RESEARCH COMMUNICATION

Human Papillomavirus Infection and Prognostic Predictors in Patients with Oropharyngeal Squamous Cell Carcinoma

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Abstract

This study focused on infection rates and subtypes of human papillomavirus (HPV) in patients with oropharyngeal squamous cell carcinoma (OSCC), and the relationship between HPV status and prognosis of the disease. We evaluated sixty-six OSCC patients who met the enrollment criteria during the period from January 1999 to December 2009. The presence or absence of oncogenic HPV types in tumors was determined using the SPF10 LiPA25 assay. Overall survival (OS) and disease specific survival (DSS) for HPV positive and HPV negative patients were estimated using Kaplan–Meier analysis. The Cox regression model was applied for multivariate analysis. HPV-DNA was detected in 11(16.7%) of all specimens. Among them, 7 were type HPV-16, while other types were HPV-16/11, HPV-35, HPV-58/52, and HPV-33/52/54. Patients with HPV positive tumors were more likely to be female, non-smokers and non-drinkers (p=0.002, 0.001 and 0.001, respectively). After a median follow-up of 24.5 months, patients with HPV positive tumors had significantly better overall survival (HR=0.106[95%CI=0.014-0.787], p=0.016,) and disease specific survival (HR=0.121[95%CI=0.016-0.906], p=0.030). Patients with HPV positive OSCC have significantly better prognosis than patients with HPV negative tumors. HPV infection is an independent prognostic factor.

Keywords: Oropharyngeal cancer - squamous cell carcinoma - human papillomavirus (HPV) - prognosis

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Introduction

Recent studies have shown an etiologic role of infection with high-risk human papillomavirus (HR-HPV) in a subset of oropharyngeal squamous cell carcinomas (OSCC) that present with a distinct biologic behavior (Gillison et al., 2000; Andrews et al., 2009; Joo et al., 2011; Klussmann et al., 2001). HPV-DNA is detected in approximately 19%-75% of all squamous cell carcinomas of oropharynx worldwide, 85%-95% of which are of the HPV-16 type (Gillison et al., 2000; Kreimer et al., 2005). HPV positive oropharyngeal cancer is more responsive to chemo/radiotherapy, and patients with HPV positive tumors show significantly improved survival outcomes than those with HPV negative tumors (Worden et al., 2008). Many studies about the relationship between HPV and OSCC have been derived from western countries, but none from mainland China (Dayyani et al., 2010).

Previous studies have demonstrated that patients with high risk HPV (HR-HPV) positive OSCC have significantly better prognosis than those with HPV negative OSCC. HR-HPV infection is an independent prognostic indicator (Weinberger et al., 2006; Kumar et al., 2007; Fakhry et al., 2008; Gillison et al., 2000; Hafkamp et al., 2008; Kuo et al., 2008; Worden et al., 2008; Lill et al., 2011; Posner et al., 2011). Kuo and colleagues reported that the 5-year overall survival rate of patients with HR-HPV positive OSCC was 89%, which was significantly higher than HR-HPV negative patients (61%, p=0.001) (Kuo et al., 2008). Hafkamp and colleagues have demonstrated that patients with HPV positive tumors had a lower loco-regional recurrence rate than those with HPV negative tumors (12% vs 27%, p=0.039) (Hafkamp et al., 2008). Tumor-related mortality in patients with HPV positive tumors was significantly lower than in those with HPV negative tumors (HR=0.41; 95%CI=0.20-0.88) (Hafkamp et al., 2008).

HPV positive OSCCs respond at a higher rate to chemo/radiotherapy compared with HPV negative OSCCs, which is considered to be a major factor leading to better prognosis. Some authors also found that patients with HPV-DNA positive tumors showed significantly improved prognosis regardless of the mode of treatment involving surgery with postoperative radiation or radiotherapy with or without chemotherapy (Licitra et al., 2006; Hong et al., 2010).

In this study, we retrospectively evaluated a series of 66 patients with OSCC treated at the Chinese Academy

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of Medical Sciences (CAMS), Cancer Hospital, Beijing. We aimed to investigate the infection rate and subtypes of HPV, as well as the differences in characteristics and survival outcomes between patients with HPV positive and HPV negative OSCC. We found that HPV-DNA was detected in eleven out of all cases. HPV positive tumors were more likely to occur in nonsmokers and nondrinkers, to be poorly differentiated. Patients with HPV positive tumors had better prognosis than those with HPV negative tumors, which is in accordance with the existing literatures.

