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Original Article

Frequency of Human Papillomavirus Genotypes in Head and Neck Epithelial Cancers in the Ear, Throat, and Nose Department of Shafa Hospital, Kerman, Iran in 2017

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ABSTRACT

Background: Human papillomavirus virus (HPV) plays an important role in some human malignancies, including head and neck squamous cell carcinoma (SCC). However, it is necessary to evaluate the role of carcinogenic agents in local settings as well. Therefore, this study aimed to evaluate the frequency of HPV genotypes in head and neck epithelial cancers.

Methods: In this case-control study, 202 patients with suspected carcinoma referred to Shafa Hospital (Kerman, Iran, 2017) for pathological evaluation were enrolled. A definitive diagnosis of carcinoma was reported for 101 patients (case group) and in the other 101 subjects, carcinoma was not diagnosed. In order to determine the presence and type of HPV in tissue samples, the PCR method was used. Data analysis was performed using Fisher's exact test, independent t-test, and logistic regression.

Results: In total, 27 head and neck SCC samples (26.7%) were positive for HPV DNA. In addition, the virus species were HPV-11 in 15 cases (14.9%), HPV-16 and HPV-18 in 12 cases (11.9%), and HPV-6 in 10 cases (9.9%). According to the results, the presence of HPV was significantly higher in patients with SCC ($P < 0.001$). Moreover, smoking ($P = 0.03$) and opioid use ($P < 0.001$) were also significantly associated with SCC.

Conclusion: The results of the present study demonstrated the presence of HPV in tissue samples of head and neck SCC patients in Kerman, Iran. Further studies are needed to investigate the preventive role of HPV vaccination in head and neck SCC.

Keywords: HPV, Head and Neck Cancers, Kerman

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Introduction

Squamous cell carcinoma (SCC) of the upper respiratory tract is the most common type of head and neck cancer (1), which can occur in the larynx, oral cavity, skin, nasopharynx, and esophagus (2). Some of the most important factors involved in the development of these cancers are smoking (3), alcohol consumption (4), chronic mucosal stimulation, low-dose radiation, occupational factors and viral factors (5), the Epstein–Barr virus (EBV) (6), and the Human Papillomavirus (HPV) (3, 7, 8). While the etiologic relation of some of these factors, such as smoking in laryngeal cancer, or EBV in nasopharyngeal cancer, is known, some other associations are still under study. The role of HPV (9, 10), an epitheliotropic virus, has now been confirmed in cervical cancer (11, 12). However, various percentages of this virus have been detected in samples of oral cavity cancer and other head and neck epithelial cancers (7).

The papillomavirus family has more than 100 viral species, each of which stimulates epithelial growth in an organ and can therefore cause cancer. The papillomaviruses are classified into three groups in terms of carcinogenesis, namely, high-risk group (HPV-16, 18), medium-risk group (HPV-30, 33), and low-risk group (HPV-6, 11). The subtypes of 6 and 11 cause benign lesions (e.g., warts and papillomas), and the genotype 16 (HPV-16) is ranked first in terms of causing cervical cancer (13, 14). According to some studies, HPV plays a significant role in oropharyngeal SCC. This is especially due to the detection of HPV in pre-cancerous and cancerous oral lesions. The HPV-containing malignant tumors are less distinctive and occur at an earlier age but have a longer lifespan, compared to non-HPV variants (15, 16).

Some studies have shown that HPV is an independent factor for the development of SCC. The mechanism of cancer formation by HPV is related to the destructive effects of E6 and E7 viral proteins on p53. The highest impact of this virus is on the development of tonsillar SCC (13, 17). The carcinogenic effect of this virus in the larynx is less than the oropharyngeal region. However, present studies have reported the involvement of HPV in more than 20% of laryngeal cancers (18, 19). A recent systematic review identified 41 publications in which laryngeal SCC was examined for the presence of HPV DNA and found that the weighted prevalence of HPV DNA in 1,712 cases of laryngeal SCC was 23.6%. This is slightly higher than the weighted prevalence of HPV DNA in oral cavity SCC, which was 20.2%. However, a significant association was found between HPV infection and laryngeal SCC. Although multiple subtypes of HPV were detected in laryngeal SCC, HPV 16 was the most common subtype identified in laryngeal SCC (20).

