Human Papilloma Virus in Oral Cavity Cancer and Relation to Change in Quality of Life Following Treatment—a Pilot Study from Northern India

Abhishek Kumar Singh¹ · Jitendra Kumar Kushwaha¹ · Akshay Anand¹ ·
Abhinav Arun Sonkar¹ · Nuzhat Husain² · Kirti Srivastava³ · Sudhir Singh³

Received: 21 June 2016 / Accepted: 6 September 2016 / Published online: 22 September 2016
© Indian Association of Surgical Oncology 2016

Abstract Human papilloma virus (HPV)-associated head and neck cancer (HNC) has generated significant amount of research interest in recent times with focus shifted to oral cavity squamous cell cancer (OCSCC) after oropharyngeal cancer. Due to high incidence of OCSCC and anecdotal reports on association of HPV infection from northern region of India, this study was conceived to investigate HPV infection and establish its association with lifestyle habits such as tobacco, alcohol consumption, oro-genital sex, number of sexual contacts, and change in quality of life posttreatment. A total of 43 primary OCSCC biopsy specimens were collected. These samples were analyzed for HPV DNA genotyping which was done by using 13 high-risk HPV real-time PCR kits. Quality of life was assessed using University of Washington questionnaire for HNC patients, which was administered pre-treatment and 3-months posttreatment. HPV presence was confirmed in only three patients (7.0 %). HPV positivity did not find any statistical correlation with age, gender, residence, addiction habit, stage, tumor size, nodal status, tumor grade, and number of sexual contacts. There was no significant ($p > 0.05$) difference in the average percent change in QOL parameters from pretreatment to posttreatment when correlated with HPV status.

Keywords Human papilloma virus · Head and neck · Oral cavity squamous cell carcinoma · Oropharyngeal carcinoma · Quality of life · Oral neoplasms

Introduction

Oral cancer is a heterogeneous group of cancers arising from different parts of the oral cavity, with different predisposing factors, prevalence, and treatment outcomes. It is the sixth most common cancer reported globally with an annual incidence of over 300,000 cases, of which 62 % arise in developing countries [1]. There is a significant difference in the incidence of oral cancer in different regions of the world, with the age-adjusted rates varying from over 20 per 100,000 of population in India. In comparison with the US population, where oral cavity cancer represents only about 3 % of malignancies, it accounts for over 30 % of all cancers in India [2].

The proven risk factors of oral cavity squamous cell cancer (OCSCC) are tobacco, smoking [3], betel quid/pan masala chewing [4], and alcohol drinking [5]. While human papilloma virus (HPV) is an important cause of head and neck cancer (HNC) especially oropharyngeal cancer (OPC), it is currently unclear whether the HPV may also have a plausible role in other HNC subsites including OCSCC. A specific role was hypothesized way back in 1983, but till date, a definite consensus still awaits the light of the day [6–8]. The healthy adult population has showed prevalence for any HPV type of 2–8 % in OCSCC [9–14] with a higher preponderance for male sex worldwide [11–13, 15] whereas previous Indian studies have reported a wide variation in HPV positivity prevalence from northeastern, eastern, and southern different regions of the country [16–20]. Further high-risk sexual behavior including oral genital sex and multiple sexual partners has been found to be associated with HPV transmission [12, 21, 22].

✉ Abhinav Arun Sonkar
abhinavarunsonkar@gmail.com

¹ Department of Surgery, King George's Medical University, Lucknow, UP 226 003, India

² Ram Manohar Lohia Institute of Medical Sciences, Lucknow, UP, India

³ Department of Radiotherapy, King George's Medical University, Lucknow, UP, India

Immunodeficiency states and smoking seem to increase the risk of HPV transmission [23]. Patients with HPV-positive HNC in general tend to be younger (<50 years) at time of diagnosis [24, 25] and generally have a better survival and favorable prognosis compared to HPV-negative patients [26–30].