Materials and Methods

Patients

Formalin-fixed, paraffin-embedded tissue blocks of 66 patients were obtained from patients diagnosed with oropharyngeal malignant tumors and treated at our institution from January 1999 to December 2009. Eligibility criteria were; primarily treated patients, pathologically confirmed SCC, availability of pathologic specimen, no distant metastases and complete medical records. Exclusion criteria included any previous chemotherapy or radiotherapy, a synchronous active cancer and discontinuation of treatment. As this study retrospectively analyzed pathologic specimens and resulted in no harm to the interest of patients, we did not apply for ethical review. Two pathologists diagnosed all the specimens independently, based on World Health Organization Classification of Tumors.

HPV Detection

A "sandwich" technique was used to cut paraffin sections for hematoxylin and eosin (H&E) staining and for HPV-DNA analysis. The first and last sections were used for confirmation of SCC after staining wth H&E, while the rest of the sections were placed in a 1.5 ml helical tube and used later for DNA extraction. After confirmation of histological diagnosis, qualified specimens were sent to the laboratory at Cancer Institute CAMS for HPV-DNA testing by PCR. For DNA extraction, 3×8 μm thick paraffin sections were incubated in dry heat blocks for 24 h with 250 μl proteinase K solution (1 mg/ml proteinase K, 45 mM Tris-HCl, 0.9 mM EDTA, and 0.45% Tween 20, pH 8.0) at 70 ± 0.5°C. The tubes were then incubated for 10 min at 95 °C to inactivate the proteinase K. DNA was diluted 1:10 prior to DNA analysis. PCR on the diluted DNA was applied to amplify HPV DNA and then the generic amplification products were detected by DNA enzyme immunoassay (DEIA). HPV positive specimens were typed by reverse hybridization line probe assay SPF10 LiPA25 (version 1) (Kleter et al., 1999; Chen et al., 2009). 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

The primary end points were, overall survival (OS), defined as the time from starting treatment to death and disease spesific survival (DSS), defined as the time from starting treatment to death caused by OSCC. Survival data were analyzed by means of the Kaplan–Meier method and survival curves were compared with the log-rank test in univariate analysis. Cox regression model was used for multivariate analysis. All statistical tests were two-sided and p values less than 0.05 were considered as statistically significant.

Results

Patient Characteristics

Among the sixty-six patients with OSCC, 61 were males and 5 were females. Mean age of the whole group was 59.18 (38 to 92) years. Twenty-seven patients presented with tumors in the palatine tonsil, 26 patients presented with tumors in tongue base, 11 tumors were seen in the soft palate and two tumors in the posterior pharyngeal wall. According to the UICC/AJCC Staging System, 46 cases were in stage IV, 11 in stage III, seven in stage II and two in stageI. Pathological differentiation of the tumors demonstrated 22 tumors as well differentiated, 32 as moderately differentiated and 12 cases as poorly differentiated. The characteristics of the two groups stratified by HPV status are summarized in Table 1.

Table 1. Clinicopathological Characteristics of Two Groups by HPV Status

Variable		HPV status (%)		Chi-square value	P value
		Positive	Negative		
Age (years)	range	46-92	38-81	-0.319*	0.751
	median	57	60		
Sex	Male	7(63.6)	54(98.2)		
	Female	4(36.4)	1(1.8)	15.624[a]	0.002
Smoking	Yes	6(54.5)	49(89.1)		
	No	4(36.4)	0(0)	21.341[a]	0.001
	unknown	1(9.1)	2(10.9)		
Drinking	Yes	5(45.5)	42(76.4)		
	No	5(45.5)	1(1.8)	14.460[a]	0.001
	unknown	1(9.1)	12(21.8)		
Differen-tiation	G1	2(18.2)	20(36.4)	1.719[a]	0.409
	G2	6(54.5)	26(47.3)		
	G3	3(27.3)	9(16.4)		
Overall stage	I, II	1(9.1)	8(14.5)	3.315[a]	0.336
	III, IV	10(90.9)	47(85.5)		
T stage	1, 2	6(54.5)	22(40)	0.310[b]	0.578
	3, 4	5(45.5)	33(60)		
Nodal status	0, 1	3(27.3)	30(54.5)	1.745[b]	0.186
	2, 3	8(72.7)	25(45.5)		
Treatment	R/CCRT	7(63.6)	26(47.3)	1.196[a]	0.807
	S	1(9.1)	11(40)		
	R+S	1(9.1)	9(16.4)		
	S+R	2(18.2)	9(16.4)		
Region	Tonsil	7(63.6)	20(36.4)	4.297[a]	0.240
	Tongue base	4(36.4)	22(40)		
	Soft palate	0(0)	11(20)		
	PPW	0(0)	2(3.6)		