Empirical and epidemiological evidence in western countries supports the role of HPV in the epidemiology of head and neck SCC. Nevertheless, the role of the virus in the development of SCC in developing countries (e.g., Iran) is still under study (8). On the other hand, conflicting results have been reported by studies about the role of this virus in the development of cancer, in different parts of the world.

Some studies have supported the role of this virus in the development of head and neck cancers, whereas others have questioned the role of this virus. For instance, Nikakhlagh et al. (2008) reported the presence of HPV in only 3.79% of the tissue samples of the patients with head and neck carcinoma (8). In addition, Zarei et al. (2007) conducted a research on patients with four different oral lesions, including oral SCC (OSCC), leukoplakia, oral lichen planus (OLP), and pyogenic granuloma (PG), and concluded that the DNA of HPV was detected in 60% of the OSCC cases, 26.7% of leukoplakia cases, 13.3% of OLP cases, and 6.7% of PG cases (21).

Considering the high prevalence of SCC in the elderlies and due to inadequate research on the role of HPV in laryngeal carcinoma, especially in our region, and given the detection of this type of cancer in some non-smokers, the present study aimed to evaluate the frequency of HPV genotypes in head and neck epithelial cancers in Kerman, Iran.

Materials and Methods

This project was approved by the Research Committee of the School of Medicine (Project No: 940693) and the Standing Ethics Committee (Ethical Code: IR.KMU.AH.REC.1394.627).

The population of this case-control study consisted of all individuals suspected of head and neck malignancies (oral cavity, oropharynx, nasopharynx, nasal cavity, paranasal sinuses and larynx) who were referred to the Otolaryngology Department of Shafa Hospital, Kerman in 2017, and underwent a diagnostic biopsy.

The tissue samples were taken from the surgical samples stored in the pathology lab. Written informed consent was previously obtained from these patients to use their tissue samples for research purposes. All participants were assured about the confidentiality of their personal information.

According to the results of pathology, the subjects were divided into two groups. The case group included patients with a definite diagnosis of SCC, and the control group encompassed participants with no signs of SCC in their pathology samples.

The sufficient sample size to estimate the difference of HPV presence in tissue samples was calculated using the equation for determining sample size for comparison of percentages and assuming an HPV frequency of 55% in the control group, and 73% in the case group, type one error = 0.10, and power = 80%. The minimum sample size required was 96. However, 101 cases were selected for each group from paraffin blocks existing in the Pathology Ward of Shafa Hospital.

Deparaffinization of samples

Paraffinized block samples were cut in 3-µm sections and 5 sections were collected in a micro-centrifuge tube. Samples were dewaxed in 800 µl xylene; all micro-centrifuge tubes were placed on a block heater at 60°C for about 10 minutes. All microtubes were centrifuged at 10,000 rpm for 1 minute and the supernatant was removed. This step was repeated three times. 500 µl pure ethanol was added to all microtubes for removing xylene, and were centrifuged at 10,000 rpm for 1 minute. The samples were then dried at 70°C on a block heater with open lids for 5-10 minutes to remove residual ethanol.

Tissue digestion

200-400 µl of Tissue Lysis Buffer was added to each tube [4 M Urea, 200 mM Tris, 20 mM NaCl, 200 mM EDTA; PH = 7.4 (25°C)]. 20-40 µl of proteinase K was added to all tubes. Samples were gently vortexed and placed for about 10 minutes on a block heater at 60°C, and all samples were subsequently incubated at 37°C overnight.

DNA Extraction

The next day, 200 µl of Binding Buffer [6 M Guanidine- Hcl, 10 mM Urea, 10 mM Tris-Hcl, 20% Tritonx-100 (v/v); PH = 4.4 (25°C)] was added to each tube with gentle vortex. DNA was isolated using a QIAamp DNA Mini kit (Qiagen, Germany), according to the manufacturer's instructions. The extracted DNA pellets were suspended in 70 µl of pre-warmed elution buffer and stored at -70°C until use.