Given the increasing incidence of HPV-related HNC, studying the factors that influence quality of life (QOL) will become more important, as these patients are younger and more likely to survive than patients with HNC unrelated to HPV. Understanding the relationship between HPV status and QOL could help clinicians customize therapy and clinical management of HNC patients to optimize both survival and QOL.

Therefore, the present study aims to explore of HPV prevalence in OCSCC and its association with various risk factors and clinical and demographic variables in Northern India. In addition, to the best of our knowledge, for the first time from India, this study relates the association of change in quality of life posttreatment with HPV status during OCSCC management.

Methodology

The study was done in the Department of Surgery, King George's Medical University UP Lucknow, in collaboration with the Department of Pathology, RML Institute of Medical Sciences Lucknow UP. A total of 43 clinically and histologically proven cases of OCSCC were included prospectively in the study from August 2013 to August 2014. Tissue biopsies were collected after obtaining approval from the Institutional Ethics Committee (vide no. 65th ECM II-C/P4) and written informed consent from the patients.

Sample Collection

Tissue biopsies were collected in 10 % buffered formalin at room temperature for histopathological diagnosis and in 1× phosphate-buffered saline (PBS, pH 7.4) and stored at −80 °C for molecular analysis. The demographic and clinical details of the patients were recorded on the prestructured questionnaire.

OCSCC patients were staged according to UICC 7th AJCC staging. Patients were treated as per standard NCCN protocols. QOL was assessed using the University of Washington (UW_QOL) questionnaire, which was filled before at admission and after 3 months of completion of treatment.

DNA Extraction

The DNA from biopsy tissues was isolated by commercially available Purelink-Invitrogen Genomic DNA Mini kit

(catalog number K1820–01) as per manufacturer's instructions. The extracted DNA was eluted in 80 µl of the elution buffer (provided in the kit) and kept at −80 °C until further use. All the DNA samples were qualitatively confirmed on 0.8 % agarose gel electrophoresis, and the concentration and purity was checked by DS-11 spectrophotometer (Denovix, USA) at 260/280 nm wavelengths.

Real-Time PCR-Based Methodology for HPV Detection

Presence of HPV in OCSCC samples was detected by real-time PCR (Biorad CFX 96TM) using 13 high-risk HPV real-time PCR kits (HybriBio Limited, China) as per manufacturer's instructions. Briefly, each PCR reaction contained 17.5 µl of PCR master mix, 0.5 µl of Taq DNA polymerase, and 2 µl of DNA. The PCR amplification protocol was as follows: initial denaturation at 95 °C for 10 min and 45 cycles of denaturation for 3 min at 95 °C, annealing for 60s at 60 °C, and extension at 72 °C for 20s. The amplification was followed by a 5-s final extension step at 38 °C. The presence or absence of HPV DNA was confirmed by the Ct values obtained for the sample.

Quality of Life Analysis

The patients received radiotherapy or surgery followed by chemoradiation as treatment modalities. QOL was assessed using the UW_QOL questionnaire [31] for head and neck cancer patients, which was administered pretreatment and 3 months post treatment.

Statistical Analysis

Data was stored and analyzed on SPSS version 17.0 using chi-squared test, paired *t* test, and Mann-Whitney *U* test.

Results

Demographic and Clinicopathological Profile

Mean age of subjects was 45.56 ± 10.04 years. Of the patients, 58.1 % were below 50 years of age and 86 % patients were males; 79.1 % patients belonged to a rural background, and 97.7 % were Hindus. The patients of OCSCC had one or more addictions viz. 58.1 % were smokers, 48.8 % chewed *pan masala*, 58.1 % chewed tobacco, and 27.9 % consumed alcohol. The most common tumor subsite was gingivo-buccal sulcus seen in 39.5 % subjects, followed by lateral border of tongue in 27.9 % subjects. Histopathological analysis showed that 93 % patients had invasive squamous cell carcinoma while 7 % had verrucous carcinoma. Of the tumors, 69.8 % were well-differentiated. Majority of the patients, i.e., 55.8 %, were well-differentiated.

belonged to TNM stage III. Of the recruited patients, 55.8 % were in a monogamous relationship and 60.5 % never reportedly had an oro-genital contact.