Note: *t=-0.319 (with T test); [a]Fisher's exact test; [b]Yate's continuity correction; G1, well-differentiated; G2, moderately-differentiated; G3, poorly-differentiated; PPW, Posterior pharyngeal wall; R, Radiotherapy; S, Surgery; R+S, Surgery with preoperative radiotherapy; S+R, Surgery with postoperative radiotherapy; CCRT, Concurrent chemo-radiotherapy

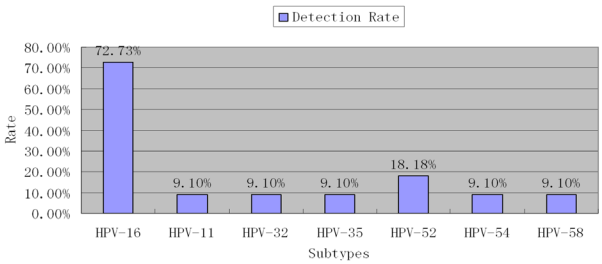

Figure 1. Detection Rate of Each Human Papillomavirus (HPV) Subtype in the HPV Positive Tumors. HPV-16 was the main subtype detected in 72.73% of the tumors

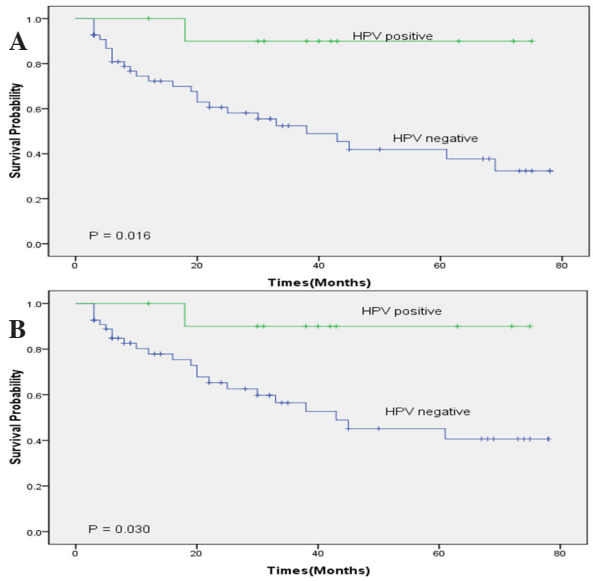

Figure 2. Kaplan-Meier Curves for Overall and Disease Specific Survival Stratified by Tumor Human Papillomavirus (HPV) Status. (A) Overall survival (OS) for the patients with oropharynx cancer. (B) Disease specific survival (DSS) for patients with oropharynx cancer. Both OS and DSS were significantly greater in patients with HPV positive tumors than in patients with HPV negative tumors. p values are shown

Treatment

Of the 66 patients with OSCC, 33 received primary radiotherapy with curative intent, six of which received concomitant chemotherapy. They were treated using the standard 3-field technique (2 opposing lateral fields for the upper neck region with an adjacent supraclavicular field for the lower neck) or by CT-planning using a 3-dimensional (3D) conformal or the intensity modulated radiotherapy technique. Seven patients terminated treatment before the end of radical radiotherapy; three for intolerable side effects with a radiation dose of 36 Gy-46 Gy, one for inability to pay (radiation dose 36 Gy) and the remaining three patients, terminated when they were referred for surgery (radiation dose 50 Gy). The average radiation dose for the other 26 patients was 69.65 Gy (56 Gy-76 Gy). Cisplatin, at a dose of 30 mg per square meter of body surface area, was administered weekly for concomitant chemotherapy, with a total dose of 250 mg-350 mg. Four patients received radical neck dissection because of residual disease in the neck after radiation.