Qualitative Real-Time PCR

After DNA extraction, for detection and screening samples positive for HPV, a qualitative real-time PCR based on SYBR Green was done. The primers used in this study were general primers from MY09 and MY011 pairs (MY09: 5'-CGT CCM AAR GGA WAC TGA TC-3' and MY011: 5'-GCM CAG GGW CAT AAY AAT GG-3'). The test was done by Rotor Gene 6000 (Corbett Research, Australia).

INNO-LiPA HPV detection and genotyping

After performing a PCR test and identifying positive samples, the INNO-LiPA test was performed to identify the types of HPV. The INNO-LiPA® HPV Genotyping Extra II kit (Fujirebio Diagnostics, Sweden) was used for this experiment, the steps of which have been described below in two steps A and B (22).

A) PCR amplification of HPV DNA

Broad-spectrum HPV DNA amplification was performed using a short PCR fragment assay (INNO-LiPA HPV detection/genotyping assay, the Netherlands). This assay amplifies a 65-bp fragment of the L1 open reading frame and allows detection of at least 43 different HPV types. The SPF10 PCR was performed with a final reaction volume of 50 µl containing 10 µl of the isolated DNA sample, 10 mmol/liter Tris-HCl (pH 9.0), 50 mmol/ liter KCl, 2.0 mmol/liter $MgCl_2$, 0.1% Triton X-100, 0.01% gelatin, 200 µmol/liter of each deoxynucleoside triphosphate, 15 pmol of each of the forward and reverse primers tagged with biotin at the 5'end, and 1.5 U of AmpliTaq Gold (Perkin-Elmer). The mixture was incubated for 9 min at 94°C, 40 cycles of 45 s at 45°C, and 40 cycles of 45 s at 72°C, with a final extension of 5 min at 72°C. Each experiment was performed with separate positive and negative PCR controls. The presence of HPV DNA was determined by hybridization of SPF10 amplimers to a mixture of general HPV probes recognizing a broad range of HPV genotypes, in a microtiter plate format, as described previously (23, 24).

B) HPV genotyping by reverse hybridization using the INNO-LiPA HPV genotyping system

A poly (dT) tail was enzymatically added to the 3' end of each of 25 oligonucleotides specific for 25 different types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74). The tailed probes were applied as horizontal lines to the membrane strips (manufactured by Labo Biomedical Products B.V., Rijswijk, the Netherlands). The HPV genotyping assay was performed as described previously. Briefly, equal volumes (10 µl each) of the biotinylated PCR products and denaturation solution (400 mmol/liter NaOH, 10 mmol/liter EDTA) were mixed in test troughs and incubated at room temperature for 5 min. Afterwards, 1 ml of pre-warmed (37°C) hybridization solution was added, followed by the addition of one strip per trough. Hybridization was performed for 1 h at 50 ± 0.5°C in a closed water bath with back-and-forth shaking. The strips were then washed twice with 1 ml of wash solution at room temperature for 20 seconds and once at 50°C for 30 minutes. Following this stringent washing, the strips were rinsed twice with 1 ml of a standard rinse solution. The strips were then incubated on a rotating platform with an alkaline phosphatase-labeled streptavidin conjugate diluted in a standard conjugate solution, at 20 to 25°C for 30 min. Afterwards, the strips were washed twice with 1 ml of rinse solution and once with standard substrate buffer; color development was initiated by the addition of 5-bromo-4-chloro-3-indolylphosphate and Nitroblue tetrazolium to 1 ml of substrate buffer. After 30 min of incubation at room temperature, the color reaction was stopped by the aspiration of the substrate buffer and addition of distilled water. After drying, the strips were visually interpreted using a grid (25, 26).

Statistical analysis

Data were analyzed using Chi-square test and t-test to compare percentages and means, by IBM SPSS Statistics software, version 20. Values less than or equal to 0.05 were considered statistically significant.

Results

From 202 patients, 152 subjects were male. Gender was significantly different between the two groups ($P < 0.001$).