HPV Status

HPV presence was confirmed in only three patients (7.0 %). Table 1 summarizes the clinical, histopathological, demographic, and behavioral characteristics of patients with reference to HPV status. HPV positivity did not find any statistical correlation with age, gender, residence, addiction habit, stage, tumor size, nodal status, tumor grade, and number of sexual contacts.

Quality of Life

There was significant change in pain, activity, swallowing, chewing, taste, saliva, mood, speech, and shoulder from pretreatment to posttreatment. However, there was no significant ($p > 0.05$) difference in the average percent change in QOL parameters from pretreatment to posttreatment when correlated with HPV status (Table 2).

Discussion

HPV has been reported as an etiological agent in the carcinogenesis of HNC especially OPC more so in western literature [1–3, 16]. These HPV-positive HNC have also been observed to have a different subset of clinicopathological characteristics and preferably good response to treatment modalities [26–30]. A few Indian studies have also reported the incidence of HPV in various head and neck cancer across different parts of India [16–20]. In India, tobacco-related OCSCC still remains the most common neoplastic lesion causing grave morbidity [1]. Although the association of HPV-positive OCSCC has been observed in quite a few studies with young age and no history of tobacco/alcohol use, this assumption does not seem to hold true for Indian OCSCC patients [16]. Moreover, numerous studies have reported HPV DNA detection in oral cancer with varying rates, the detection techniques included in situ hybridization and southern blot hybridization (lower sensitivity) or PCR (high sensitivity methods) [32].

The overall prevalence of HPV-associated OCSCC worldwide varies from 0 to 30 %. Mentioning region wise, Indian studies have reported a wide variation in expression of prevalence of HPV-associated OCSCC between 15 and 70.6 % in northeast, eastern, and southern parts [16–20]. Our study stated positivity rate of 4 % in Northern India, which is low, compared to other parts of India.

In other parts of the body such as genital tract, HPV infects exclusively the basal cells of the epithelium, where the virus can remain latent. There is evidence that HPV also infects the

Table 1 Prevalence of HPV status with clinical, histopathological, demographic, and behavioral characteristics of patients

	No. of patients	HPV status				<i>p</i> value ¹
		Positive		Negative		
		No.	%	No.	%	
<hr/>						
Age (years)						
<50	25	3	12.0	22	88.0	0.12
≥50	18	0	0.0	18	100.0	
Gender						
Male	37	3	8.1	34	91.9	0.47
Female	6	0	0.0	6	100.0	
Residence						
Rural	34	3	8.8	31	91.2	0.35
Urban	9	0	0.0	9	100.0	
Smoking						
Yes	25	2	8.0	23	92.0	0.75
No	18	1	5.6	17	94.4	
Pan masala						
Yes	21	1	4.8	20	95.2	0.57
No	22	2	9.1	20	90.0	
Tobacco						
Yes	25	2	8.0	23	92.0	0.75
No	18	1	5.6	17	94.4	
Alcohol						
Yes	12	1	8.3	11	91.7	0.82
No	31	2	6.5	29	93.5	
Histology grade						
Well differentiated	30	3	10.0	27	90.0	0.49
Moderately differentiated	10	0	0.0	10	100.0	
Poorly differentiated	3	0	0.0	3	100.0	
Tumor size						
T1	6	0	0.0	6	100.0	0.76
T2	26	2	7.7	24	92.3	
T3	11	1	9.1	10	90.9	
Nodal status						
N0	4	0	0.0	4	100.0	0.84
N1	25	2	8.0	23	92.0	
N2	14	1	7.1	13	92.9	
AJCC stage						
I	2	0	0.0	2	100.0	0.55
II	2	0	0.0	2	100.0	
III	26	1	3.8	25	96.2	
IV	13	2	15.4	11	84.6	
No. of sexual contacts						
1	24	2	8.3	22	91.7	0.86
2	6	0	0.0	6	100.0	
3	6	1	16.7	5	83.3	
4	3	0	0.0	3	100.0	
≥5	4	0	0.0	4	100.0	

gingival tissue with periodontal pocket; the only location that is exposed to the external environment [33–35].

