Int J Clin Exp Med 2019;12(8):10689-10695
www.ijcem.com /ISSN:1940-5901/IJCEM0092395

Original Article

Correlation between HPV infection and tumor recurrence in patients with head and neck squmaous cell carcinoma in Tangshan, China

Jun-Quan Yang[1], Meng Wu[2], Yi-Fei Liu[3], Su-Mei Yang[4], Feng-Yan Han[5], Ling Zhang[5], Hong-Xia Liu[5]

Departments of [1]Radio-Chemotherapy Oncology, [4]Pneumology, Tangshan People's Hospital, Tangshan, China; [2]Pathological Teaching and Research Division, Department of Basic Medicine, Tangshan Vocational and Technical College, Tangshan, China; Departments of [3]Neurology, [5]Pathology, Tangshan Union Hospital, Tangshan, China

Received February 11, 2019; Accepted July 10, 2019; Epub August 15, 2019; Published August 30, 2019

Abstract: To explore the HPV infection status in head and neck carcinoma tissues, clinical features of HPV positive patients and its correlation with tumor recurrence in Tangshan area, China. In total, 418 cases of head and neck carcinoma specimens were selected from January 1, 2003 to December 31, 2013 in Tangshan Union Hospital. PCR-reverse dot blot hybridization was used to detect HPV DNA. The clinical pathological features of HPV positive patients with head and neck cancer and its relationship with tumor recurrence were analyzed. Results showed that the detection rate of HPV in head and neck carcinoma tissues was 20.8% (87/418) in the Tangshan area. Most common subtype was HPV16, and a few were HPV11, HPV35 or HPV58. Among them, the detection rates in the carcinoma tissues of larynx, oral, hypopharynx and oropharynx were 19% (19/100), 29.3% (24/82), 26.1% (12/46) and 28.6% (32/112), respectively. There was no HPV detected in the adenoid cystic carcinoma of salivary glands (0/78). There were significant differences in HPV detection rates between different tumor tissues ($P<0.05$). The HPV detection rate in the smoking group was 13.1%, which was lower than that of the non-smoking group (33.3%), and the difference was statistically significant ($P<0.05$). The HPV detection rate in the recurrent group was 3.9%, lower than that in the non-recurrence group (30.7%), and the difference was statistically significant ($P<0.05$). There was no significant correlation between HPV detection rate and gender, age, classification and staging of the patients ($P>0.05$). Multivariate logistic regression analysis showed that tumor differentiation (R = 1.549, 95% CI = 1.131~2.122) and HPV infection (OR = 0.089, 95% CI = 0.038~0.210) were the main factors affecting the recurrence ($P<0.01$). Our findings support that the detection rate of HPV is low in the head and neck carcinoma tissues in the Tangshan area. HPV positive patients are mostly non-smokers. The head and neck carcinoma patients with poor differentiation and HPV negative status have a high recurrence rate. Routine HPV detection is necessary for patients with head and neck cancer in order to warn of early tumor recurrence.

Keywords: Head and neck neoplasm, HPV, recurrence, prognosis

Introduction

Human papillomavirus is related to the occurrence of a variety of human diseases, and its role in the pathogenesis of tumors was previously limited to the study of cervical cancer. Since Gissmann *et al,* [1] detected HPV DNA in human head and neck carcinoma tissues for the first time, more and more data showed that persistent exposure to HPV infection is an important factor in the progress of head and neck tumors. In order to investigate the state of the HPV gene in head and neck cancer tissues in the Tangshan area of China, we used PCR - reverse dot blot analysis to detect HPV DNA in 418 tissue specimens removed from head and neck carcinoma cases from 2003 to 2013 in the Tangshan Disease Pathology Research Base, to explore HPV infection rate in head and neck carcinoma tissues, and to analyze the pathological characteristics of HPV-positive patients and their correlation with tumor recurrence.