In the SCC and control groups, 72 (71.3%) and 19 (18.8%) subjects were smokers, respectively, and the difference was significant ($P < 0.001$). Moreover, 72 subjects in the SCC group (71.3%) and 18 subjects in the control group (17.8%) were opioid users, and the difference was significant ($P < 0.001$) as well. But, no significant relation was observed between alcohol consumption and the incidence of SCC ($P = 1$). The mean age of patients in the SCC and control groups was significantly different ($P < 0.001$) (Table 1).

In total, 202 subjects were assessed in terms of the presence of HPV. From 101 patients with SCC, 27 (26.7%) had HPV, whereas, from the 101 individuals without cancer, only 3 subjects (3.0%) had HPV. The prevalence of HPV was significantly higher in patients with SCC ($P < 0.001$) (Table 1).

The prevalence of site-specific HPV in the SCC and control groups is presented in Tables 2 and 3. In the SCC group, the most common HPV-positive location was the larynx; whereas, in the control group, it was the nasopharynx. The frequency of HPV subtypes HPV11, 16, 18, and 6 was 15 (14.9%), 12 (11.9%), 12 (11.9%), and 10 (9.9%), respectively. Some samples contained more than one type of virus.

Logistic regression showed that the risk of SCC incidence was 9.19 and 2.66 times higher in drug users and smokers compared to others. In HPV-positive patients, the risk of SCC incidence was 11.8 times higher, compared to those with negative HPV tests (Table 4).

Table 1. Age, gender, cigarette smoking, opium use, and alcohol consumption in the case (SCC) and control groups

Group	Control No (%)	SCC No (%)	Fisher's Exact Test P-value
Male	64 (63.4)	88 (87.1)	0.001
Female	37 (36.6)	13 (12.9)	
Smokers	19 (18.8)	72 (71.3)	0.001
Non-smokers	82 (81.2)	29 (28.7)	
Opium-dependent	18 (17.8)	79 (78.2)	0.001
Non-opium-dependent	82 (18.2)	22 (21.8)	
Alcohol users	6 (5.9)	6 (5.9)	1.0
Non-users	95 (94.1)	95 (94.1)	
HPV$^+$	3 (3.0)	27 (26.7)	<0.001
HPV$^-$	98 (97.0)	74 (73.3)	
	Mean ± SD	**Mean ± SD**	**Independent t-test P-value**
Age (year)	37.66 ± 16.81	59.83 ± 13.61	0.001

Table 2. The distribution of HPV subtypes in the head and neck sub-sites in patients with SCC

	SCC	HPV Positive	
	No (%)	**Type**	**No (%)**
Oral cavity	14 (13.8)	6, 11	3 (11.11)
Oropharynx	7 (6.9)	-	-
Nasal cavity and PNS	7 (6.9)	-	-
Nasopharynx	2 (1.9)	11, 16	1 (3.0)
Larynx	71 (70.0)	6, 11, 16, 18, 31, 33, 56	23 (85.19)
Total	101 (100.0)	-	27 (100.0)

Table 3. The distribution of HPV subtypes in the head and neck sub-sites in the controls

	Control	HPV Positive	
	No (%)	Type	No (%)
Oral cavity	16 (15.8)	-	-
Oropharynx	16 (15.8)	-	-
Nasal cavity and PNS	38 (37.6)	-	-
Nasopharynx	14 (13.8)	6,11	3 (100.0)
Larynx	17 (16.8)	-	-
Total	101 (100.0)	-	3 (100.0)

Table 4. The results of adjusted logistic regression for the association of risk factors with SCC

Characteristics	OR	CI95%	P-value
HPV test			
Positive	11.8	2.99-47.05	<0.001
Negative	1		
Smoking			
Yes	2.66	1.06-6.67	0.03
No	1		
Opioid abuse			
Yes	9.19	3.66-23.06	<0.001
No	1		

Discussion

In the present study, different types of HPV were detected in 26.7% of the patients with head and neck SCC. In this respect, conflicting results have been obtained by various studies conducted in different parts of the world. For instance, Nikakhlagh et al. (2008) performed a research in Ahvaz, Iran, and concluded that 3.97% of the cases with SCC were positive for HPV DNA (8). Nonetheless, the mentioned rate was zero and 46% in the study done by Li et al. (2003) on Chinese and Australian patients with SCC of the tonsil, respectively (9).