Twelve patients received surgery as the only mode of treatment, five of which were T1-2 and N0-1 with no indication of postoperative radiotherapy. Four patients were reluctant to undergo radiotherapy while the remaining 3 never returned. Combined surgery and radiation treatments were provided for 21 patients, out of which 10 received preoperative radiotherapy (dose of 50 Gy) while 11 received postoperative radiotherapy (dose of 60 Gy, except one with 36 Gy).

Primary tumors were resected en bloc using the lower lip and mandible split technique associated with the transoral approach. Free margins were confirmed pathologically by intra-operative frozen biopsy. The methods of dissection included selective, modified and comprehensive neck dissections. Repair and constructive procedures were performed for the defects after radical excision of primary tumors, mostly with pectoralis major musculocutaneous flap, while the other procedures included the submental flap, free forearm flap and free rectus abdominis myocutaneous flap. There were no deaths due to surgery.

Table 2. Overall and Disease-specific Survival Analyzed in Univariate Analysis with Log-rank Test

Variable		No. of patients	Overall Survival		Disease-specific Survival	
			3-year(%)	P value	3-year(%)	P value
Age	>60ys	32	59.8	0.773	62.3	0.942
	≤60ys	34	60.2		65.2	
Sex	male	61	56.0	0.073	62.5	0.096
	female	5	100		100	
Smoking	Yes	55	54.9	0.261	59.1	0.283
	No	4	100		100	
	Unknown	7	68.6		68.6	
Drinking	Yes	47	57.1	0.323	62.1	0.446
	No	6	83.3		83.3	
	Unknown	13	57.7		57.7	
Differen-tiation	G1	22	55.5	0.692	58.6	0.885
	G2	32	57.5		62.0	
	G3	12	73.3		73.3	
Overall stage	I, II	9	88.9	0.320	88.9	0.185
	III, IV	57	56.7		60.5	
T stage	1, 2	28	66.8	0.401	70.1	0.342
	3, 4	38	54.2		57.7	
Nodal status	2, 3	33	48.8	0.086	52.6	0.071
	0, 1	33	72.8		75.6	
Treatment	R or CCRT	33	54.7	0.199	57.2	0.239
	S	12	53.6		53.6	
	R+S	10	50		63.5	
	S+R	11	87.5		87.5	
HPV status	Positive	11	90.0	0.016	90.0	0.030
	negative	55	52.4		56.4	

Note: G1, well-differentiated; G2, moderately-differentiated; G3, poorly-differentiated; PPW, Posterior pharyngeal wall; R, Radiotherapy; S, Surgery; R+S, Surgery with preoperative radiotherapy; S+R, Surgery with postoperative radiotherapy; CCRT, Concurrent chemoradiotherapy

HPV-DNA Infection Rate and Subtypes

HPV-DNA was detected in 11 (16.7%) of the tumor specimens, 72.73% of which were of the HPV-16 subtype (seven HPV-16 and one HPV-16/11) (Figure 1). The other types included HPV-35, HPV-58/52 and HPV-33/52/54. The HPV-DNA positive tumors included those in the palatine tonsil (7 cases) and tongue base (4 cases). The infection rate of HPV was 25.9% in the palatine tonsil and 15.5% in the tongue base. Both, the soft palate and

Table 3. Overall and Disease-specific Survival Analyzed by Means of Cox Regression Model

Variable		Overall Survival		Disease-specific Survival	
		HR (95%CI)	P value	HR (95%CI)	P value
Nodal status	N2/3 vs N0/1	2.346(1.075-5.118)	0.032	2.575(1.095-6.058)	0.030
HPV status	Positive vs Negative	0.106(0.014-0.787)	0.028	0.121(0.016-0.906)	0.040

Notes: HR, Hazard Ratio; CI, Confidence Interval

posterior pharyngeal wall did not present with HPV infections. HPV-DNA was not detected in negative paraffin control specimens and was detected in 87.5% (7/8) positive control specimens.

Clinico-pathological Features of HPV positive and HPV negative OSCC

Sixty-six patients with OSCC were analyzed, 11 of which were HPV positive. Tumors in patients with HPV positive tumors were more likely to be diagnosed in the tongue base or palatine tonsil primary tumors than those with HPV negative tumors (100% vs 76.4%, respectively). However, no statistically significant difference was noted (p=0.240). There were more female patients with HPV positive tumors than with HPV negative tumors (36.4% and 1.8%, respectively, p=0.002) (Table 1). HPV positive OSCCs were more likely to occur in non-smokers (36.4%, p=0.001) and non-drinkers (45.5%, p=0.001). However, other factors such as tumor stage, nodal status and overall TNM stage did not differ with HPV status (Table 1).