Patients and methods

Cases description

From January 1st 2003 to December 31st 2013; 418 surgically-removed specimens of head and

neck carcinoma, from patients aged 37 to 75 years, were selected at Tangshan Head and Neck Disease Pathological Research Base. Lesioned locations were at larynx (100 cases), gingiva (40 cases), oral floor (15 cases), upper jaw (11 cases), buccal mucosa (16 cases), pear-shaped fossa (27 cases), posterior cricoid region (13 cases), side and posterior wall of hypopharynx (6 cases), soft palate (40 cases), tongue root (36 cases), tonsil (21 cases), lateral pharyngeal wall (15 cases) and salivary gland (78 cases), respectively. All surgical specimens were reviewed by two senior pathologists.

HPV DNA detection by PCR-DNA reverse dot blot hybridization

Human Papillomavirus Subtype Nucleic Acid Detection Kit (Shenzhen Yaneng Biotechnology Co., Ltd., China) was used to detect the HPV infection with PCR-DNA reverse dot blot hybridization. All specimens were fixed in 4% neutral formalin, dehydrated and embedded in paraffin, and one representative block from each patient was sectioned at 4 μm. Paraffin sections were placed in EP tubes for xylene dewaxing, 0.5 ml samples were centrifuged at 14000 rpm for 1 min (the centrifugal radius was 5 cm). Supernatant was discarded and cell lysis was carried out using human papillomavirus nucleic acid detection kit to extract DNA, and 1 μl (about 100 ng) extracted DNA samples were taken as templates for PCR amplification. Hybridization, filter washing, and color development were carried out according to kit operation instructions. The positive result showed blue signals at the corresponding HPV genotype and IC membrane sites, while other sites were not colored. The negative results were only colored at IC sites. In accordance with the order of the probe sequence and the color on the membrane the HPV genotypes were determined.

Statistical analysis

The statistical analyses were performed with PASW Statistics 24.0 (SPSS Inc., Chicago, IL, USA). Categorical data are described by relative numbers. The differences in age, gender, smoking history, tumor location, differentiation, TNM stage and recurrence between HPV-positive and HPV-negative were analyzed by chi-square test or nonparametric rank test. Unconditional logistic regression model was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) to analyze and compare the correlation between different factors and tumor recurrence. ORs values were adjusted by age, gender and smoking status and statistically significant level was considered as $\alpha = 0.05$.

Results

Infection status of HPV in the head and neck carcinoma tissues

PCR-DNA reverse dot blot hybridization showed that among the 418 head and neck carcinoma patients 87 cases were HPV positive, the overall prevalence rate of HPV was 20.8%. The most prevalent genotype was HPV16, followed by HPV HPV11, HPV35 and HPV58. In laryngeal squamous cell carcinoma tissues the detection rate of HPV was 19.0% (19/100). The overall detection rate of HPV in oral carcinoma tissues was 29.3% (24/82), including 27.5% (11/40) in gingiva, 26.7% (4/15) in oral floor, 27.3% (3/11) in upper jaw, and 37.5% (6/16) in buccal mucosa, respectively. The overall detection rate of HPV in oropharyngeal squamous cell carcinoma tissues was 28.6% (32/112), including 22.5% (9/40) in soft palate, 33.3% (12/36) in tongue root, 42.9% (9/21) in tonsil, and 13.3% (2/15) in lateral pharyngeal wall. The overall detection rate of HPV in hypopharyngeal carcinoma tissues was 26.1% (12/46), including 22.2% (6/27) in pear-shaped fossa, 30.8% (4/13) in posterior cricoid region, 33.3% (2/6) in side and posterior wall of hypopharynx and 0 (0/78) in salivary adenoid cystic carcinoma tissues (**Figures 1** and **2**).