Moreover, Bishop et al. (2013) performed a research on sinonasal tract SCC samples archived at the Johns Hopkins Hospital, and reported that from 161 patients diagnosed with this disease, 21% had positive HPV (7). This rate was 14% in the study by Furuta et al. (1992) on the patients with SCC of the nasal cavity and paranasal sinus treated at Hokkaido University hospital in Japan (27). In another study by Correnti et al. (2004) conducted in Venezuela to determine HPV in patients with SCC of the oral cavity, 50% of the subjects had positive HPV (28). In a study by Zarei et al. (2007) in Kerman,

Iran, 60% of the patients with oral cavity SCC had positive HPV (21). In a review study, Torrente et al. (2011) reported that HPV infections was present in 25% of the patients with laryngeal SCC, and the most common subtypes were 16 and 18 (18). In the study of Hernandez et al. (2014) in the U.S., HPV DNA was detected in 21% of the laryngeal cancer cases and HPV 16 and 33 were the most commonly detected genotypes (19). A meta-analysis by Li et al. (2013) based on 12 case-control studies also suggested a strong association between HPV infection and laryngeal SCC, with a significant pooled odds ratio of 5.39. This association was even stronger for HPV-16, and the pooled odds ratio was significant (6.07) (29). Researchers reported that HPV infection causes laryngeal cancer via genomic integration, activating proto-oncogenes, and inhibiting anti-oncogenes (30).

Meanwhile, researchers from Turkey used nonisotopic in situ hybridization (NISH) technique, and showed signals of HPV 6/11, 16/18, and 31/33 infection in 80%, 40%, 30% of the laryngeal carcinomas, respectively (10). In a review study by Ragin et al. (2007), the mean prevalence of HPV in the samples for all oropharyngeal tumors was 28% (31).

In the present research, HPV-11, 16, 18, and 6 were about 15%, 12%, 12%, and 10%, respectively. The majority of the virus types detected in the samples tested by Nikakhlagh et al. were 16 and 18 (8) as well. In a study by Bishop et al. (2013), the most and least common types were 16 (82%) and 18 (6%), respectively (7). Also, these two types were the most common types found in the study by Furuta et al. (1992) (27). In the study of Reimers et al. (2007) (32), the most common types were 5, 16, and 33 (32). But in the studies by Na et al. (2006) in patients with head and neck SCC in Korea (33), Sugiyama et al. (2007) in patients with oral SCC in Japan (34), Licitra et al. (2006) in patients with surgically treated oropharyngeal SCC in Milan, Italy (35), Weinberger et al. (2006) in patients with oropharyngeal SCC in New Haven, CT, the USA (36), Báez et al. (2004) in patients with head and neck SCC in Puerto Rico (37), Ringström et al. (2002) in patients with head and neck SCC in Massachusetts, the USA (38), and Mellin et al. (2002) in patients with tonsillar cancer in Stockholm, Sweden (39), the dominant HPV type was 16.

The diagnosis of HPV in most above-mentioned studies (33-39), as well as the present study was done by PCR. The sensitivity and specificity of PCR is close to 100% (40).

Recently, researchers have mentioned that HPV positive oropharyngeal SCC is an epidemic not easily recognized by many physicians, and this results in delays in diagnosis and management of this type of cancer (41). Other researchers have reported that in addition to tobacco and alcohol, HPV plays a significant role in the development of some head and neck SCC, and more notably, oropharynx SCC. But, HPV-induced SCC tumors have a better prognosis compared to non-HPV-induced tumors. Therefore, determining the HPV status in patients with SCC is essential for treatment and predicting prognosis (42).

Although HPV has been present in other cancerous lesions such as cervical cancer in Kerman province (43) as well, but its role has been denied in prostate cancer (44). Therefore, more research is needed to determine the cancer types that might be related to HPV in Kerman, Iran.