Table 2 Average percentage change from pretreatment to posttreatment in QOL parameters according to HPV status

QOL parameters	HPV		<i>p</i> value ^a
	Positive (<i>n</i> = 3)	Negative (<i>n</i> = 40)	
Pain	27.4 ± 40.5	11.1 ± 19.2	0.49
Appearance	3.00 ± 33.1	0.00 ± 0.00	NA
Activity	13.0 ± 38.3	0.00 ± 0.00	NA
Recreation	11.7 ± 34.0	0.00 ± 0.00	NA
Swallowing	17.7 ± 32.2	11.1 ± 19.2	0.72
Chewing	10.8 ± 45.3	0.00 ± 0.00	NA
Taste	32.0 ± 32.0	11.1 ± 19.2	0.27
Saliva	36.2 ± 18.5	16.6 ± 28.8	0.09
Mood	40.1 ± 36.7	38.8 ± 34.6	0.95
Anxiety	33.7 ± 80.1	44.4 ± 9.6	0.10
Speech	31.3 ± 59.2	33.3 ± 76.3	0.95
Shoulder	14.7 ± 17.8	0.00 ± 0.00	NA

^a Mann-Whitney *U* test

Wide-ranging studies analyzing large number of patients and describing anatomic subsites, as well HPV DNA frequency per site, indicate that HPV can be found in any of the subsites [8, 10, 22, 36–40]. Subsite frequencies are varying between studies, but floor of mouth (9–42 %) and the tongue (8–25 %) seem to be predominantly infected by HPV. Though HPV DNA is present in a reasonable subgroup of OCSCC, it has been shown that not all of HPV DNA-positive tumors can be regarded as etiologically HPV driven [7, 41, 42].

Literature is present for the association of HPV-positive OPC with younger age and sexual contact. HPV-associated OPC has distinct clinical, pathological, molecular, and epidemiologic features [43]. Our data show that HPV-related OCSCC similarly has predilection for younger age (all positive were <50 years of age) and male gender. Pathologically, all HPV-positive tumors were well differentiated. The HPV positivity in OCSCC was minute in the study that may have confounded the role of sexual contact, pan masala, tobacco, smoking, and alcohol as risk factors for HPV infection.

There is conflicting data on the association of tobacco with HPV infection [7, 44]. We observed in our data that tobacco consumption in any form is a risk factor, and offenders have higher rate of HPV infection. Nicotine modifying antigen-mediated signaling pathways leading to alterations in immune functions may lead to increased chances of viral infection [45]. Conversely, in the present study, we could not clearly show the association of HPV positivity with alcohol consumption. But, in recent studies, we have shown that alcohol intake was associated with significantly increased risk of HPV infection in men [46, 47]. We therefore suggest that consumption of tobacco and alcohol leads to increased vulnerability for HPV infection in Indians, same as worldwide.

Some research groups have advocated that HPV-positive and negative HNC cases should not only be considered on the basis of clinicopathological entities but also on the basis of response to different treatment modalities [32, 43, 48]. Several studies have shown that patients with HPV-positive OPC have a significantly improved overall and disease-free survival compared to patients with HPV-negative OPC patients [7, 49–55]. HPV-positive OPC patients have a better prognosis because of varied reasons including harboring fewer or different genetic alterations, which can be associated with better response to therapy [56, 57], having higher radiosensitivity, probably due to intact apoptotic response to radiation [53, 58], absence of field cancerization [49], and immunologic response playing a role in the improved response to radiotherapy and chemotherapy [59, 60], and lastly, association of younger age, good performance status, and fewer comorbidities may also contribute to improved survival [61]. All these data suggest that HPV status can be used in the clinical decision-making practices to less intensify the treatment offered to this particular subset of patients. In contrast to the clear picture in OPC [7, 51, 62], where the prognostic relevance of biologically active HPV infection is established, no such clear association has been found in OCSCC. Published literature is divided between the improved likelihood for HPV-positive OCSCC on one hand [63, 64] and worst outcome on the other [65–67] with no clout on patient survival [10, 37, 68].