Clinical data of HPV-positive and HPV-negative carcinoma

The detection rate of HPV-DNA was different in different head and neck carcinoma tissues. Chi-square segmentation treatment method showed that the difference detection rate of HPV was mainly due to the difference between salivary adenoid cystic carcinoma tissue (HPV detection rate was null) and laryngeal cancer, oral cancer, oropharyngeal cancer and hypopharyngeal cancer groups ($P<0.001$), while the results of pairwise comparison showed that there was little difference in HPV detection rate among laryngeal, oral, oropharyngeal and hypopharyngeal carcinoma groups ($P>0.05$). The detection rate of HPV in the smoking group was higher than that in the non-smoking group, with

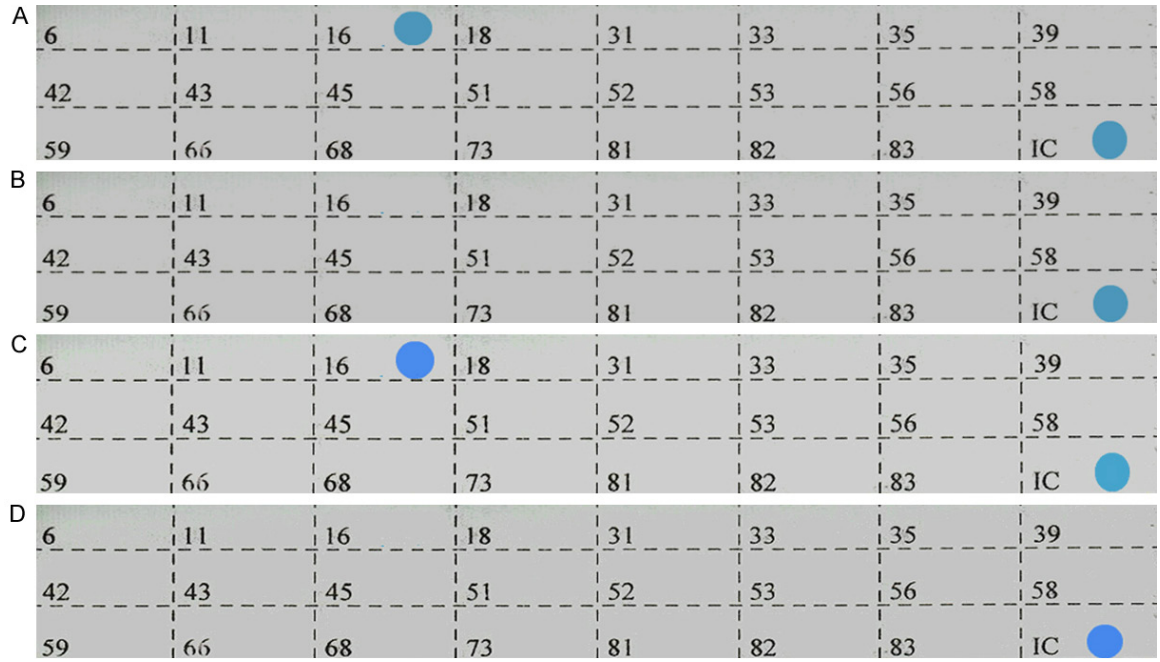

Figure 1. HPV genotype detection by PCR-DNA reverse dot hybridization. A. The positive control displayed a signal at the corresponding HPV genotype site and control site (blue spot). B. Negative control showed blue signal at the control site. C. Blue signals on hybridized membrane strips show HPV type 16 infection in tumor tissues. D. The blue signal only appeared at the quality control site indicate that the HPV test were negative.