Conclusion

The results of this study showed that in approximately one-fourth of the head and neck carcinomas under study, HPV was detected. It seems that in Kerman, this virus is a predisposing factor, or a risk factor that causes head and neck SCC. The role of other factors, such as smoking and opioid use should also be considered in Kerman. More clinical studies should be conducted in different geographical areas to further investigate the role of HPV in head and neck SCC.

References

1. Ragin CC, Modugno F, Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. J Dent Res 2007; 86(2):104-14. doi: 10.1177/154405910708600202.
2. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. JAMA Oncol 2017; 3(4):524-48. doi:

10.1001/jamaoncol.2016.5688.

3. Koyanagi YN, Matsuo K, Ito H, Wakai K, Nagata C, Nakayama T, et al. Cigarette smoking and the risk of head and neck cancer in the Japanese population: a systematic review and meta-analysis. Jpn J Clin Oncol 2016; 46(6):580-95. doi: 10.1093/jjco/hyw027.
4. Kawakita D, Matsuo K. Alcohol and head and neck cancer. Cancer Metastasis Rev 2017; 36(3):425-34. doi: 10.1007/s10555-017-9690-0.
5. Nikakhlagh S, Saki N, Shoar MH, Sartipipor A, Saki S. Incidence of etiologic factors in squamous cell carcinoma of head and neck in ahvaz. Iran J Otorhinolaryngol 2012; 24(67):85-90.
6. Prabhu SR, Wilson DF. Evidence of Epstein–Barr virus association with head and neck cancers: a review. J Can Dent Assoc 2016; 82:g2.
7. Bishop JA, Guo TW, Smith DF, Wang H, Ogawa T, Pai SI, et al. Human papillomavirus-related carcinomas of the sinonasal tract. Am J Surg Pathol 2013; 37(2):185-92. doi: 10.1097/PAS.0b013e3182698673.
8. Nikakhlagh S, Saki N, Mokvandi M, Jahanshahi J, Emadmostofi N. Detection and typing of human papilloma virus DNA by PCR in head and neck squamous cell carcinoma in E.N.T. Ward of Ahwaz Imam Hospital. Avicenna Journal of Clinical Medicine 2008; 15(2):19-22. [In Persian].
9. Li W, Thompson CH, Xin D, Cossart YE, O'Brien CJ, McNeil EB, et al. Absence of human papillomavirus in tonsillar squamous cell carcinomas from Chinese patients. Am J Pathol 2003; 163(6):2185-9. doi: 10.1016/S0002-9440(10)63576-6.
10. Kaya H, Kotiloğlu E, Inanli S, Ekicioğlu G, Bozkurt SU, Tutkun A, et al. Prevalence of human papillomavirus (HPV) DNA in larynx and lung carcinomas. Pathologica 2001; 93(5):531-4.
11. Sohrabi A, Rahnamaye Farzami M, Mirab Samiee S, Modares MH. An overview on papillomaviruses as the main cause of cervical cancer. Iranian Journal of Obstetrics, Gynecology and Infertility 2015; 18(145):14-25. [In Persian].