Survival Analysis

We evaluated the association between HPV status and improved survival. Median follow-up time for the entire study population was 24.5 months (range 3-79 months). During this time, only one patient with HPV positive OSCC died due to tumor recurrence (mediastinal metastasis). Among the patients with HPV negative tumors, 23 died from the disease, including 7 with distant metastases, 3 with local recurrence, 6 with neck recurrence and 7 with uncontrolled loco-regional disease. Four other patients died of natural or unknown causes. More patients with HPV negative OSCC died because of recrudescent or uncontrolled tumor than those with HPV positive tumors.

Based on Kaplan-Meier estimates, both, overall survival and disease specific survival for patients with HPV positive tumors improved significantly compared with that of patients with HPV negative tumors (p=0.016 and 0.030, log-rank test). The estimated 3-year overall and disease specific survival rates among HPV positive patients were 90.0% and 90.0%, respectively. On the contrary, the 3-year overall and disease specific survival rates among HPV negative patients were 52.4% and 56.4%, respectively (Figure 2A, B).

Univariate analysis was performed to evaluate other factors potentially associated with OS and DSS with the log-rank test. There were no significant differences in survival rates stratified by other factors, such as age, sex, smoking, drinking, tumor differentiation, tumor stage, nodal status and overall TNM stage (Table 2).

Multivariable analysis was performed to estimate the association of tumor HPV status with survival outcomes. Tumor HPV status was independently associated with mortality risk after adjusting for other factors. Patients with HPV positive tumors had an 89.4% (95% CI=0.014-0.787) lower risk of death than patients with HPV negative tumors. In this analysis, nodal status was also independently associated with elevated mortality risk (N2/N3 vs N0/N1, HR=2.346, 95% CI=1.075 to 5.118, p=0.032) (Table 2).

Discussion

This was the first study conducted in mainland China that retrospectively investigated the relationship between tumor HPV status and clinical outcomes of patients with oropharyngeal squamous cell carcinoma (OSCC). Our results provide evidence that HPV positive OSCC is a distinct entity and tumor HPV status is an independent prognostic factor for patients with OSCC, which is consistent with the hypothesis that has been reported in several other studies (Gillison et al., 2000; Klussmann et al., 2001; Weinberger et al., 2006; Hafkamp et al., 2008; Worden et al., 2008; Andrews et al., 2009; Dayyani et al., 2010). On the basis of our results, we believe that future clinical trials should be designed for patients nationwide with OSCC and further information could be obtained to determine whether treatment affects the survival outcomes stratified by tumor HPV status.

There was a significant increase in the incidence of oropharyngeal cancer with the prevalence of HPV positive OSCC almost doubling each decade between 1970 and 2007, followed by a decline of HPV negative tumors (Shiboski et al., 2005; Nasman et al., 2009). Human papillomavirus has been established as an important etiologic factor in a subset of OSCC and plays a role in carcinogenesis (Gillison et al., 2000). HPV infection has been detected in 19%-75% of all oropharyngeal cancers with HPV-16 as the main subtype accounting for 85%-95% of the tumors (Gillison et al., 2000; Herrero et al., 2003; Kreimer et al., 2005; Tachezy et al., 2009 St Guily et al., 2011). The HPV infection rate in our study was 16.7%, a little lower than that reported previously. HPV-16 was detected in 72.73% of all the HPV positive tumors (Figure 1), which was also lower than that reported in the literature (Gillison et al., 2000; Herrero et al., 2003; Kreimer et al., 2005).

HPV infection in the oral cavity and oropharynx is directly related to sexual habits, especially oral sex, which is considered to be the reason for the high detection rate of HPV-positive tumors in western countries (Gillison et al., 2008). Chinese people, especially in mainland China, have significantly different sexual lifestyles compared to the westerners wherein, oral sex is not traditionally practiced. This may explain the lower overall infection rate of HPV and the varying distribution of the different subtypes. Of the various sub sites, palatine tonsil has been reported with the highest rate of tumor incidence (Mak-Kregar et al.,

1995; St Guily et al., 2011). Due to the small sample size in our study, we observed a low incidence rate of tumors in the palatine tonsil (27/66), while other sub sites such as the tongue base and soft palate presented with higher rates of tumor incidence. Thus, there might exist a selection bias introduced during criteria determination, which is an important factor that could lead to variations in detection rates and subtypes of HPV. Nevertheless, in accordance with previous reports, we found a high infection rate of HPV in the palatine tonsil (7/27, 25.9%), whereas none were found in the soft palate and pharyngeal wall.