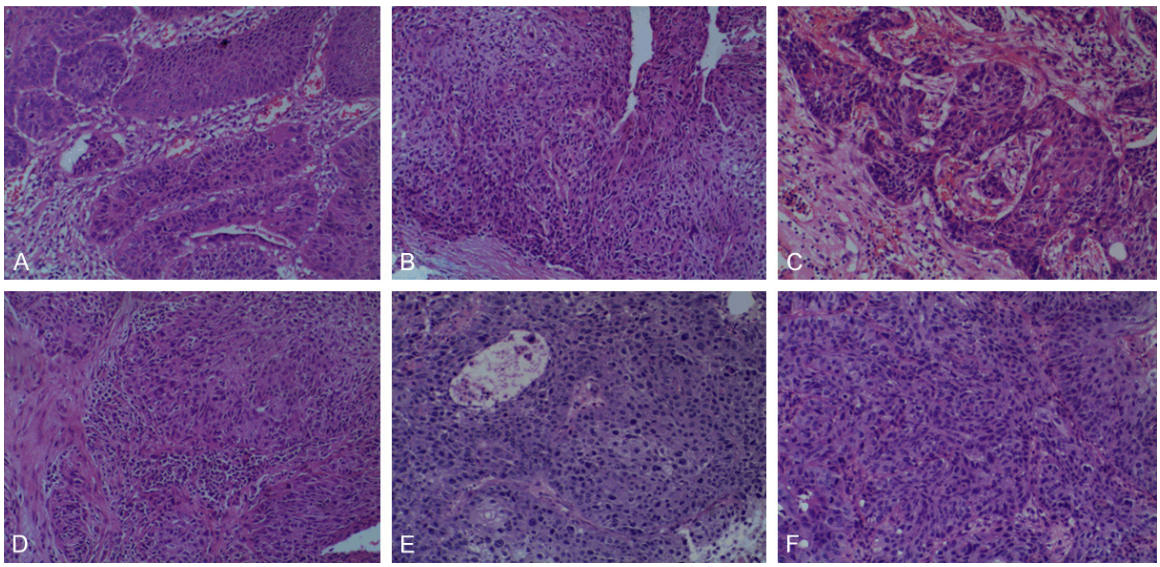

Figure 2. HPV-positive head and neck squamous cell carcinomas (hematoxylin-eosin staining). A. Larynx; B. Pear-shaped fossa; C. Oral floor; D. Soft palate; E. Tongue root; F. Tonsil.

statistical significance ($P<0.05$). The detection rate of HPV in the recurrent group was lower than that in the non-recurrent group, and the difference was also statistically significant ($P<0.01$). The detection rate of HPV was not related to patients' gender, age, tumor differentiation and stage ($P>0.05$, **Table 1**).

Multivariate analysis of variance affecting recurrence of head and neck cancer

A total of 418 patients with head and neck carcinoma were included in analysis. In logistic regression model with recurrence as the dependent variable and sex, age, smoking, tumor dif-

Table 1. Relationship between HPV and clinical characteristics in 418 patients with head and neck cancer in Tangshan area [n (%)]

Characteristics	n	HPV+	HPV-	χ^2/Z	*P*
Gender					
Male	261 (62.4)	49 (18.8)	212 (81.2)	2.904	0.088
Female	157 (37.6)	38 (24.2)	119 (75.8)		
Age (years)					
≤50 y	184 (44.0)	32 (17.4)	152 (82.6)	3.534	0.060
>50 y	234 (56.0)	55 (23.5)	179 (76.5)		
Smoking					
Yes	259 (62.0)	34 (13.1)	225 (86.9)	17.675	<0.001
No	159 (38.0)	53 (33.3)	106 (66.7)		
Localization					
Larynx	100 (23.9)	19 (19.0)	81 (81.0)		
Oral cavity	82 (19.6)	24 (29.3)	58 (70.7)		
Oropharynx	112 (26.8)	32 (28.6)	80 (71.4)	25.245	<0.001
Hypopharynx	46 (11.0)	12 (26.1)	34 (73.9)		
Salivary gland	78 (0)	0 (0)	78 (100.0)		
Differentiation					
Well	87 (20.8)	16 (18.4)	71 (81.6)		
Moderate	224 (53.6)	51 (22.8)	173 (77.2)	14350.5	0.958
Poor	107 (25.6)	20 (18.7)	87 (81.3)		
TNM stage					
I and II	171 (40.9)	39 (22.8)	132 (77.2)	2.206	0.137
III and IV	247 (59.1)	48 (19.4)	199 (80.6)		
Relapse					
Yes	154 (36.8)	6 (3.9)	148 (96.1)	30.164	<0.001
No	264 (63.2)	81 (30.7)	183 (69.3)		