Patients with HPV-related HNC have higher rates of survival, which has been assumed to be at least partially due to an increased responsiveness in this subset with unique tumor biology. Given the improved survival in patients with HPV-related HNC, it has been suggested that patients with HPV-positive tumors would have better QOL trails. Surprisingly, the published literature seems not to fall in line with this understanding. In a principal report, Sharma et al. [69] did not find a difference in 1-year posttreatment QOL or change in QOL from pretreatment to 1-year posttreatment by HPV status in patients of OCSCC and OPC combined. However, they found that patient with HPV tumors had higher pre treatment QOL and a greater decrease in QOL from pretreatment to immediate posttreatment. On multivariate analysis controlling for tumor site, this association did not remain significant. Lately, Maxwell et al. [70] determined the posttreatment QOL (using same questionnaire as our study) in the head and neck cancer (including OCSCC and OPC) patients, classifying by HPV status and primary treatment modality (surgical vs nonsurgical) and showed that HPV patients had better QOL scores in speech ($P = 0.0009$), chewing ($P = 0.0004$), and swallowing ($P = 0.021$) after 1 year posttreatment compared to HPV-negative patients. Primary treatment modality did not affect overall QOL or any of the QOL categories in HPV-positive patients. In our study though, there was no statistical difference in QOL parameters (pain, appearance, activity, etc.) between HPV-positive and negative OCSCC patients

after 3 months posttreatment but nearly all parameters showed more average percentage change in HPV-positive cases. Some patients were missing QOL data in the report by Sharma et al. at certain time points, which could be biasing the results, and the majority of the study population was white. Therefore, the results may not be representative of more diverse populations of HNC patients especially in India. To the best of our knowledge, this study for the first time reports a comparison between HPV-positive and HPV-negative cases in terms of quality of life to various modalities in Indian population.

Compliance with Ethical Standards Tissue biopsies were collected after obtaining approval from the Institutional Ethics Committee (vide no. 65th ECM II-C/P4) and written informed consent from the patients.

Conflicts of Interest The authors declare that they have no conflict of interest.