12. Onsory K, Ahmadi AR, Jalilvand E. Epidemiology of human papilloma virus (HPV) type 16 and 18 in the patients with cervical cancer in Tehran. Journal of Microbial World 2016; 8(4):281-9. [In Persian].
13. Tomaić V. Functional roles of E6 and E7 oncoproteins in HPV-Induced malignancies at diverse anatomical sites. Cancers (Basel) 2016; 8(10):95. doi: 10.3390/cancers8100095.
14. Fakhraei F, Haghshenas MR. Human papillomaviruses and cancer. Journal of Mazandaran University of Medical Sciences 2013; 23(98):340-60. [In Persian].
15. Spence T, Bruce J, Yip KW, Liu FF. HPV associated head and neck cancer. Cancers (Basel) 2016; 8(8):75. doi: 10.3390/cancers8080075.
16. D'Souza G, Dempsey A. The role of HPV in head and neck cancer and review of the HPV vaccine. Prev Med 2011; 53(Suppl 1):S5-S11. doi: 10.1016/j.ypmed.2011.08.001.
17. Johnson ME, Cantalupo PG, Pipas JM. Identification of head and neck cancer subtypes based on human papillomavirus presence and E2F-Regulated gene expression. mSphere 2018; 3(1):e00580-17. doi: 10.1128/mSphere.00580-17.
18. Torrente MC, Rodrigo JP, Haigentz Jr M, Dikkers FG, Rinaldo A, Takes RP, et al. Human papillomavirus infections in laryngeal cancer. Head Neck 2011; 33(4):581-6. doi: 10.1002/hed.21421.
19. Hernandez BY, Goodman MT, Lynch CF, Cozen W, Unger ER, Steinau M, et al. Human papillomavirus prevalence in invasive laryngeal cancer in the United States. PLoS One 2014; 9(12):e115931. doi: 10.1371/journal.pone.0115931.
20. Flint PW, Haughey BH, Robbins KT, Thomas JR, Niparko JK, Lund VJ, et al. 7th ed. Cummings Otolaryngology E-Book: Head and Neck Surgery. Elsevier; 2020.
21. Zarei MR, Moradi A, Chamani G, Kakouei S, Hamkar R, Mohammad AS, et al. Detection of human papillomavirus DNA sequences in oral lesions using polymerase chain reaction. Acta Medica Iranica. 2007; 5(3):177-182.
22. Afshar RM, Mollaie HR, Fazlalipour M, Arabzadeh SA. Prevalence and type distribution of human papillomavirus infection using the INNo-Lipa assay, Kerman, Southeast Iran. Asian Pac J Cancer Prev 2013; 14(9):5287-91. doi: 10.7314/apjcp.2013.14.9.5287.
23. Ghasemian E, Monavari SH, Irajian GR, Jalali Nodoshan MR, Roudsari RV, Yahyapour Y. Evaluation of human papillomavirus infections in prostatic disease: a cross-sectional study in Iran. Asian Pac J Cancer Prev 2013; 14(5):3305-8. doi: 10.7314/apjcp.2013.14.5.3305.
24. Eftekhaar NS, Karbalaie Niya MH, Izadi F, Teaghinezhad-S S, Keyvani H. Human papillomavirus (HPV) genotype distribution in

