. Although TNM classification can predict prognosis correctly in some patients, loco-regional relapse after standard treatment in early stage diseases does produce conflicts.

Research in genomic features of tumors which can affect prognosis presents a new horizon and introduces tumor markers in malignant diseases. One of the well-studied tumor markers is TP53 gene, which plays a role as a tumor suppressor gene by initiating cell cycle arrest in G1 and giving an opportunity for DNA repair or apoptotic cell death.

If TP53 gene is silenced by mutation or aberration, damaged cells then have the opportunity to grow and become cancerous. Environmental factors, such as tobacco, alcohol, and viral infection, can produce malignant degeneration by cell alteration as a result of gene aberration [3,14].

The true relationship between HPV infection and head and neck cancers has yet to be determined, but the estimated incidence for high risk HPV is around 25–30% [9]. Different results in the literature are due to tumor sites, sample size, geographic differences, detection methods, and genetic variation, such as TP53 polymorphism [14,17]. Kreimer et al. reviewed 60 studies to determine the prevalence of HPV in oral cavity and oro-pharyngeal cancers. The average rate of infection was 35.6% for oro-pharyngeal cancers and 23.5% for oral cavity cancers. The predominant type was HPV 16, which involved 87% of HPV-infected oro-pharyngeal and 68% of HPV-infected oral cavity cancers [19].

Shiboski et al. found that between 1973 and 2001, 7% of oral cavity SCC and 5% of pharyngeal SCC cases reported by the SEER program were between 20 and 44 years old. Also, in this group, oral tongue and tonsil had the greatest proportion of involved areas in young adults [15].

The increase in the incidence of tongue and tonsil SCC in some countries, without parallel increment of their main risk factors (smoking and drinking), may be due to other probable risk factors, such as oncogenic HPV infection, which concurrently exhibits its own rapid growing pattern [8,12].

However, in the study of Siebers et al., conducted in the Netherlands, it was found that none of their seven non-smoking and non-drinking oral tongue SCC patients were high risk (HR)-HPV-positive [16]. This result is similar to that of Kantola and Dahlstrom's studies, in which there was no association between HR-HPV infection and oral tongue SCC [5,10]. On the other hand, da Silva reported a higher risk (25.6%) of oncogenic HPV infection in tongue SCC in Brazil [6]. HPV infection incidence, in the study by Dalhstrom, was 40% for the base of tongue and 2.4% for the mobile tongue [4].

In cervical cancer, HPV-E6 oncoprotein disrupts the p53 pathway. Although aberration and loss of function of p53 gene may be seen in 80% of oral cavity SCC, its silencing may be different from that occurring in cervical cancer, and viral integration with host DNA is not common [7,14,15].

Kumar et al. reported a 31% incidence of HPV-16-positive oropharyngeal SCC cancer in India.

Seventy-four percent of these patients had overexpression of TP53, but there was no correlation between viral infection and TP53 aberration with other tumoral statuses, such as tumor stage, histological grade, and lymph node metastasis [11].

Although HPV infection can be detected in oral premalignant and malignant lesions, only a small percentage of exposed cases developed oral cancer over an extended period of time. This pattern in oropharyngeal cancer was also explained by Chen and Elamin [3,7]. This particular study (4) emphasizes that it is related to the variants of p53, which may be responsible for different genetic susceptibility to the HPV onco-proteins carcinogenesis.

According to the results of Mukhopadhyay's study, lack of p53 expression in Indian patients is more evident in poorly differentiated tumor and in stage II/III. In addition, 88% of overexpression occurred in well-differentiated OSCC. As the amount of HPV antigen increases in tumor mass, detection of p53 decreased, which may be due to E6 onco-protein. Therefore, the expression of p53 is greater in a smaller tumor [13].

In our study, 25% of the patients were infected with either HPV 16 or HPV 18. Unexpectedly, in 60% of positive samples, HPV 18 was detected. Campisi et al. also found HPV 18 as the most frequent genotype in their OSCC patients [2]. The major risk factors for our patients were tobacco and betel nut as opposed to alcohol consumption. It must be considered that high risk sexual habits are not common in Iran, and this may explain the low number of positive samples.

In this study, the incidence of overall positive rate is similar to that of da Silva's and Kumar's findings. Parallel to the studies of Kumar, Brachman and Chang, HPV status and p53 expression did not have any significant correlation with tumor stage, nodal metastasis, and tumor differentiation in our patients [1,11].

The results of the present study do not support the role speculated for HPV infection, as well as TP53 in carcinogenesis or nodal metastasis in oral tongue SCC. The possible distinct natures of oral tongue SCC, as well as environmental and host genetic factors, should be respected.

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