Financial Support None

References

- Coelho KR (2012) Challenges of the oral cancer burden in India. *J Cancer Epidemiol* 2012:701932
- Sankaranarayanan R, Ramadas K, Thomas G et al (2005) Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial. *Lancet* 365(9475):1927–1933
- IARC (2004) Tobacco smoke and involuntary smoking. *IARC Monogr Eval Carcinog Risks Hum* 83:1–1438
- IARC (2007) Smokeless tobacco and some tobacco-specific N-nitrosamines. *IARC Monogr Eval Carcinog Risks Hum* 89:1–592
- IARC (1988) Alcohol drinking. *Epidemiological studies of cancer in humans. IARC Monogr Eval Carcinog Risks Hum* 44:153–250
- Syrjanen K, Syrjanen S, Lamberg M et al (1983) Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *Int J Oral Surg* 12:418–424
- Ang KK, Harris J, Wheeler R et al (2010) Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 363:24–35
- Syrjanen S, Lodi G, von Bultzingslowen I et al (2011) Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. *Oral Dis* 17(Suppl 1):58–72
- Kreimer AR, Villa A, Nyitray AG et al (2011) The epidemiology of oral HPV infection among a multinational sample of healthy men. *Cancer Epidemiol Biomark Prev* 20:172–182
- Isayeva T, Li Y, Maswahu D, Brandwein-Gensler M (2012) Human papillomavirus in non-oropharyngeal head and neck cancers: a systematic literature review. *Head Neck Pathol* 6(Suppl 1):S104–S120
- Gillison ML, Broutian T, Pickard RK et al (2012) Prevalence of oral HPV infection in the United States, 2009–2010. *JAMA* 307:693–703
- Pickard RK, Xiao W, Broutian TR et al (2012) The prevalence and incidence of oral human papillomavirus infection among young men and women, aged 18–30 years. *Sex Transm Dis* 39:559–566
- Bui TC, Markham CM, Ross MW, Mullen PD (2013) Examining the association between oral health and oral HPV infection. *Cancer Prevent Res* 6:917–924
- Lang Kuhs KA, Gonzalez P, Struijk L et al (2013) Prevalence and risk factors for oral human papillomavirus among young women in Costa Rica. *J Infect Dis* 208:1643–1652
- Kero K, Rautava J, Syrjanen K, Grenman S, Syrjanen S (2012) Oral mucosa as a reservoir of human papillomavirus: point prevalence, genotype distribution, and incident infections among males in a 7-year prospective study. *Eur Urol* 62:1063–1070
- Kumar R, Rai AK, Das D et al (2015) Alcohol and tobacco increases risk of high risk HPV infection in head and neck cancer patients: study from north-east region of India. *PLoS One* 10(10):e0140700
- Koppikar P, deVilliers EM, Mulherkar R (2005) Identification of human papillomaviruses in tumors of the oral cavity in an Indian community. *Int J Cancer* 113(6):946–950
- Mishra A, Bharti AC, Varghese P et al (2006) Differential expression and activation of NF- κ B family proteins during oral carcinogenesis: role of high risk human papilloma infection. *Int J Cancer* 119:2840–2850
- Chaudhary AK, Pandya S, Singh M et al (2013) Identification of high-risk human papillomavirus (HPV-16 & 18) infection by multiplex PCR (MPX-PCR) and its expression in oral sub mucous fibrosis and oral squamous cell carcinoma. *Head Neck Oncol* 5(1):4
- Kulkarni SS, Kulkarni SS, Vastrad PP et al (2011) Prevalence and distribution of high risk human papillomavirus (HPV) types 16 and 18 in carcinoma of cervix, saliva of patients with oral squamous cell carcinoma and in the general population in Karnataka, India. *Asian Pac J Cancer Prev* 12:645–648
- D'Souza G, Agrawal Y, Halpern J et al (2009) Oral sexual behaviors associated with prevalent oral human papillomavirus infection. *J Infect Dis* 199:1263–1269
- Chung CH, Bagheri A, D'Souza G (2014) Epidemiology of oral human papillomavirus infection. *Oral Oncol* 50:364–369
- Beachler DC, D'Souza G (2013) Oral human papillomavirus infection and head and neck cancers in HIV-infected individuals. *Curr Opin Oncol* 25:503–510
- Varnai AD, Bollmann M, Bankfalvi A et al (2009) The prevalence and distribution of human papillomavirus genotypes in oral epithelial hyperplasia: proposal of a concept. *J Oral Pathol Med* 38:181–187
- Garnaes E, Kiss K, Andersen L et al (2015) A high and increasing HPV prevalence in tonsillar cancers in Eastern Denmark, 2000–2010: the largest registry-based study to date. *Int J Cancer* 136:2196–2203
- Koyama K, Uobe K, Tanaka A (2007) Highly sensitive detection of HPV-DNA in paraffin sections of human oral carcinomas. *J Oral Pathol Med* 36:18–24
- Tural D, Eliçin O, Batur S et al (2013) Human papillomavirus is independent prognostic factor on outcome of oropharyngeal squamous cell carcinoma. *Tumour Biol* 34:3363–3369
- Argiris A, Li S, Ghebremichael M, Egloff AM et al (2014) Prognostic significance of human papillomavirus in recurrent or metastatic head and neck cancer: an analysis of Eastern Cooperative Oncology Group trials. *Ann Oncol* 25:1410–1416
- Vainshtein JM, Spector ME, McHugh JB et al (2014) Refining risk stratification for locoregional failure after chemoradiotherapy in human papillomavirus-associated oropharyngeal cancer. *Oral Oncol* 50:513–519
- Liang C, Marsit CJ, McClean MD et al (2012) Biomarkers of HPV in head and neck squamous cell carcinoma. *Cancer Res* 72:5004–5013
- D'Cruz AK, Yueh B, Das AK et al (2007) Validation of the University of Washington quality of life questionnaires for head and neck cancer patients in India. *Indian J Cancer* 44(4):147–154
- Goon PK, Stanley MA, Ebmeyer J et al (2009) HPV & head and neck cancer: a descriptive update. *Head & Neck Oncol* 1:36