patients with recurrent respiratory papillomatosis (RRP) in Iran. Asian Pac J Cancer Prev 2017; 18(7):1973-6. doi: 10.22034/APJCP.2017.18.7.1973.

25. Yahyapour Y, Shamsi-Shahrabadi M, Mahmoudi M, Siadati S, Shahryar SS, Shokri-Shirvani J, et al. Evaluation of human papilloma virus infection in patients with esophageal squamous cell carcinoma from the Caspian Sea area, north of Iran. Asian Pac J Cancer Prev 2012; 13(4):1261-6. doi: 10.7314/apjcp.2012.13.4.1261.
26. Karbalaie Niya MH, Safarnezhad Tameshkel F, Panahi M, Bokharaei Salim F, Monavari SH, Keyvani H. Human papillomavirus investigation in head and neck squamous cell carcinoma: initial report from the low risk HPV types associations. Asian Pac J Cancer Prev 2017; 18(9):2573-9. doi: 10.22034/APJCP.2017.18.9.2573.
27. Furuta Y, Takasu T, Asai T, Shinohara T, Sawa H, Nagashima K, et al. Detection of human papillomavirus DNA in carcinomas of the nasal cavities and paranasal sinuses by polymerase chain reaction. Cancer 1992; 69(2):353-7. doi: 10.1002/1097-0142(19920115)69:2<353::aid-cncr2820690213>3.0.co;2-j.
28. Correnti M, Rivera H, Cavazza ME. Detection of human papillomaviruses of high oncogenic potential in oral squamous cell carcinoma in a Venezuelan population. Oral Dis 2004; 10(3):163-6. doi: 10.1046/j.1601-0825.2003.00989.x.
29. Li X, Gao L, Li H, Gao J, Yang Y, Zhou F, et al. Human papillomavirus infection and laryngeal cancer risk: a systematic review and meta-analysis. J Infect Dis 2013; 207(3):479-88. doi: 10.1093/infdis/jis698.
30. Yang D, Shi Y, Tang Y, Yin H, Guo Y, Wen S, et al. Effect of HPV infection on the occurrence and development of laryngeal cancer: a review. J Cancer 2019; 10(19):4455-62. doi: 10.7150/jca.34016.
31. Ragin CC, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. Int J Cancer 2007; 121(8):1813-20. doi: 10.1002/ijc.22851.
32. Reimers N, Kasper HU, Weissenborn SJ, Stützer H, Preuss SF, Hoffmann TK, et al. Combined analysis of HPV-DNA, p16 and EGFR expression to predict prognosis in oropharyngeal cancer. Int J Cancer 2007; 120(8):1731-8. doi: 10.1002/ijc.22355.
33. Na I, Kang HJ, Cho SY, Koh JS, Lee JK, Lee BC, et al. EGFR mutations and human papillomavirus in squamous cell carcinoma of tongue and tonsil. EJC 2006; 43(3):520-26.
34. Sugiyama M, Bhawal UK, Kawamura M, Ishioka Y, Shigeishi H, Higashikawa K, et al. Human papillomavirus-16 in oral squamous cell carcinoma: clinical correlates and 5-year survival. Br J Oral Maxillofac Surg 2007; 45(2):116-22. doi: 10.1016/j.bjoms.2006.04.012.
35. Licitra L, Perrone F, Bossi P, Suardi S, Mariani L, Artusi R, et al. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J Clin Oncol 2006; 24(36):5630-6. doi: 10.1200/JCO.2005.04.6136.
36. Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, Brandsma J, et al. Molecular classification identifies a subset of human papillomavirus-associated oropharyngeal cancers with favorable prognosis. J Clin Oncol 2006; 24(5):736-47. doi: 10.1200/JCO.2004.00.3335.
37. Báez A, Almodovar JI, Cantor A, Celestin F, Cruz-Cruz L, Fonseca S, et al. High frequency of HPV16-associated head and neck squamous cell carcinoma in the Puerto Rican population. Head Neck 2004; 26(9):778-84. doi: 10.1002/hed.20046.
38. Ringström E, Peters E, Hasegawa M, Posner M, Liu M, Kelsey KT. Human papillomavirus type 16 and squamous cell carcinoma of the head and neck. Clin cancer Res 2002; 8(10):3187-92.
39. Mellin H, Dahlgren L, Munck-Wikland E, Lindholm J, Rabbani H, Kalantari M, et al. Human papillomavirus type 16 is episomal and a high viral load may be correlated to better prognosis in tonsillar cancer. Int J Cancer 2002; 102(2):152-8. doi: 10.1002/ijc.10669.
40. Harlé, A., Guillet, J., Thomas, J. Sastre-Garau X,Rouyer M,Ramacci c,et al. Evaluation and validation of HPV real-time PCR assay for the detection of HPV DNA in oral cytobrush and FFPE samples. Sci Rep 2018; 11313(8). .doi.org/10.1038/s41598-2018-29790-z.
41. Economopoulou P, Kotsantis I, Psyrri A. Special issue about head and neck cancers: HPV positive cancers. Int J Mol Sci 2020; 21(9):3388. doi: 10.3390/ijms21093388.
42. Augustin JG, Lepine C, Morini A, Brunet A, Veyer D, Brochard C, et al. HPV detection in head and neck squamous cell carcinomas: what is the issue? Front Oncol 2020; s10:1751. doi: 10.3389/fonc.2020.01751.
43. Monsfi N, Dabiri SH, Abbaszadeh M, Safizadeh H, Fotouhi-Ardakani R, et al.

Frequency of Dysplastic and Cancerous Pap Smear and Genotyping of Human Papillomavirus by DNA Probetechniques in Kerman, Iran. Journal of Kerman University of Medical Sciences, 2013; 20(5): 450-459.

44. Jafari E, Dabiri SH, Mortazaeizadeh A, Sayadi AR, Amir Poor Rostami S. Non- Detection of HPV DNA in Prostatic Cancer and Benign Prostatic Hyperplasia: a case- control study in Kerman. Journal of Kerman University of Medical Sciences, 2018; 25(1): 77-83.