Table 2. Univariate logistic regression analysis influencing tumor recurrence

Factors	B	S.E.	Wald χ^2	DF	*P*	OR	95% CI
Gender	-0.104	0.227	0.208	1	0.648	0.901	0.577~1.408
Age	-0.301	0.216	1.939	1	0.164	0.740	0.485~1.130
Smoking	0.266	0.237	1.268	1	0.260	1.305	0.821~2.075
Differentiation	0.415	0.163	6.460	1	0.011	1.515	1.100~2.087
TNM stage	0.274	0.228	1.439	1	0.230	1.315	0.841~2.085
HPV	-2.341	0.446	27.550	1	<0.001	0.096	0.040~0.231

Table 3. Factors affecting tumor recurrence and their evaluation

Variable		Assignment
X_1	Gender	Male = 0, Female = 1
X_2	Age	≤50 y = 0, >50 y = 1
X_3	Smoking	Yes = 0, No = 1
X_4	Differentiation	Well = 1, Moderate = 2, Poor = 3
X_5	TNM stage	I + II = 0, III + IV = 1
X_6	HPV	HPV- = 0, HPV+ = 1
Y	Relapse	unrelapse = 0, relapse = 1

ferentiation degree and stage as the independent variables, statistically significant independent variables (*P*< 0.1, **Table 2**) were selected and included in a multivariate logistic regression analysis. Results showed that the regression coefficient of tumor differentiation was positive (OR = 1.515, 95% CI = 1.100~2.087), indicating that patients with poor differentiation were prone to recurrence. The HPV regression coefficient was negative (OR = 0.096, 95% CI = 0.040~0.231), suggesting that HPV-negative patients were more likely to relapse than HPV-positive ones (**Tables 3** and **4**).

Discussion

Head and neck squamous cell carcinomas (HNSCC) mainly include oral cancer, oropharyngeal cancer, hypopharyngeal cancer and laryngeal cancer. Previous studies have suggested that the occurrence of HNSCC is related to smoking, drinking and other factors [2]. Recent research data show that HPV infection is closely related to the incidence of HNSCC [3]. The research on the relationship between HPV and head and neck tumors can be traced back to 1983. Since Gissmann *et al,* [1] first detected HPV in head and neck specimens, more and more epidemiological, clinical and molecular biological evidence showed that HPV, especially HPV16 and other high-risk virus infections are related to the occurrence of various HNSCC [4-6]. Since 2000, the research on HPV and HNSCC has made remarkable progress, and it has been found that specific anatomical sites have an important relationship with HPV susceptibility. The tumor site most closely related to HPV infection is located at the bottom of the tonsils

Table 4. Multivariate logistic regression analysis influencing tumor recurrence

Factors	B	S.E.	Wald χ^2	DF	*P*	OR	95% CI
Differentiation	0.438	0.160	7.450	1	0.006	1.549	1.131~2.122
HPV	-2.420	0.439	30.358	1	<0.001	0.089	0.038~0.210