33. Madinier I, Doglio A, Cagnon L et al (1992) Southern blot detection of human papillomaviruses (HPVs) DNA sequences in gingival tissues. *J Periodontol* 63:667–673
34. Hormia M, Willberg J, Ruokonen H, Syrjanen S (2005) Marginal periodontium as a potential reservoir of human papillomavirus in oral mucosa. *J Periodontol* 76:358–363
35. Tezal M (2012) Interaction between chronic inflammation and oral HPV infection in the etiology of head and neck cancers. *Int J Otolaryngol* 2012:575242
36. Reuschenbach M, Kansy K, Garbe K et al (2013) Lack of evidence of human papillomavirus-induced squamous cell carcinomas of the oral cavity in southern Germany. *Oral Oncol* 49:937–942
37. Lingen MW, Xiao W, Schmitt A et al (2013) Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. *Oral Oncol* 49:1–8
38. Duncan LD, Winkler M, Carlson ER et al (2013) p16 immunohistochemistry can be used to detect human papillomavirus in oral cavity squamous cell carcinoma. *J Oral Maxillofac Surg* 71:1367–1375
39. Walline HM, Komarck C, McHugh JB et al (2013) High-risk human papillomavirus detection in oropharyngeal, nasopharyngeal, and oral cavity cancers: comparison of multiple methods. *JAMA otolaryngol– head Neck Surg* 139:1320–1327
40. Upile NS, Shaw RJ, Jones TM et al (2014) Squamous cell carcinoma of the head and neck outside the oropharynx is rarely human papillomavirus related. *Laryngoscope* 124(12):2739–2744
41. van Houten VM, Snijders PJ, van den Brekel MW et al (2001) Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. *Int J Cancer* 93:232–235
42. Wiest T, Schwarz E, Enders C et al (2002) Involvement of intact HPV16 E6/E7 gene expression in head and neck cancers with unaltered p53 status and perturbed pRb cell cycle control. *Oncogene* 21:1510–1517
43. Chau NG, Rabinowits G, Haddad RI (2014) Human papillomavirus-associated oropharynx cancer (HPV-OPC): treatment options. *Curr Treat Options in Oncol* 15:595–610
44. Jamaly S, Khanekhenari MR, Rao R et al (2014) Relationship between p53 overexpression, human papillomavirus infection, and lifestyle in Indian patients with head and neck cancers. *Tumour Biol* 33:543–550
45. Mehta H, Nazzal K, Sadikot RT (2008) Cigarette smoking and innate immunity. *Inflamm Res* 57:497–503
46. HY O, Seo SS, Kim MK et al (2014) Synergistic effect of viral load and alcohol consumption on the risk of persistent high-risk human papillomavirus infection. *PLoS One* 9:e104374
47. Schabath MB, Thompson ZJ, Egan KM et al (2015) Alcohol consumption and prevalence of human papillomavirus (HPV) infection among US men in the HPV in men (HIM) study. *Sex Transm Infect* 91(1):61–67
48. Kumar RV, Kadkol SS, Daniel R et al (2003) Human papillomavirus, p53 and cyclin D1 expression in oropharyngeal carcinoma. *Int J Oral Maxillofac Surg* 32:539–543
49. Chaturvedi AK (2012) Epidemiology and clinical aspects of HPV in head and neck cancers. *Head and Neck Pathol* 6:S16–S24
50. Olshan AF (ed) (2010) Epidemiology, pathogenesis, and prevention of head and neck cancer. Springer, New York
51. Marur S, D'Souza G, Westra WH et al (2010) HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol* 11: 781–789