In a recent study, HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 were classified as high risk viruses, while HPV- 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and 89 were considered as viruses with low oncogenic risk (Munoz et al., 2003). In our study, each of the eleven specimens with HPV-DNA contained high-risk viruses. HPV-18, a high- risk type virus, common in cervical cancers, especially in adenocarcinomas is not frequently detected in oropharyngeal squamous cell carcinoma (2.9%) 5, and was not detected in our study.

Consistent with the literature (Klussmann et al., 2001; Hafkamp et al., 2008; Andrews et al., 2009), HPV positive tumors in this study were more likely to occur in female patients, nonsmokers and non-drinkers (p=0.002, 0.001 and 0.001, respectively). HPV positive tumors trended towards being poorly differentiated, though we did not find any statistically significant difference. This study provides further evidence that HPV positive tumors are a unique clinical entity distinct from HPV negative tumors. However, there were no significant differences in age, tumor stage, nodal status and overall stage between patients with HPV positive and -negative tumors. This is rather inconsistent with the existing literature where HPV positive tumors have been shown to be mostly poorly-differentiated, presenting with lymph node metastasis and advanced disease (stages III-IV) (Gillison et al., 2000; Smith et al., 2004; Joo et al., 2011).

Risk of death is redued by 60-80% in patients with HPV positive tumors when compared to those with HPV negative tumors (Gillison et al., 2000). In this study, patients with HPV positive tumors had significantly improved overall and disease specific survival rates than with HPV negative tumors (p value - 0.016 and 0.030, respectively). Only one patient with HPV positive tumor died from the disease, whereas, among the patients with HPV negative tumors, 23 died from the disease.

Compared to patients with HPV negative tumors, those with HPV positive tumors have a better response rate to induction chemotherapy and concomitant chemoradiotherapy, and thus have improved overall survival and disease related survival (Weinberger et al., 2006; Fakhry et al., 2008; Worden et al., 2008; Lill et al., 2011). It is notable that patients in majority of the reported studies primarily received chemo/radiotherapy with curative intent and salvage surgery if necessary. In this study, half of the patients received non-radiation as the curative treatment (33/66), with no impact on the survival outcomes when analyzed by univariate analysis (p>0.05).

None of the factors such as age, sex, smoking, drinking, tumor differentiation, tumor stage and overall stage had impact on survival. The overall survival (HR=2.346[95% CI=1.075-5.118]) and disease specific survival (HR=2.575 [95% CI=1.095-6.058]) reduced significantly when nodal status was upgraded (N2/N3 vs N0/N1), and this was also independently prognostic in the multivariate analysis.

In this study, we successfully investigated the infection rate of HPV in OSCC in our institution and demonstrated its relationship with clinical outcomes. However, there were some limitations, one of which was the small simple size. There were only 27 tonsil cancers in our study, much less than the general incidence rate that has been reported which is 54%-60% (D'Souza et al., 2007; Worden et al., 2008; St Guily et al., 2011), and we chose only those patients initially treated in our hospital with available pathological specimens, not including all cases during the period, resulting in selection bias. However, this is the first study to investigate HPV infections in OSCC among the Chinese population in China mainland.

In summary, the HPV infection rate of OSCC in our study was 16.7%, which is slightly lower than that reported in previous studies. HPV positive OSCC is a unique clinical entity distinct from HPV negative tumors. Patients with HPV positive OSCC have improved prognosis than those with HPV negative tumors, and HR-HPV infection is an independent prognostic indicator. HPV infection in oropharyngeal carcinoma in the Chinese population warrants further investigation with larger clinical trials at the national level.

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References

Andrews E, Seaman WT, Webster-Cyriaque J (2009). Oropharyngeal carcinoma in non-smokers and non-drinkers: a role for HPV. *Oral Oncol*, **45**, 486-91.

Chen W, Zhang X, Molijn A, et al (2009). Human papillomavirus type-distribution in cervical cancer in China: the importance of HPV 16 and 18. *Cancer Causes Control*, **20**, 1705-13.

D'Souza G, Kreimer AR, Viscidi R, et al (2007). Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med*, **356**, 1944-56.