of the tongue and palate, followed by the oral, laryngeal and nasal mucosa. However, there are also huge geographical differences between HPV infection and HNSCC. Kreimer *et al*, [7] found the infection rates of HPV were 51% and 24% in tonsillar squamous cell carcinoma and in laryngeal carcinoma tissues, respectively; while it was 19% in normal laryngeal mucosa. It is speculated that HPV infection is closely related to the incidence of HNSCC, especially oropharyngeal carcinoma, and the role of HPV in the development of laryngeal carcinoma is still in doubt. Chernock *et al,* [8] detected HPV infection in 156 cases of oropharyngeal squamous cell carcinoma by *in situ* hybridization. The positive rate of HPV in oropharyngeal keratinized squamous cell carcinoma and non-keratinized squamous cell carcinoma was 8% (2/25) and 69% (29/42), respectively; while in mixed squamous cell carcinoma was 46.1% (41/89). In this study we found that in 418 head and neck carcinoma tissues collected from the Tangshan Head and Neck Disease Pathological Research Base (the municipal pathological research base approved by Tangshan Health Planning Commission and listed in Tangshan Union Hospital Pathological Examination and Training Center) the average detection rate of HPV was 20.8%. Among them, HPV was not detected in salivary adenoid cystic carcinoma tissue; while in the larynx, oral cavity, oropharynx and hypopharynx, there was a certain detection rate, but there was no statistical difference. Whether these data are representative or not needs a further increase of sample size and long-term follow-up for an in-depth study. Compared with the HPV detection rate reported in Europe, America and other countries, it was low. This may be related to the differences in individual behavior patterns with regional cultural variety, as well as the differences in detection methods in different regions.

At present, the study on the relationship between HPV and HNSCC mainly focuses on two aspects: on the one hand, the role of HPV in the occurrence and development of HNSCC; on the other hand, the prognosis of HPV-positive patients with HNSCC. After HPV invades the human body, it is often characterized by transient and latent infection, and the main factors leading to the HPV from latent state to active state are the HPV subtype and the immune status of the host [9]. Most HPV viruses in the head and neck mucosa are present in the epithelial cells in a latent infection state. When the patient's own immunity declines, it is activated to cause abnormal epithelial cell proliferation and causes tumors. Most studies suggest that the tumorigenic effect of HPV is mainly due to the infection of the mucosal epithelium, and the viral DNA can be integrated into the host cell genomic DNA, resulting in host chromosomal aberrations. In addition, HPV can also integrate into the periphery of proto-oncogenes, activate proto-oncogenes or disable tumor suppressor genes, leading to cell cancer [10, 11]. Data analysis of Swedish malignant tumors epidemiological study from 1958 to 1996 showed that women with cervical cancer had a significantly increased risk of developing tonsillar and tongue cancer. Researchers believe that this condition is associated with changes in sexual behavior, including oral sex history, multiple sexual partners, genital warts infection and cannabis use [12, 13]. Other studies have shown that the incidence of tongue squamous cell carcinoma in young adults under the age of 45 in the United States and Europe has increased significantly over the past 20 years [14, 15]. Data from more than 5,000 cases of tongue squamous cell carcinoma registered by Danish, Swedish and Finnish oncology institutions from 1960-1994 also showed that 276 (5.5%) cases were young people aged 20 to 39 years, where the incidence of tongue squamous cell carcinomas in young men increased five times, and that of tongue squamous cell carcinomas in young women increased six times, while incidence of tongue squamous cell carcinomas in elderly patients increased only twice [16]. Yang *et al,* reported that HPV is not related to patient age, gender, location, differentiation, and TNM stage in 46 cases of hypopharyngeal carcinoma tissues and the median progression-free survival of HPV-positive pa-