52. Chung CH, Gillison ML (2009) Human papillomavirus in head and neck cancer: its role in pathogenesis and clinical implications. *Clin Cancer Res* 15:6758–6762
53. 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* 100:261–269
54. Rischin D, Young RJ, Fisher R et al (2010) Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. *J Clin Oncol* 28:4142–4148
55. Lassen P, Eriksen JG, Krogdahl A et al (2011) The influence of HPV associated p16 expression on accelerated fractionated radiotherapy in head and neck cancer: evaluation of the randomized DAHANCA 6&7 trial. *Radiother Oncol* 100:49–55
56. Klussmann JP, Mooren JJ, Lehnen M et al (2009) Genetic signatures of HPV-related and unrelated oropharyngeal carcinoma and their prognostic implications. *Clin Cancer Res* 15:1779–1786
57. Stransky N, Egloff AM, Tward AD et al (2011) The mutational landscape of head and neck squamous cell carcinoma. *Science* 333(6046):1157–1160
58. Lindel K, Beer KT, Laissue J et al (2001) Human papillomavirus positive squamous cell carcinoma of the oropharynx: a radiosensitive subgroup of head and neck carcinoma. *Cancer* 92:805–813
59. Sorensen BS, Busk M, Horsman MR et al (2014) Effect of radiation on cell proliferation and tumor hypoxia in HPV positive head and neck cancer in vivo models. *Anticancer Res* 34:6297–6304
60. Won HS, Lee YS, Jeon EK et al (2014) Clinical outcome of induction chemotherapy in locally advanced head and neck squamous cell carcinoma. *Anticancer Res* 34:5709–5714
61. Ang KK, Sturgis EM (2012) Human papillomavirus as a marker of the natural history and response to therapy of head and neck squamous cell carcinoma. *Semin Radiat Oncol* 22:128–142
62. 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* 92:709–720
63. Schwartz SR, Yueh B, McDougall JK et al (2001) Human papillomavirus infection and survival in oral squamous cell cancer: a population based study. *Otolaryngol Head Neck Surg* 125:1–9
64. Elango KJ, Suresh A, Erode EM et al (2011) Role of human papilloma virus in oral tongue squamous cell carcinoma. *Asian Pac J Cancer Prev* 12:889–896
65. Kozomara R, Jovic N, Magic Z et al (2005) p53 mutations and human papillomavirus infection in oral squamous cell carcinomas: correlation with overall survival. *J Craniomaxillofac Surg* 33:342–348
66. Duray A, Descamps G, Decaestecker C et al (2012) Human papillomavirus DNA strongly correlates with a poorer prognosis in oral cavity carcinoma. *Laryngoscope* 122:1558–1565
67. Lee LA, Huang CG, Liao CT et al (2012) Human papillomavirus-16 infection in advanced oral cavity cancer patients is related to an increased risk of distant metastases and poor survival. *PLoS One* 7: e40767
68. Annertz K, Rosenquist K, Andersson G et al (2014) High-risk HPV and survival in patients with oral and oropharyngeal squamous cell carcinoma - 5-year follow up of a population-based study. *Acta Otolaryngol* 134:843–851
69. Sharma A, Méndez E, Yueh B et al (2012) Human papillomavirus-positive oral cavity and oropharyngeal cancer patients do not have better quality-of-life trajectories. *Otolaryngol Head Neck Surg* 146(5):739–745
70. Maxwell JH, Mehta V, Wang H et al (2014) Quality of life in head and neck cancer patients: impact of HPV and primary treatment modality. *Laryngoscope* 124(7):1592–1597