Dayyani F, Etzel CJ, Liu M, et al (2010). Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). *Head Neck Oncol*, **2**, 15.

Fakhry C, Westra WH, Li S, et al (2008). Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst*, **100**, 261-9.

Gillison ML, D'Souza G, Westra W, et al (2008). Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst*, **100**, 407-20.

Gillison ML, Koch WM, Capone RB, et al (2000). Evidence for a causal association between human papillomavirus

and a subset of head and neck cancers. *J Natl Cancer Inst*, **92**, 709-20.

Hafkamp HC, Manni JJ, Haesevoets A, et al (2008). Marked differences in survival rate between smokers and nonsmokers with HPV 16-associated tonsillar carcinomas. *Int J Cancer*, **122**, 2656-64.

Herrero R, Castellsague X, Pawlita M, et al (2003). Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *J Natl Cancer Inst*, **95**, 1772-83.

Hong AM, Dobbins TA, Lee CS, et al (2010). Human papillomavirus predicts outcome in oropharyngeal cancer in patients treated primarily with surgery or radiation therapy. *Br J Cancer*, **103**, 1510-7.

Joo YH, Jung CK, Sun DI, et al (2011). High-risk human papillomavirus and cervical lymph node metastasis in patients with oropharyngeal cancer. Head Neck.

Kleter B, van Doorn LJ, Schrauwen L, et al (1999). Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. *J Clin Microbiol*, **37**, 2508-17.

Klussmann JP, Weissenborn SJ, Wieland U, et al (2001). Prevalence, distribution, and viral load of human papillomavirus 16 DNA in tonsillar carcinomas. *Cancer*, **92**, 2875-84.

Kreimer AR, Clifford GM, Boyle P, Franceschi S (2005). Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev*, **14**, 467-75.

Kumar B, Cordell KG, Lee JS, et al (2007). Response to therapy and outcomes in oropharyngeal cancer are associated with biomarkers including human papillomavirus, epidermal growth factor receptor, gender, and smoking. *Int J Radiat Oncol Biol Phys*, **69**, S109-11.

Kuo KT, Hsiao CH, Lin CH, et al (2008). The biomarkers of human papillomavirus infection in tonsillar squamous cell carcinoma-molecular basis and predicting favorable outcome. *Mod Pathol*, **21**, 376-86.

Licitra L, Perrone F, Bossi P, et al (2006). High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. *J Clin Oncol,* **24**, 5630-6.

Lill C, Kornek G, Bachtiary B, et al (2011). Survival of patients with HPV-positive oropharyngeal cancer after radiochemotherapy is significantly enhanced. *Wien Klin Wochenschr*, **123**, 215-21.

Mak-Kregar S, Hilgers FJ, Levendag PC, et al (1995). A nationwide study of the epidemiology, treatment and survival of oropharyngeal carcinoma in The Netherlands. *Eur Arch Otorhinolaryngol*, **252**, 133-8.

Munoz N, Bosch FX, de Sanjose S, et al (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*, **348**, 518-27.

Nasman A, Attner P, Hammarstedt L, et al (2009). Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? *Int J Cancer*, **125**, 362-6.

Posner MR, Lorch JH, Goloubeva O, et al (2011). Survival and human papillomavirus in oropharynx cancer in TAX 324: a subset analysis from an international phase III trial. *Ann Oncol*, **22**, 1071-7.

Shiboski CH, Schmidt BL, Jordan RC (2005). Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 20-44 years. *Cancer*, **103**, 1843-9.

Smith EM, Ritchie JM, Summersgill KF, et al (2004). Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. *Int J Cancer,* **108**, 766-72.

St Guily JL, Jacquard AC, Pretet JL, et al (2011). Human papillomavirus genotype distribution in oropharynx and oral cavity cancer in France--The EDiTH VI study. *J Clin Virol*, **51**, 100-4.

Tachezy R, Klozar J, Rubenstein L, et al (2009). Demographic and risk factors in patients with head and neck tumors. *J Med Virol*, **81**, 878-87.

Weinberger PM, Yu Z, Haffty BG, et al (2006). Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol*, **24**, 736-47.

Worden FP, Kumar B, Lee JS, et al (2008). Chemoselection as a strategy for organ preservation in advanced oropharynx cancer: response and survival positively associated with HPV16 copy number. *J Clin Oncol,* **26**, 3138-46.