tients was also higher than that of HPV-negative ones [17]. This study found that there was no significant difference in HPV detection rate among head and neck carcinoma patients of different gender, age, differentiation and stage, but it was related to smoking status. The HPV detection rate in non-smoking head and neck cancer patients was higher than that in smoking ones, and the difference was statistically significant. In addition, the detection rate of HPV in relapsed patients was lower than that in non-relapsed patients, and the difference was statistically significant. Further regression analysis confirmed that recurrence of HNSCC patients was associated with tumor differentiation and HPV infection. The recurrence rate of poorly-differentiated patients was higher than that of well-differentiated ones. The tumor differentiation was a risk factor for recurrence of head and neck cancer. The recurrence rate of HPV-positive patients was lower than that of HPV-negative ones, and the HPV detection in tumor tissues was an independent protective factor for recurrence. The possible explanation is that HPV-positive HNSCC patients are more sensitive to radiotherapy and chemotherapy. This is mainly due to the different carcinogenic pathways between HPV-positive HNSCC and HPV-negative individuals, in which p53 plays an important role in tumor formation. After HPV infection, the viral E6 protein can cause the tumor suppressor gene p53 activity to decrease or even inactivate, while in the HPV-negative HNSCC, there is a mutation in the p53 gene. Therefore, HPV-positive HNSCC patients have complete apoptotic responses to radiotherapy and chemotherapy, which may be one of the reasons for better prognosis of HPV-positive head and neck cancer patients [18, 19]. In addition, long interspersed nuclear elements (LINE-1) hypomethylation is also common in HNSCC. Richards *et al*, [20] found that hypomethylation is higher in HPV-negative HNSCC patients than in HPV-positive ones. The instability of tumor genes with high hypomethylation level is also high, and gene instability often predicts poor prognosis of tumors. These factors may be the reason for better prognosis of HPV-positive HNSCC patients. Due to the limited sample size, the conclusions of this study still need to be further validated. In the future, a follow-up study will be carried out to provide support for existing findings. Clinically, patients with head and neck cancer can be routinely tested for HPV, which has certain guiding significance for the judgment of tumor prognosis.

Since HPV detection has been used in cervical cancer screening, great progress has been made in the detection of HPV. We speculate that the difference in HPV infection rates in different regions is related to the lifestyle of people in the region and HPV testing methods. In addition, the quality and type of samples are also important factors affecting HPV detection results. At present, the detection methods of HPV mainly include cytological examination, multiplex polymerase chain reaction (PCR), RT-PCR, Southern blot, dot blot and fluorescence in situ hybridization (FISH). Among them, PCR is the most commonly used detection method. It is mainly used to detect viral DNA with strong sensitivity and is also suitable for detection of highly degraded DNA samples, such as formalin-fixed paraffin-embedded tissue sections. *In situ* hybridization (ISH) is the direct observation of HPV-DNA by microscopy and its localization in tissues and cells is by means of fluorescent probes or other markers. It is helpful to determine the state of HPV-DNA in host cells. Isolated HPV-DNA in the cells under microscope is often a diffuse nuclear signal, while HPV-DNA integrated into the host genome is mostly a dotted signal. Although ISH is intuitive, its sensitivity is lower than that of PCR, and its cost is relatively high. The method of HPV detection used in this study is PCR-reverse dot blot hybrid method, which has the characteristics of high sensitivity and specificity. It is reliable to detect HPV. In conclusion, selecting an objective and effective detection method according to different research purposes and specific sample conditions is a prerequisite for evaluating the correlation between HPV infection and HNSCC correctly.

HPV-related head and neck carcinoma has specific epidemiological, pathological characteristics and prognosis. Through the analysis of HPV detection in head and neck carcinoma specimens in the Tangshan area of China, this study has a better understanding of HPV-related HNSCC. Patients with poorly-differentiated and HPV-negative cancer should be followed up. This conclusion has certain early warning value for the diagnosis and treatment of some cases of HNSCC. The role of HPV in HNSCC needs further study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hong-Xia Liu, Department of Pathology, Tangshan Union Hospital, Tangshan 063004, China. Tel: +86-13503257696; E-mail: lhx1lmq@126.com

References

[1] Gissmann L, Wolnik L, Ikenberg H, Koldovsky U, Schnürch HG, zur Hausen H. Human papillomavirus types 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. Proc Natl Acad Sci U S A 1983; 80: 560-3.

[2] Osei-Sarfo K, Tang XH, Urvalek AM, Scognamiglio T, Gudas LJ. The molecular features of tongue epithelium treated with the carcinogen 4-nitroquinoline-1-oxide and alcohol as a model for HNSCC. Carcinogenesis 2013; 34: 2673-2781.

[3] Souza GD, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, Westra WH, Gillison ML. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007; 356: 1944-1956.

[4] Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000; 9: 709-720.

[5] Tomar S, Graves CA, Altomare D, Kowli S, Kassler S, Sutkowski N, Gillespie MB, Creek KE, Pirisi L. Human papillomavirus status and gene expression profiles of oropharyngeal and oral cancers from European American and African American patients. Head Neck 2016; 38 Suppl 1: E694-E704.

[6] Anastasios KM. Role of human papillomavirus in the pathogenesis of oral squamous cell carcinoma. World J Exp Med 2012; 2: 65-69.

[7] Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005; 14: 467-475.

[8] Chernock D, El-Mofty SK, Thorstad WL, Parvin CA, Lewis JS Jr. HPV-related non-keratinizing squamous cell carcinoma of the oropharynx: utility of microscopic features in predicting patient outcome. Head Neck Pathol 2009; 3: 186-194.

[9] Vidya A, Harish S, Subhas B, Veena S, Shruthi H. Human papilloma virus associated squamous cell carcinoma of the head and neck. J Sex Transm Dis 2015; 2015: 791024.

[10] Meulendijks D, Tomasoa NB, Dewit L, Smits PH, Bakker R, van Velthuysen ML, Rosenberg EH, Beijnen JH, Schellens JH, Cats A. HPV-negative squamous cell carcinoma of the anal canal is unresponsive to standard treatment and frequently carries disruptive mutations in TP53. Br J Cancer 2015; 112: 1358-1366.

[11] Allie KA, Trisha MW, Susanne IW. Human papillomavirus induced transformation in cervical and head and neck cancers. Cancers (Basel) 2014; 6: 1793-1820.

[12] Shiboski CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma increasing trends in the U.S population ages 20-44 years. Cancer 2005; 103: 1843-1849.

[13] Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, Viscidi R. Distinct risk factor profiles for human papillomavirus type 16 - positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 2008; 100: 407-420.

[14] Schantz S, Guo PY. Head and neck cancer incidence trends in young American, 1973-1997, with a special analysis for tongue cancer. Arch Otolaryngol Head Neck Surg 2002; 12: 268-274.

[15] Sonia NW, Maria F, Penelope DH. Recent progress in therapeutic treatments and screening strategies for the prevention and treatment of HPV-associated head and neck cancer. Viruses 2015; 7: 5040-5065.

[16] Faraji F, Eisele DW, Fakhry C. Emerging insights into recurrent and metastatic human papilloma virus-related oropharyngeal squamous cell carcinoma. Laryngoscope Investigative Otolaryngology 2017; 2: 10-18.

[17] Yang JQ, Wang HB, Wu M, Sun YM, Liu HX. Correlation of HPV16 infection and p16 expression with prognosis in patients with hypopharyngeal carcinoma. Int J Clin Exp Pathol 2016; 9: 4978-4985.

[18] Daniel M, Martin CA, Alfredo AM, Vitale-Cross L, Wang Z, Zaida M, Delic NC, Samuels Y, Lyons JG, Gutkind JS. The head and neck cancer cell oncogenome: a platform for the development of precision molecular therapies. Oncotarget 2014; 5: 8906-8923.

[19] Dong HC, Cui XB, Wang LH, Li M, Shen YY, Zhu JB, Li CF, Hu JM, Li SG, Yang L, Zhang WJ, Chen YZ, Li F. Type-specific detection of human papillomaviruses in Kazakh esophageal squamous cell carcinoma by genotyping both E6 and L1 genes with MALDI-TOF mass spectrometry. Int J Clin Exp Pathol 2015; 8: 13156-13165.

[20] Richards KL, Zhang B, Baggerly KA, Colella S, Lang JC, Schuller DE, Krahe R. Genome-wide hypomethylation in head and neck cancer is more pronounced in HPV-negative tumors and is associated with genomic instability. PLoS One 2009; 4: e4941.